SURAT PERJANJIAN PENUGASAN PELAKSANAAN HIBAH PENELITIAN BAGI DOSEN PERGURUAN TINGGI SWASTA DI LINGKUNGAN KOPERTIS WILAYAH IV TAHUN ANGGARAN 2016

Nomor	:2403 /K4/KM/2016
Tanggal	: 27 Mei 2016

Antara

KOORDINASI PERGURUAN TINGGI SWASTA WILAYAH IV
DIREKTORAT JENDERAL RISET DAN PENGEMBANGAN
KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI

Dengan

Ketua LPPM
Universitas Kristen Maranatha

Jumlah dana sebesar Rp. 1.494.000.000,-
Satu milyar empat ratus sembilan puluh empat juta

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
KOORDINASI PERGURUAN TINGGI SWASTA WILAYAH IV
Jalan Penghulu Haji Hasan Mustafa No. 38 Bandung - 40124
Telp. (022) 7275630, 7274377, Fax. (022) 7207812
Laman: www.kopertis4.or.id – email: sisinfo_kopwil4@yahoo.com

SURAT PERJANJIAN PENUGASAN PELAKSANAAN HIBAH PENELITIAN BAGI DOSEN PERGURUAN TINGGI SWASTA TAHUN ANGGARAN 2016
Nomor : 2983 /K4/KM/2016

Pada hari ini **Jumat** tanggal **27** bulan **Mei** tahun **Dua Ribu Enam Belas**, kami yang bertandatangan dibawah ini :

1. Prof. Dr. Ir. Abdul Hakim Halim, M.Sc : Kuasa Pengguna Anggaran Kopertis Wilayah IV Kementerian Riset, Teknologi dan Pendidikan Tinggi yang berkedudukan di Bandung berdasarkan Keputusan Menteri Pendidikan dan Kebudayaan Republik Indonesia Nomor No. 222/MPK.A4/KP/2013 tanggal 24 Juni 2013 untuk selanjutnya disebut **PIHAK PERTAMA**;

2. Prof. Dr. dr. Susy Tjahjani, M.Kes. : Ketua LPPM **Universitas Kristen Maranatha** yang berkedudukan di **Bandung** dalam hal ini bertindak untuk dan atas nama Perguruan Tinggi tersebut untuk selanjutnya disebut **PIHAK KEDUA**.

Perjanjian penugasan ini berdasarkan kepada :
1. Undang-Undang Republik Indonesia Nomor 17 Tahun 2003, tentang Keuangan Negara.
2. Undang-Undang Republik Indonesia Nomor 20 Tahun 2003, tentang Sistem Pendidikan Nasional.
3. Undang-Undang Republik Indonesia Nomor 01 Tahun 2004, tentang Perbendaharaan Negara.
4. Undang-Undang Republik Indonesia Nomor 15 Tahun 2004, tentang Pemeriksaan dan Tanggung Jawab Keuangan Negara.
5. Undang-Undang Republik Indonesia Nomor 12 Tahun 2012 tentang Pendidikan Tinggi.
6. Undang-Undang Nomor 39 Tahun 2008 tentang kementerian Negara (Lembaga Negara Republik Indonesia Tahun 2008 Nomor 166, Tambahan Lembaran Negara Republik Indonesia Nomor 4916):
7. Peraturan Presiden Nomor 7 Tahun 2015 tentang Organisasi Kementerian Negara (Lembaran Negara Republik Indonesia Tahun 2015 Nomor 8);
8. Peraturan Presiden Nomor 13 Tahun 2015 tentang Kementerian Riset, Teknologiu, dan Pendidikan Tinggi (Lembaran NegaraRepublik Indonesia Tahun 2015 Nomor 14);
9. Keputusan Presiden Nomor 121/P Tahun 2014 tentang Pembentukan Kementerian dan Pengangkatan Menteri Kabinet Kerja Periode 2014-2019;
10. Peraturan Menteri Riset, Teknologi dan Pendidikan Tinggi Republik Indonesia Nomor 15 Tahun 2015, tentang Organisasi dan Tata Kerja Kementerian Riset, Teknologi dan Pendidikan Tinggi;
11. Keputusan Direktur Riset dan Pengabdian Kepada Masyarakat Nomor 0299/E3/2016 tentang Penerima Hibah Penelitian dan Pengabdian Masyarakat di Perguruan Tinggi Tahun 2016;
12. Daftar Isian Pelaksanaan Anggaran (DIPA) Nomor SP DIPA-042.06.1.401516/2016 tanggal 7 Desember 2015.

PIHAK PERTAMA dan **PIHAK KEDUA** secara bersama-sama bersepakat mengikatkan diri dalam suatu Perjanjian Pelaksanaan Hibah Penelitian dengan ketentuan dan syarat-syarat diatur dalam Pasal-Pasal berikut :

Pasal 1

(1) **PIHAK PERTAMA** memberi tugas kepada **PIHAK KEDUA**, dan **PIHAK KEDUA** menerima tugas tersebut untuk mengkoordinir dan sebagai penanggungjawab Penugasan Pelaksanaan Hibah Penelitian yang dilakukan oleh dosen perguruan tinggi di **Universitas Kristen Maranatha.**

(2) **PIHAK KEDUA** bertanggungjawab penuh atas pelaksanaan, administrasi dan keuangan atas pekerjaan/kegiatan sebagaimana dimaksud pada ayat (1) dan berkewajiban menyimpan semua bukti-bukti pengeluaran serta dokumen pelaksanaan lainnya.

(3) Penugasan Pelaksanaan Hibah Penelitian sebagaimana dimaksud pada ayat (1) sebanyak 27 (Dua puluh tujuh) judul dengan total dana sebesar **Rp. 1.494.000.000,- (Satu milyar empat ratus sembilan puluh empat juta)** dibebankan pada Daftar Isian Pelaksanaan Anggaran (DIPA) Nomor SP DIPA-042.06.1.401516/2016 tanggal 7 Desember 2015.

(4) Daftar nama ketua pelaksana, judul, dan besarnya biaya setiap judul yang telah disetujui untuk didanai tercantum dalam Lampiran yang merupakan bagian yang tidak terpisahkan dari Surat Perjanjian ini.

Pasal 2

(1) **PIHAK PERTAMA** memberikan dana untuk kegiatan sebagaimana dimaksud dalam Pasal 1 sebesar **Rp. 1.494.000.000,-** (Satu milyar empat ratus sembilan puluh empat juta) yang dibebankan kepada DIPA Nomor SP DIPA-042.06.1.401516/2016 tanggal 7 Desember 2015.

(2) Dana pelaksanaan Hibah sebagaimana dimaksud pada ayat (1) dibayarkan oleh **PIHAK PERTAMA** kepada **PIHAK KEDUA** secara bertahap dengan ketentuan sebagai berikut:

a) Pembayaran Tahap Pertama sebesar 70% dari total bantuan dana kegiatan yaitu 70% X **Rp. 1.494.000.000,- = Rp. 1.045.800.000,- (Satu milyar empat puluh lima juta delapan ratus ribu)**

b) Pembayaran Tahap Kedua/Terakhir sebesar 30% dari total bantuan dana kegiatan yaitu 30% X **Rp. 1.494.000.000,- = Rp. 448.200.000,- (Empat ratus empat puluh delapan juta dua ratus ribu),** dibayarkan setelah **PIHAK KEDUA** mengunggah ke **SIM-LITABMAS** selambat-lambatnya tanggal **15 Juli 2016** dokumen di bawah ini:
   1. Catatan harian dan laporan penggunaan anggaran 70%
   2. Laporan kemajuan pelaksanaan pekerjaan

(3) **PIHAK KEDUA** bertanggungjawab mutlak dalam pembelanjaan dana tersebut pada ayat (1) sesuai dengan proposal kegiatan yang telah disetujui dan berkewajiban untuk menyimpan semua bukti-bukti pengeluaran sesuai dengan jumlah dana yang diberikan oleh **PIHAK PERTAMA.**

(4) **PIHAK KEDUA** berkewajiban mengembalikan sisa dana yang tidak dibelanjakan ke Kas Negara

(5) **PIHAK KEDUA** berkewajiban menyampaikan foto copy bukti pengembalian Dana ke Kas Negara yang telah divalidasi oleh KPPN setempat kepada **PIHAK PERTAMA**.

Pasal 3

(1) Dana Hibah Penelitian sebagaimana dimaksud Pasal 2 ayat (1) dibayarkan kepada Institusi/Lembaga Perguruan Tinggi sebagai berikut :

Nama Perguruan Tinggi	:	**Universitas Kristen Maranatha**
Nomor Rekening	:	**0023653578**
Nama penerima pada rekening	:	**YPTK Maranatha**
Nama Bank	:	**BNI**
Alamat Bank	:	**Jl. Perintis Kemerdekaan No. 3**
Kota	:	**Bandung**
NPWP Perguruan Tinggi	:	**01.210.846.0-441.000**

(2) **PIHAK PERTAMA** tidak bertanggungjawab atas keterlambatan dan/atau tidak terbayarnya sejumlah dana sebagaimana dimaksud dalam Pasal 2 ayat (1) yang disebabkan karena kesalahan **PIHAK KEDUA** dalam mengisi data lembaga, nama bank, nomor rekening, alamat, dan persyaratan lainnya yang tidak sesuai dengan ketentuan.

Pasal 4

(1) **PIHAK KEDUA** berkewajiban untuk membuat Surat Perjanjian Penugasan Pelaksanaan Hibah Penelitian dengan masing-masing ketua pelaksana untuk pengaturan hak dan kewajiban setiap pelaksana di lingkungan perguruan tingginya yang berisi antara lain: nama pelaksana, judul penelitian, jumlah dana hibah, tatacara/termin pembayaran, waktu pelaksanaan, batas akhir pelaporan penugasan dan penggunaan keuangan, dan sanksi.

(2) Penilaian kemajuan pelaksanaan hibah penelitian sebagaimana dimaksud pada ayat (1) dilakukan oleh **PIHAK KEDUA**, setelah ketua pelaksana mengunggah laporan kemajuan pelaksanaan kegiatan ke SIM-LITABMAS, dengan berpedoman kepada prinsip-prinsip dan atau kaidah Program Penelitian.

(3) Perubahan terhadap susunan tim pelaksana dan substansi pelaksanaan hibah penelitian dapat dibenarkan apabila telah mendapat persetujuan tertulis dari Direktur Riset dan Pengabdian Masyarakat Direktorat Jenderal Penguatan Riset dan Pengembangan

Pasal 5

(1) **PIHAK KEDUA** berkewajiban untuk menindaklanjuti dan mengupayakan Hibah Penelitian yang dilakukan dosen untuk memperoleh paten dan/atau publikasi ilmiah untuk setiap judul-judul Hibah Penelitian sebagaimana dimaksud Pasal 1 ayat (1).

(2) Perolehan sebagaimana dimaksud pada ayat (1) dimanfaatkan sebesar-besarnya untuk pelaksanaan tridharma perguruan tinggi;

(3) **PIHAK KEDUA** berkewajiban untuk melaporkan perkembangan perolehan paten dan/atau publikasi ilmiah seperti yang dimaksud pada ayat (1) secara berkala kepada **PIHAK PERTAMA** pada setiap akhir Tahun Anggaran berjalan.

Pasal 6

(1) **PIHAK KEDUA** harus menyampaikan Surat Pernyataan telah menyelesaikan seluruh pekerjaan yang dibuktikan dengan pengunggahan pada SIMLITABMAS.
   a. Catatan harian dan penggunaan dana 30%, pada tanggal **15 Oktober 2016**
   b. Catatan akhir, laporan keuangan 100%, capaian hasil, poster, artikel ilmiah dan profile, pada tanggal **31 Oktober 2016**

(2) Apabila sampai dengan batas waktu yang telah ditetapkan untuk melaksanakan Hibah Program Penelitian telah berakhir, **PIHAK KEDUA** belum menyelesaikan tugasnya dan atau terlambat mengirim laporan Kemajuan dan atau terlambat mengirim laporan akhir, maka **PIHAK KEDUA** dikenakan sanksi denda sebesar 1 ‰ (satu permil) setiap hari keterlambatan sampai dengan setinggi-tingginya 5% (lima persen), terhitung dari tanggal jatuh tempo sebagaimana tersebut pada ayat (1),(2) dan (3), yang terdapat dalam Surat Perjanjian Penugasan Pelaksanaan Hibah Penelitian bagi Dosen Perguruan Tinggi Swasta Tahun Anggaran 2016.

(3) Peneliti/Pelaksana Hibah Penelitian yang tidak hadir dalam kegiatan Monitoring dan Evaluasi serta Seminar Hasil Hibah Penelitian tanpa pemberitahuan sebelumnya ke Direktur Riset dan Pengabdian Masyarakat, maka Pelaksana Hibah Penelitian tidak berhak menerima sisa dana penugasan tahap kedua sebesar 30%. PIHAK KEDUA harus mengembalikan dana penugasan 30% yang telah diterima ke Kas Negara.

(4) Denda sebagaimana dimaksud pada ayat (2) disetorkan ke Kas Negara dan menyerahkan foto copy bukti pengembalian Dana ke Kas Negara yang telah divalidasi oleh KPPN setempat kepada **PIHAK PERTAMA.**

Pasal 7

(1) Laporan hasil Hibah Penelitian sebagaimana tersebut pada pasal 6 ayat (1) harus memenuhi ketentuan sebagai berikut:
   1. Bentuk/ukuran kertas A4;
   2. Warna cover (disesuaikan dengan ketentuan di perguruan tinggi masing-masing);
   3. Di bawah bagian kulit ditulis:

Dibiayai oleh
Direktur Riset dan Pengabdian Masyarakat
Direktorat Jenderal Penguatan Riset dan Pengembangan
Kementerian Riset, Teknologi, dan Pendidikan Tinggi
Sesuai dengan Surat Perjanjian Penugasan Pelaksanaan Hibah Penelitian
Nomor: 105/SP2H/PPM/DRPM/II/2016, tanggal 17 Februari 2016

(2) Softcopy laporan hasil Hibah Penelitian sebagaimana tersebut pada ayat (1) harus diunggah ke SIM-LITABMAS sedangkan hardcopy wajib disimpan oleh **PIHAK KEDUA**.

Pasal 8

(1) Apabila **PIHAK KEDUA** berhenti dari jabatannya, sebelum pelaksanaan perjanjian ini selesai, maka **PIHAK KEDUA** wajib menyerah terimakan tanggung jawabnya kepada pejabat baru yang menggantikannya.

(2) Apabila setiap ketua pelaksana penelitian di perguruan tinggi sebagaimana dimaksud dalam Pasal 4 ayat (1) tidak dapat melaksanakan Penelitian ini, maka **PIHAK KEDUA** wajib menunjuk pengganti ketua pelaksana penelitian yang merupakan salah satu anggota tim setelah mendapat persetujuan tertulis dari Direktur Riset dan Pengabdian Masyarakat Direktorat Jenderal Penguatan Riset dan Pengembangan;

(3) Apabila **PIHAK KEDUA** tidak dapat melaksanakan tugas sebagaimana dimaksud dalam Pasal 1 maka harus mengembalikan dana yang telah diterimanya ke Kas Negara serta menyerahkan fotocopy bukti pengembalian ke kas Negara yang telah divalidasi oleh KPPN setempat kepada **PIHAK PERTAMA.**

(4) Apabila dikemudian hari terbukti bahwa judul-judul Penelitian sebagaimana dimaksud dalam Pasal 1 dijumpai adanya duplikasi dengan Penelitian lain dan/atau diperoleh indikasi ketidak jujuran/itikad kurang baik yang tidak sesuai dengan kaidah ilmiah, maka kegiatan Penelitian tersebut dinyatakan batal dan **PIHAK KEDUA** wajib melaporkan ke **PIHAK PERTAMA** dan mengembalikan dana Penelitian yang telah diterima ke Kas Negara serta menyerahkan fotocopy bukti pengembalian ke kas Negara yang telah divalidasi oleh KPPN setempat kepada **PIHAK PERTAMA.**

Pasal 9

PIHAK KEDUA berkewajiban memungut dan menyetor pajak ke kantor pelayanan pajak setempat yang berkenaan dengan kewajiban pajak berupa:

1. pembelian barang dan jasa dikenai PPN sebesar 10% dan PPh 22 sebesar 1,5%;
2. belanja honorarium dikenai PPh Pasal 21 dengan ketentuan:
   a. 5% bagi yang memiliki NPWP untuk golongan III, serta 6% bagi yang tidak memiliki NPWP;
   b. untuk golongan IV sebesar 15%; dan
3. pajak–pajak lain sesuai ketentuan yang berlaku.

Pasal 10

(1) Hak Kekayaan Intelektual yang dihasilkan dari pelaksanaan Penelitian tersebut diatur dan dikelola sesuai dengan peraturan dan perundang-undangan yang berlaku.

(2) Hasil Penelitian berupa peralatan dan/atau alat yang dibeli dari kegiatan ini adalah milik negara yang dapat dihibahkan kepada institusi/lembaga/masyarakat melalui Surat Keterangan Hibah.

Pasal 11

(1) Apabila terjadi perselisihan antara **PIHAK PERTAMA** dan **PIHAK KEDUA** dalam pelaksanaan perjanjian ini akan dilakukan penyelesaian secara musyawarah dan mufakat dan apabila tidak tercapai penyelesaian secara musyawarah dan mufakat maka penyelesaian dilakukan melalui proses Hukum yang berlaku dengan memilih domisili Hukum di Pengadilan Bandung.

(2) Hal-hal yang belum diatur dalam perjanjian ini akan diatur kemudian oleh kedua belah pihak.

Pasal 12

Surat Perjanjian Pelaksanaan ini dibuat rangkap 3 (tiga) bermaterai cukup sesuai dengan ketentuan yang berlaku, dan biaya materai dibebankan kepada **PIHAK KEDUA**.

PIHAK PERTAMA	**PIHAK KEDUA**
METERAI TEMPEL 6000 ENAM RIBU RUPIAH	UNIVERSITAS KRISTEN MARANATHA BANDUNG
Prof. Dr. Ir. Abdul Hakim Halim, M.Sc	Prof. Dr. dr. Susy Tjahjani, M.Kes.
NIP. 195609141986011001	

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
KOORDINASI PERGURUAN TINGGI SWASTA WILAYAH IV

Tahun Anggaran : 2016
Nomor Bukti :
Mata Anggaran :

KUITANSI

Sudah terima dari : KUASA PENGGUNA ANGGARAN KOPERTIS WILAYAH IV

Uang sebesar (dengan huruf) : **== Rp. 1.045.800.000,-==**
Satu milyar empat puluh lima juta delapan ratus ribu rupiah.

Untuk pembayaran : Biaya Penugasan Pelaksanaan Hibah Penelitian Tahun 2016 Tahap I (Satu) sebesar 70%, sesuai SP3 No.2403/K4/KM//2016

Rp. 1.045.800.000,-

Bandung, 27 Mei 2016

Kuasa Pengguna Anggaran Kopertis Wilayah IV,	Bendahara Pengeluaran Kopertis Wilayah IV,	Ketua LPPM Universitas Kristen Maranatha,
Prof. Dr. Ir. Abdul Hakim Halim, M.Sc.	Tarya Sutaryo, S.Sos.	Prof. Dr. dr. Susy Tjahjani, M.Kes.
NIP. 195609141986011001	NIP. 196402101987021001	

METERAI TEMPEL 4DDB5ADF495602292 6000 ENAM RIBU RUPIAH

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
KOORDINASI PERGURUAN TINGGI SWASTA WILAYAH IV

Tahun Anggaran : 2016
Nomor Bukti :
Mata Anggaran :

KUITANSI

Sudah terima dari : KUASA PENGGUNA ANGGARAN KOPERTIS WILAYAH IV

Uang sebesar (dengan huruf) : **== Rp. 448.200.000,-==**
Empat ratus empat puluh delapan juta dua ratus ribu rupiah.

Untuk pembayaran : Biaya Penugasan Pelaksanaan Hibah Penelitian Tahun 2016 Tahap II (Dua) sebesar 30%, sesuai SP3 No.2403/K4/KM//2016

Rp. 448.200.000,-

Bandung, 27 Mei 2016

Kuasa Pengguna Anggaran Kopertis Wilayah IV,	Bendahara Pengeluaran Kopertis Wilayah IV,	Ketua LPPM Universitas Kristen Maranatha,
Prof. Dr. Ir. Abdul Hakim Halim, M.Sc.	Tarya Sutaryo, S.Sos.	Prof. Dr. dr. Susy Tjahjani, M.Kes.
NIP. 19560914198601100	NIP. 196402101987021001	

METERAI TEMPEL 6000 ENAM RIBU RUPIAH

LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT

Berita Acara ini dibuat rangkap 3 (tiga) untuk dipergunakan sesuai dengan keperluan.

PIHAK PERTAMA
Kuasa Pengguna Anggaran
Kopertis Wilayah IV,

METERAI TEMPEL
BED90ADF816424524
ENAM RIBU RUPIAH

Prof. Dr. Ir. Abdul Hakim Halim, M.Sc.
NIP. 195609141986011001

PIHAK KEDUA
Ketua LPPM
Universitas Kristen Maranatha,

LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT

Prof. Dr. dr. Susy Tjahjani, M.Kes.

SURAT PERNYATAAN TANGGUNGJAWAB MUTLAK
PENUGASAN PELAKSANAAN HIBAH PENELITIAN TAHUN 2016

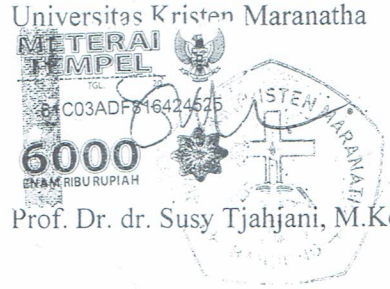
Yang bertanda tangan di bawah ini:

Nama	:	Prof. Dr. dr. Susy Tjahjani, M.Kes.
Jabatan	:	Ketua LPPM
Institusi	:	Universitas Kristen Maranatha
No. SP3	:	/K4/KM/2016
Jumlah Judul	:	27 Judul
Jumlah Dana	:	Rp. 1.494.000.000,-

Menyatakan dengan sesungguhnya bahwa:

1. Bertanggungjawab mutlak dalam pembelanjaan dana Penugasan Pelaksanaan Hibah Penelitian Tahun 2016 dan berkewajiban untuk menyimpan semua bukti-bukti pengeluaran sesuai dengan jumlah dana yang diberikan;
2. Berkewajiban mengembalikan sisa dana yang tidak dibelanjakan ke Kas Negara;
3. Bertanggungjawab penuh atas data adminisitrasi pelaksana penerima dana Penugasan Pelaksanaan Hibah Penelitian Tahun 2016.
4. Berkewajiban untuk menindaklanjuti dan mengupayakan hasil Penugasan Pelaksanaan Hibah Penelitian Tahun 2016 yang dilakukan terlaksana secara efektif dan efisien;
5. Berkewajiban untuk menyimpan hardcopy dan softcopy Laporan Kemajuan dan Laporan Akhir Penugasan Pelaksanaan Hibah Penelitian Tahun 2016.

Bandung,
Ketua LPPM
Universitas Kristen Maranatha



Prof. Dr. dr. Susy Tjahjani, M.Kes.

Lampiran Surat Perjanjian Penugasan Pelaksanaan Hibah Penelitian Tahun Anggaran 2016

Nomor SP3 : 2403 /K4/KM/2016
Perguruan Tinggi : Universitas Kristen Maranatha
Nomor DIPA : SP DIPA-042.06.1.401516/2016
Tanggal DIPA : 07 Desember 2015
Unit Organisasi : Koordinasi Perguruan Tinggi Swasta Wilayah IV
Lembaga : Kementerian Riset, Teknologi, dan Pendidikan Tinggi

NO	NAMA	JUDUL	SKEMA	100%	70%	30%
1	DANIEL SETIADIKARUNIA	Perancangan Deskriptor Ciri-Lokal Baru untuk Ekstraksi Ciri Citra Wajah dan Citra Pembuluh Darah dalam Mengenali Identitas Seseorang	Penelitian Fundamental	50.000.000	35.000.000	15.000.000
2	WAHYU WIDOWATI	Human Mesenchymal Stem Cells-Condition Medium (hMSCs-CM)dari Wharton's Jelly sebagai Induksi Apoptosis pada Cancer Stem Cells (CSCs)	Penelitian Kompetensi	112.000.000	78.400.000	33.600.000
3	P LINDAWATI SEWU	PERLINDUNGAN HUKUM BAGI PARA PIHAK DALAM WARALABA MENYONGSONG TERBENTUKNYA MASYARAKAT EKONOMI ASEAN	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
4	JOHANNES IBRAHIM KOSASIH	Penerapan Dual Banking System dalam Praktik Perbankan di Indonesia Menyongsong Terbentuknya Masyarakat Ekonomi ASEAN	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
5	RITA TJOKROPRANOTO	MEKANISME SINERGISME Bacillus thuringiensis.var.israeliensis DENGAN Bacillus sphaericus 2362 SEBAGAI KONTROL VEKTOR FILARIASIS LIMFATIK MENGATASI MUTASI GEN Cqm1	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
6	TAN LIE ING	Pengaruh Penggunaan Ps Ball Terhadap Balok Beton Bertulang Dengan Pembebanan Monotonik Dan Pembebanan Siklik	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
7	SUSY TJAHJANI	PENGEMBANGAN OBAT ANTIMALARIA KOMBINASI BERBASIS HERBAL CURCUMIN DAN EKSTRAK/ FRAKSI KULIT MANGGIS DALAM TERAPI MALARIA UNTUK MENCEGAH RESISTENSI OBAT	Penelitian Kompetensi	112.000.000	78.400.000	33.600.000
8	ANANG KRISTIANTO	Perkuatan Kolom Beton Bertulang Bangunan Rumah Tinggal Tahan Gempa dengan Menggunakan Pen-Binder	Penelitian Fundamental	60.000.000	42.000.000	18.000.000
9	HENKY LISAN SUWARNO	Intensi Kewirausahaan Mahasiswa Pada Universitas-Universitas Di Indonesia	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
10	OLGA CATHERINA PATTIPAWAEJ	Pemodelan Bangunan Pemecah Gelombang Sisi Miring	Penelitian Fundamental	60.000.000	42.000.000	18.000.000
11	NOVIE THERESIA BR PASARIBU	PENGENALAN MANUSKRIP AKSARA BATAK TOBA MENGGUNAKAN JARINGAN SYARAF TIRUAN	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
12	LUSIANA DARSONO	Ekstrak Kulit Manggis dan Senyawa-senyawa Xanthone Sebagai Bahan Antiinflamasi Secara Ex Vivo Pada Sel RAW 264.7Antidislipidemia Secara In Vivo Pada Tikus Aterosklerosis	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
13	ROSIDA TIURMA MANURUNG	Pemerolehan Bahasa pada Anak 4–5 Tahun dengan Terapi Hi - Tech	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
14	MEILINAH HIDAYAT	AKTIVITAS EKSTRAK ETANOL BIJI KEDELAI (Glycine max L.merr) VARIETAS DETAM 1, DAUN JATI BELANDA (Guazuma ulmifolia) dan KOMBINASINYA SEBAGAI ANTIOBESITAS MELALUI INHIBISI ENZIM LIPASE PANKREAS DAN ANTIADIPOGENESIS SECARA IN	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
15	ANNY NURBASARI	Analisis Branding Kota Bandung	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
16	TIMBUL HAMONANGAN SIMANJUNTAK	Pencapaian Keadilan Sosial Melalui Penguatan Kapasitas Fiskal dan Kinerja Keuangan Daerah Berbasis Desentralisasi Fiskal pada Kabupaten/Kota di Jawa Timur	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
17	RATNA WIDIASTUTI	FAKTOR KEPRIBADIAN PENENTU PERILAKU CYBERLOAFING PADA MAHASISWA PERGURUAN TINGGI DI INDONESIA	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
18	CINDRAWATY LESMANA	Pengujian tekan elemen struktur kayu dengan inovasi pembalik beban sebagai alat tambahan Universal Testing Machine (UTM)	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
19	SE TIN	PENGARUH KEMAMPUAN MANAJERIAL, KEMAMPUAN PENGENDALIAN INTERNAL DAN KONDISI EKONOMI MAKRO TERHADAP KUALITAS LABA: STUDI PADA PERUSAHAAN GO PUBLIC DI INDONESIA	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
20	ROBBY YUSSAC TALLAR	STUDI PERBANDINGAN PERILAKU KUAT GESER CAMPURAN PASIR DAN TANAH RESIDUAL TROPIS YANG DIPADATKAN	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
21	MARCELLIA SUSAN	Kualitas Pelayanan Kesehatan Rumah Sakit Umum Kelas A di Bandung sebagai Bagian Pelayanan Masyarakat	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
22	DIANA KRISANTI JASAPUTRA	POTENSI PENGEMBANGAN BROKOLI SEBAGAI AGEN ANTIMUTAGENIK RESEPTOR NKG2DUNTUK MENCEGAH KANKER	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
23	HENNDY GINTING	PERAN FAKTOR-FAKTOR KEPRIBADIAN DALAM HUBUNGAN ANTARA RELIGIOSITAS DAN MAKNA HIDUP	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
24	NOVI	ANALISIS PENGARUH GETARAN TERHADAP KONSENTRASI PEKERJA	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
25	YOSAFAT AJI PRANATA	PENGEMBANGAN STRUKTUR KOMPOSIT KAYU-BETON BERTULANG UNTUK BANGUNAN TAHAN GEMPA	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
26	ERWANI MERRY SARTIKA	PERANCANGAN DAN REALISASI ROBOT HUMANOID AUTONOMOUS	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
27	MULIADY	Prototip UAV untuk Pencarian Korban Bencana dan Pemetaan Wilayah Bencana	Penelitian Hibah Bersaing	50.000.000	35.000.000	15.000.000
				1.494.000.000	1.045.800.000	448.200.000

Bandung, Mei 2016
Kuasa Pengguna Anggaran

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LAPORAN AKHIR
HIBAH KOMPETENSI

Human Mesenchymal Stem Cells-Condition Medium (hMSCs-CM) Dari Wharton's Jelly Sebagai Induksi Apoptosis Pada Cancer Stem Cells (CSCs)

Tahun ke 2 dari rencana 3 tahun

Oleh :

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OKTOBER 2016

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DAFTAR ISI

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RINGKASAN

Pemanfaatan limbah Wharton's Jelly sebagai sumber stem cells yang memiliki karakteristik MSCs yang biasanya diisolasi dari human bone marrow (hBM) diharapkan dapat menghasilkan metode isolasi yang tepat serta meningkatkan karakteristik hMSCs pada lingkungan mikro hipoksia berdasarkan karakteristik fenotipe (mengekspresikan marker CD73, CD90 dan CD105; CD34, CD14 dan CD45 serta HLA-DR), karakeristik isotip (19E1, PE, FITC, 192a PE), memiliki kemampuan berdiferensiasi menjadi *osteocyte*, *chondrocyte* dan *adipocyte*, serta menurunkan doubling time. hMSCs-*condition medium* yang dihasilkan MSCs dari Wharton's Jelly memiliki aktivitas antikanker pada berbagai jenis kanker.

Rencana penelitian tahun ke dua meliputi : a). Induksi interleukin (IL2) terhadap viabilitas sel NK, b). Induksi IL2 pada sel NK terhadap kadar TNFα, IFNγ, c). Induksi IL2 pada sel NK (IL2-NK) terhadap kadar TNFα, IFNγ, perforin, granzim pada CM kokultur hWJMSCs dan sel NK, d). Induksi IL2 pada sel NK (IL2-NK) terhadap kadar TNFα, IFNγ, perforin, granzim pada CM kokultur MCF7 dan sel NK, e). Induksi IL2 pada sel NK (IL2-NK) terhadap kadar TNFα, IFNγ, perforin, granzim pada CM kokultur hWJMSCs dan sel NK

Hasil penelitian tahun ke 2 menunjukkan : a). IL2 meningkatkan proliferasi sel NK, semakin lama inkubasi semkain meningkat proliferasi; b). IL2 meningkatkan kadar TNFα, IFNγ sel NK, c). Sel NK yang diinduksi IL2 (IL2-NK) dapat meningkatkan produksi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK, d). IL2-NK dapat menginduksi apoptosis pada sel MCF7, d). Sel NK yang diinduksi IL2 (IL2-NK) dapat meningkatkan produksi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL2-NK, e). IL2-NK dapat menginduksi apoptosis dan meningkatkan ekspresi gen BAX, P53 dan menurunkan ekspresi gen BCL-2 pada sel MCF7.

Rencana penelitian tahun 3 meliputi : a). Induksi interleukin (IL15) terhadap viabilitas sel NK, b). Induksi IL15 pada sel NK terhadap kadar TNFα, IFNγ, c). Induksi IL15 pada sel NK (IL15-NK) terhadap kadar TNFα, IFNγ, perforin, granzim pada CM kokultur hWJMSCs dan sel NK, d). Induksi IL15 pada sel NK (IL15-NK) terhadap kadar TNFα, IFNγ, perforin, granzim pada CM kokultur MCF7 dan sel NK, e). Induksi IL15 pada sel NK (IL15-NK) terhadap kadar TNFα, IFNγ, perforin, granzim pada CM kokultur hWJMSCs dan sel NK

Kata kunci : mesenchymal stem cells, Wharton's Jelly, Condition Medium, hMSCs-condition medium, antikanker, apoptosis

PRAKATA

Puji syukur penulis panjatkan kehadirat Tuhan Yang Maha Esa atas rahmatNya sehingga penelitian dan laporan Hibah Kompetensi Tahun Anggaran 2015 dapat diselesaikan.

Berdasarkan hasil penelitian tahun 2 menunjukkan IL2 meningkatkan proliferasi sel NK, semakin lama inkubasi semkain meningkat proliferasi; b). IL2 meningkatkan kadar TNFα, IFNγ sel NK, c). Sel NK yang diinduksi IL2 (IL2-NK) dapat meningkatkan produksi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK, d). IL2-NK dapat menginduksi apoptosis pada sel MCF7, d). Sel NK yang diinduksi IL2 (IL2-NK) dapat meningkatkan produksi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL2-NK, e). IL2-NK dapat menginduksi apoptosis dan meningkatkan ekspresi gen BAX, P53 dan menurunkan ekspresi gen BCL-2 pada sel MCF7

Terima kasih dan rasa hormat tim peneliti sampaikan kepada Kepala Lembaga dan Pusat Penelitian Pada Masyarakat Universitas Kristen Maranatha Bandung yang telah memberikan pembinaan selama ini.

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Bandung, Oktober 2016

BAB I

PENDAHULUAN

1.1. Latar belakang

Sebesar 7% angka kematian di seluruh dunia pada tahun 2005 disebabkan oleh karsinoma dan 1% dari kematian oleh karsinoma adalah akibat karsinoma mammae yaitu sebanyak 502.000 jiwa. Kanker payudara merupakan kanker paling umum baik di negara maju maupun negara bekembang yaitu sebesar 690.000 kasus untuk setiap wilayah negara maju maupun negara berkembang dengan perbandingan populasi 1:4. Kasus kanker payudara bervariasi antara 19,3 per 100.000 wanita di wilayah Afika timur, 89,9 per 100.000 wanita di Erpoa Barat dan lebih 80 per 100.000 wanita di negara maju didunia (terkecuali Jepang) dan kurang dari 40 per 100.000 wanita di berbagai wilayah negara berkembang (Ferlay *et al*., 2010). Sebesar 7% angka kematian di seluruh dunia pada tahun 2005 disebabkan oleh kanker dan 1% dari kematian adalah akibat kanker payudara yaitu sebanyak 502.000 jiwa. Saat ini 20% dari wanita yang didiagnosis menderita kanker payudara meninggal dunia dan tiap tahun jumlah penderita kanker payudara bertambah sekitar 7 juta. Di dunia setiap tiga menit ditemukan penderita kanker payudara dan setiap 11 menit ditemukan seorang wanita meninggal dunia akibat kanker payudara. (WHO, 2006; *American Cancer Society*, 2010).

Hingga saat ini, terapi kanker payudara dengan pembedahan, radioterapi, terapi hormon dan kemoterapi. Beberpa macam cara penanganan kanker antara lain pembedahan, penyinaran, kemoterapi, terapi hormon, imunoterapi dan hipertermi. Seringkali cara-cara tersebut dikombinasikan. Pembedahan dan radiasi dapat mencapai penyembuhan lengkap (kuratif) bila belum terjadi metastasis dan dilakukan sedini mungkin, kemoterapi dengan sitostatika hanya dapat menyembuhkan sejumlah kecil jenis kanker. Pengobatan kanketr yang sudah menyebar bersifat paliatif yaitu meringankan gejala tanpa menyembuhkan penyakit. Berbagai usaha terapi baik kuratif maupun paliatif termasuk pencarian dari berbagai senyawa yang berasal dari bahan alam sebagai bahan antikanker, namun belum menemukan hasil yang memuaskan.

Upaya mencari jalan keluar penyakit kanker, memberikan harapan baru sebagai terapi alternatif setelah ditemukan sel punca dapat digunakan sebagai bahan terapi penyakit degeneratif termasuk penyakit kanker.

Akhir-akhir ini telah diterapkan suatu pilihan terapi dalam upaya mengatasi penyakit degeneratif dengan cara pengobatan terapi sel punca (*stem cell).* Sel punca banyak terdapat

dalam sumsum tulang, namun pada penelitian lebih lanjut ditemukan juga bahwa ternyata sel punca dapat pula diisolasi dari darah tali pusat, darah perifer, hepar, kulit, maupun dari pulpa gigi, dan bahkan dari jaringan lemak yang pada umumnya merupakan limbah buangan sisa operasi *liposuccion* (Hester, 2000), serta dari *human embryonic stem cell* (hESC) (Aleckovic dan Simon, 2008).

Human umbilical cord (HUC) atau tali pusat yang berasal dari embrio, adalah membran ekstra embrio dan Wharton's Jelly berada dalam plasenta yang merupakan sumber stem cell. Belum diketahui secara pasti apakah Wharton's jelly (WJ) memiliki sel punca embrionik (human embryonic stem cells:hESCs), sel punca mesenkimal (human mesenchymal stem cells : hMSC) atau keduanya. Wharton's jelly memiliki keunikan yaitu laju proliferasi yang tinggi, multipotent, hipoimun ogenitas, tidak menyebabkan teratoma dan memiliki sifat antikanker, sehingga sangat penting dan sangat bermanfaat untuk terapi dan tindakan penyakit kanker. Sel punca asal Wharton's jelly (WJSCs) menunjukkan kadar yang rendah marker POUF 1, NANOG, SOX2 dan LIN28 sehingga tidak bersifat teratoma. Beberapa sitokin secara signifikan meningkatkan ILA 2A yang dapat menginduksi apoptosis, sehingga memiliki aktivitas antikanker. Sel punca Wharton's jelly menunjukkan peningkatan ekspresi gen yang terkait sistem imun, kemotaksis dan kematian sel. Kemampuan WJSCS dalam mengatur imunitas mengakibatkan WJSCS merupakan sumber sel punca yang sesuai untuk terapi *allogenic* tanpa terjadi *immunorejection* (Fong *et al*., 2010).

Pemanfaatan limbah *Wharton's Jelly* bagian dari *human umbilical cord* (HUC) sebagai sumber *stem cells* yang memiliki karakteristik MSCs yang biasanya diisolasi dari human bone marrow (hBM) diharapkan dapat menghasilkan metode isolasi yang tepat serta meningkatkan karakteristik (mengekspresikan marker CD73, Cd90 dan CD105; CD34, CD14 dan CD45 serta HLA-DR), karakeristik isotip (19E1, PE, FITC, 192a PE), memiliki kemampuan berdiferensiasi menjadi *osteocyte, chondrocyte dan adipocyte*, serta menurunkan *doubling time. hMSCs-condition medium* (hMSCs-CM) yang dihasilkan MSCs dari *Wharton's Jelly* memiliki aktivitas antikanker pada pada cancer stem cells (CSCs) dari sel kanker payudara.

BAB II

TINJAUAN PUSTAKA

2.1. Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) merupakan sumber sel punca yang lebih baik yaitu dapat memperbaharui (*self-renew*) dan bersifat multipoten, MSCs yang digunakan banyak berasal dari *human bone marrow* (*human bone marrow mesenchymal stem cells*: hBMMSCs), namun sumber hBMMSCs memerlukan tindakan invasif dan menghasilkan jumlah MSCs sedikit, tidak mampu bertahan lama pada kultur *in vitro* sehingga penggunaan hBMMSCs masih kurang menguntungkan. hBMMSCs merupakan sumber MSCs yang umum, namun menghasilkan jumlah MSCs yang sedikit dan berkurang secara signifikan dengan bertambahnya usia donor, sehingga perlu dicarikan sumber alternatif MSCs seperti jaringan adipose, *umbilical cord blood* (UCB), cairan amnion, tali pusat dan Wharton's Jelly (Rao and Mattson, 2001; Romanov et al., 2003).

Sumber *embryonic stem cell* lain yaitu berasal dari *Human umbilical cord* (HUC) atau tali pusat yang berasal dari embrio, yaitu berupa membran ekstra embrio dan Wharton's Jelly berada dalam tali pusat. Wharton's Jelly (WJ) memiliki keunikan yaitu memiliki laju proliferasi yang tinggi, bersifat multipoten, hipoimun ogenitas, tidak menyebabkan teratoma dan memiliki sifat antikanker, sehingga sangat penting dan sangat bermanfaat untuk terapi dan tindakan penyakit kanker. Sel punca asal Wharton's jelly (Wharton's Jelly Stem Cells: WJSCs) menunjukkan kadar yang rendah marker POUF 1, NANOG, SOX2 dan LIN28 sehingga tidak bersifat teratoma. Kemampuan WJSCs dalam mengatur imunitas mengakibatkan WJSCs merupakan sumber sel punca yang sesuai untuk terapi *allogenic* tanpa terjadi *immunorejection* (Fong *et al*., 2010).

Berdasarkan konsensus dari *The International Society of Cellular Therapy*, sebuah sel digolongakn sebagai *stem cell* mesenkimal (MSCs) harus memiliki karakteristik : 1). Sel menempel pada permukaan cawan; 2). Memiliki molekul protein permukaan (*cluster of differentation*, CD) : CD73, Cd90 dan CD105. Stem cell mesenkimal (MSCs) tidak mengekspresikan CD34, CD14 dan CD45 serta HLA-DR; 3). Mampu berdiferensiasi sesuai 3 jalur diferensiasi mesenkimal yaitu osteogenik (menjadi sel tulang), kondrogenik (menjadi sel tulang rawan), adipogenik (menjadi sel lemak) (Weiss *et al*., 2008; Halim dkk, 2010) bersifat multipoten (berdiferensiasi menjadi tulang, lemak, kartilago dan lain-lain). (Weiss *et al*., 2008).

2.2. Human Umbilical Cord

Human umbilical cord (HUC) atau tali pusat yang berasal dari embrio, adalah membran ekstra embrio dan Wharton's Jelly berada dalam plasenta yang merupakan sumber stem cell. Belum diketahui secara pasti apakah Wharton's jelly (WJ) memiliki sel punca embrionik (human embryonic stem cells:hESCs), sel punca mesenkimal (human mesenchymal stem cells : hMSC) atau keduanya. Wharton's jelly memiliki keunikan yaitu laju proliferasi yang tinggi, multipotent, hipoimun ogenitas, tidak menyebabkan teratoma dan memiliki sifat antikanker, sehingga sangat penting dan sangat bermanfaat untuk terapi dan tindakan penyakit kanker. Sel punca asal Wharton's jelly (WJSCs) menunjukkan kadar yang rendah marker POUF 1, NANOG, SOX2 dan LIN28 sehingga tidak bersifat teratoma. Beberapa sitokin secara signifikan meningkatkan ILA 2A yang dapat mennginduksi apoptosis, sehingga memiliki aktivitas antikanker. Sel punca Wharton's jelly menunjukkan peningkatan ekspresi gen yang terkait sistem imun, kemotaksis dan kematian sel. Kemampuan WJSCS dalam mengatur imunitas mengakibatkan WJSCS merupakan sumber sel punca yang sesuai untuk terapi *allogenic* tanpa terjadi *immunorejection* (Fong *et al*., 2010).

MSCs dari Wharton's Jelly (MSCs-WJ) bersifat multipoten dan proliferasi serta kapasitas memperbaharui (*self renewal*) yang lebih tinggi dibanding MSCs dari stem sel dewasa (*adult stem cells*) (Nekanti *et al*., 2010).

Berdasarkan hasil penelitian pemberian WJSCs tikus yang diberikan secara intravena atau intratumor pada tikus yang menderita adenokarsinoma mammae menunjukkan pada hari ke 14 terjadi perbaikan dan hal ini menunjukkan bahwa WJSCS memiliki aktivitas antikanker. Pada hari ke 34 sampai 38 menunjukkan tidak terjadi metastasis pada hari ke 100 setealh inokulasi WJSCs. Mekanisme antikanker dari WJSCs belum diketahui secara jelas, diduga adanya kontak antar sel atau kontak secara bebas (Ganta et al., 2009). Berdasarkan hasil penelitian bahwa hWJSCs (human wharton's jelly stem cell) secara signifikan dapat mengurangi, melemahkan pertumbuah kanker payudara pada mencit, Kultur hWJSCs dengan seeding 100.000 sel dapat menginduksi apoptosis pada kultur jumlah sel yang sama (100.000 sel) pada cell line kanker payudara (MCF-7), kolorektak (HT-29) dan kanker hati (HepG2) dan berdasarkan perubahan morfologi serta berdasarkan uji Annexin-V. Conditioned medium (CM) dari hWJSCs (hWJSC-CM) juga dapat menekan pertumbuhan sel HepG2 berdasarkan uji *in vitro* Green Fluorescent Protein (Hep-G2-GFP), hal ini menunjukkan bahwa aktivitas antikanker dari hWJSCs dikarekan kontak antar sel melalui faktor difusi yang disekresikal oleh hWJSCs. Berdasarkan identifikasi, isolasi dan karakterisasi baik hWJScs atau hWJSCs

yang memiliki aktivitas antikanker sangat memungkinkan aplikasi klinis hWJScs atau hWJSCs untuk terapi kanker (Ayuzawa *et al*., 2009).

Stem cells suatu sel harus memiliki karakteristik antara lain belum berdiferensiasi (*undifferentiated)*, mampu memperbanyak diri (*self renewal*) dan dapat berdiferensiasi menjadi > 1 jenis sel (multipoten/pluripoten) (Halim dkk, 2010).

2.3. Rekayasa Mesenchymal Stem Cells Sebagai Bahan Terapi Kanker

Keterlibatan sitokin pro-inflamasi, makrofag *migration inhibitory factor* (MIF) dalam migrasi MSCs. CD74 mengatur migrasi dan *homing* sehingga merupakan strategi yang bermanfaat meningkatkan daya, aktivitas biologis termasuk antikanker (Song *et al*., 2006). MSCs akan tertarik pada lokasi iradiasi dan lokal radiasi akan memicu migrasi MSCs dan pencangkokan sehingga MSCs dapat tumbuh dengan cepat (Francois *et al*., 2006). Sel punca mesenkim tanpa rekayasa menunjukkan efek antikanker baik secara *in vitro* serta *in vivo* pada berbagai jenis kanker. Berbagai jenis faktor yang dihasilkan oleh MSCs memiliki aktivitas antikanker, mengurangi proliferasi pada berbagai jenis kanker glioma, melanoma, paru-paru, hepatoma, payudara (Nakamura *et al*., 2004; Qiao *et al*., 2008). MSCs sebagai bahan terapi kanker dapat dikombinasikan dengan berbagai strategi untuk meningkatkan aktivitas antikanker yaitu dengan transduksi, transfeksi yang mengekspresikan interleukin (IL-2, IL-12), interferon (IFN-α, IFN-β, IFN-γ), CX3CL1 (Fractalkine), TRAIL, TNF (Shah *et al*., 2012), untuk meningkatkan aktivitas MSCs dapat dilakukan dengan induksi interleukin (Thomas *et al*., 2014).

MSCs dimodifikasi secara genetis untuk mengekspresikan berbagai gen eksogenous untuk ekspresi/sekresi bagi terapi tertentu yang diinginkan seperti untuk berbagai jenis kanker (Shah, 2012). MSCs digunakan sebagai pengiriman berbagai bahan, molekul yang dapat menghambat pertumbuhan kanker, IFN-β memiliki aktivitas antiproliferasi dan proapoptosis (Chawla-Sarkar *et al*., 2001; Johns, *et al*. 1992). hMSCs yang direkayasa mengekspresikan IFN-β digunakan untuk pengiriman bahan yang dapat menghambat metastasis kanker payudara, melanoma, glioma, dan paru-paru (Studeny *et al*., 2002; Shah, 2012). Efek antikanker MSCs yang mengekspresikan IFN-β dapat menghambat pertumbuahan kanker melanoma mencit, kanker sistem syaraf pusat serta dapat meningkatkan daya tahan hidup rodensia (Studeny *et al*., 2002). MSCs yang mengekspresikan IFN-β dapat meningkatkan *homing* MSCs, menghambat pertumbuhan kanker prostat, paru-paru dan menghambat metastasis (Ren *et al*., 2008a). MSCs yang mengekspresikan IFN-α dapat menghambat metastasis kanker paru-paru, melanoma,

meningkatkan daya tahan hidup hewan model dkarenakan meningkatnya apoptosis sel kanker (Ren *et al*., 2008b; Shah, 2012). Koinjeksi MSCs-IFN-β dan sel kanker melanoma A375SM pada mencit menunjukkan MSCs-IFN-β mampu menghambat pertumbuhan kanker dan memperpanjang usia mencit (Studeny *et al*., 2002). Pemberian secara intravena MSCs-IFN-β secara signifikan meningkatkan umur mencit dan mengurangi metastasis paru-paru baik pada hewan yang dicangkok sel kanker payudara (MDA231) maupun sel kanker melanoma (A375SM), hal ini dikarenakan efek toksik dari IFN-β yang diekspresikan oleh MSCs (Studeny *et al*., 2002). Terapi sel menggunakan hMSCs-IFN-β dapat meningkatkan ekspresi IFN-β pada sel kanker dan jaringan sekitarnya sehingga dapat mengendalikan pertumbuhan malignansi sel. Pendekatkan yang sesuai bagi berbagai jenis kanker dikarenakan efek antiproliferatif dan proapoptosis dan IFN-β (Menon *et al*., 2007).

TNF-α diketahui memiliki potensi antikanker baik secara *in vitro* maupun in vivo (Muenchen *et al*., 2000). TNF-α adalah mediator dari berbagai respon seluler meliputi apoptosis dan nekrosis, sel lisis dan diferensiasi (Muenchen *et al*., 2000). TNF-α memicu sejumlah sinyal transduksi yang akan memicu terjadinya apoptosis, proliferasi berdasarkan *threshold* dari sel terhadap TNF-α. Sebagian besar sel tidak mengalami apoptosis ketika terpapar TNF-α dalam kadar rendah sebagai mekaisme protektif sel melalui induksi TNF-α sebagai antiapoptosis dengan melibatkan gen seperti A20, Bcl-2, MnSOD, penghambat apoptosis, NF-kB sehingga sel tidak sensitif terhadap TNF-α. Apoptsosi akan terinduksi oleh TNF-α dosis tinggi berikatan dengan reseptor (TNF-R1) dan mengaktifkan caspase (Muenchen *et al*., 2000).

Berbagai penelitian menunjukkan bahwa IFN-α memiliki efek antiproliferatif, antikanker, imunomodulator. IFN-α merupakan sitokin regulator yang multifungsional. MSCs yang ditransduksi adenovirus yang mengekspresikan IFN-α pada mencit menderita kanker melanoma, metastasis kanker paru-paru (B16F10) menunjukkan MSCs mampu *homing* pada lokasi kanker paru-paru dan mampu meningkatkan daya tahan hidup mencit (Ren *et al*., 2008b). Imunosupresan dari MSCs dapat ditingkatkan melalui aksi sitokin inflamasi seperti IFN-γ ditambah TNF-α yang akan meningkatkan produksi kemokin juga faktor imunosupresan seperti nitrit oksida (NO) dan indoleamine 2,3-dioxygenase sehingga sel memiliki auto antigen tumor (Romieu-Mourez *et al*., 2010).

IFN-γ sebagai bahan antikanker yang dapat dikirimkan melalui rekayasa MSCs yaitu sebagai imunostimulator, induksi apoptosis, antiangiogenik pada model metastasis melanoma, kolon (Loebinger and Janes, 2010).

TRAIL adalah anggota keluarga *tumor necrosis factor-α* (TNF-α) yang dapat menginduksi apoptosis pada berbagai jenis kanker dan dapat memperbanyak pada sel normal (Kagawa *et al*., 2001). TRAIL menginduksi apoptosis melalui interaksi dengan reseptor kematian dan inisiasi kematian sel yang diinduksi oleh caspase (Mohr *et al*., 2008). Rekayasa menggunakan gen spesifik antikanker, sehingga MSCs mampu memproduksi bahan antikanker secara konstan. TRAIL diidentifikasi dan cloned pada basis *sequence homology* pada domain ekstraseluler dari ligand CD95 (CD95L) dan TNF (Pitti *et al*., 1996; Sun *et al*., 2011).

TNF-α sebagai antitumor meliputi : 1). penghambatan angiogenesis pada dosis tinggi secara *in vitro*, *in vivo*; 2). menginduksi apoptosis sel lini kanker prostat (LNCaP); 3). merangsang imunitas antitumor melalui peningkatan pembentukan dan proliferasi sel T sitotoksik (*cytotoxic T cells* : CTL), mencegah penghambatan pembentukan CTL yang dimediasi TGF-β; 4). menginduksi produksi berbagai sitokin (IL-1, IL-6, IL-8 dan IFN-γ) dan faktor sitotoksik (NO, ROS) oleh makrofag dan sel NK; 5). Melindungi sel dendritik dari apoptosis yang diinduksi tumor (Tse *et al*., 2012). TNF-α dan anggotanya keluarganya memiliki aktivitas antikanker antara lain TRAIL sebagai antikanker melalui induksi apoptosis pada sel kanker dan tidak menginduksi apoptosis pada sel normal (Aggrawal *et al*., 2012).

Sitokin IFN-γ, TNF-α sebagai bahan antikanker dikarenakan efek antikanker berupa : 1). meningkatnya antigen oleh sel dendrit, 2). meningkatnya molekul ko-stimulator, 3). memicu diferensiasi sel limfoid, 4). merangsang aktivasi sel efektor antara lain makrofag sitotoksik, sel NK, sel LAK dan sel CTLs, 5). induksi apoptosis, 6). menghambat angiogenesis (Ren *et al*., 2008a).

2.4. Sel NK Sebagai Antikanker

Gangguan sistem imun antitumor pada kanker payudara diakibatkan oleh defisiensi produksi IFN-γ pada mencit yang secara spontan dapat memicu perkembangan kanker payudara (Shankaran *et al*., 2001; Mamessier *et al*., 2011). Pada penderita kanker payudara ditemukan adanya gangguan maturasi sel NK darah perifer dan fungsi sitotoksik sel NK (Caras *et al*., 2004). Karakterisasi ekspresi gen menunjukkan bahwa inflitrasi sel NK bersifat sitotoksik sehingga dapat membunuh sel kanker (Mamessier *et al*., 2011).

Sel NK membunuh sel target melalui jalur yaitu kontak secara langsung sel NK dan sel target. Jalur pertama yaitu toksin granul sitoplasma yaitu protein yang merusak membran yang disebut perforin serta protease serin (granzim) dengan berbagai substrat lainnya yang spesifik yang disekresikan melalui eksositosis dan secara bersama menginduksi apoptosis sel

target. Jalur eksositosis granul mengaktivasi secara potensial mekanisme kematian sel melalui aktivasi protease sistein apoptotik (caspase) tetapi dapat pula menyebakan kematian sel tanpa aktivasi caspase. Jalur ke dua melibatkan *Death Receptor* (Fas/CD95) pada sel target oleh ligand (FasL) pada sel NK sehingga menghasilkan apoptosis *dependent-apoptosis*. Fungsi efektor sel NK dikendalikan oleh interaksi reseptor sel NK dan ligand baik sel target atau sel lain pada sistem imun (Smyth *et al*., 2005).

Aktivitas antitumor dari sel NK dapat mengarah kepada penurunan massa kanker melaui mekanisme sitolisis atau sekresi INF-γ, namun juga secara tidak langsung berperan dalam mengontrol sel tumor dengan menginduksi aktivitas antitumor sel T (Mocikat *et al*., 2003; Adam *et al*., 2005).

Ekspresi reseptor aktivasi sel NK mengalami penurunan secara signifikan yaitu NKp30, NKp44, NKp46, NKG2D, dan NKG2C pada penderita *acute myeloid leukemia* (AML) dibanding sel NK pada orang normal (Szczepanski *et al*., 2010; Levy *et al*., 2011). Karakteristik sel NK yang mengalami kelemahan pada penderita kanker sehingga tidak mampu mengeliminasi sel kanker (Levy *et al*., 2011).

Sel NK membunuh sel target dengan membebaskan, menghasilkan perforin dan granzim dari granul sitotoksik atau ekspresi permukaan Fas ligan atau TRAIL. NK yang diaktivasi potensial menghasilkan IFNγ, TNFα dan berbagai sitokin, kemokin yang secara siginifkan berperan pada respon imun (James *et al*., 2013). Sekresi sitokin di limfosit dan sel NK yang diaktivasi berperan penting dalam supresi sel kanker (Hwang *et al*., 2012). TNF-α berperan penting mengaktivasi sel T dan menolak sel kanker (Tanigawa *et al*., 2000; Gillett *et al*., 2010) dan IFN-γ menghambat pertumbuhan dan metastasis sel kanker melalui aktivasi sel NK dan sel T limfosit (Hwang *et al*., 2012). TNF-α dan IFN-γ adalah sitokin yang memodulasi sistem imun, membantu mengaktivasi sel T, sel B limfosit dan sel NK sebagai antikanker (Hwang *et al*., 2012).

2.5. Stem Cells-Condition Medium Sebagai Bahan Terapi Kanker

Berdasarkan hasil penelitian *human mesenchymal stem cells-condition medium* (hMSCs-CM) yang diisolasi dari *bone marrow* mampu menghambat proliferasi kanker paru-paru SK-MES-1 (*lung cancer cell lines squamous carcinoma cells*) dan A549 (*adenoma carcima cells*), dapat menginduksi apoptosis, meningkatkan aktivitas *caspase*-3 (Li *et al*., 2010). Secara *in vivo hMSCs-condition medium* yang diinjeksikan pada sel tumor mencit

BALB menunjukkan bahwa terjadinya penurunan ekspresi VEGF pada sel tumor mencit dibanding ko ntrol (Li *et al*., 2010).

Sel punca mesenkim (*Mesenchymal Stem Cells* : MSCs) yang memiliki sifat mampu memperbanyak diri dengan tingkat proliferasi yang tinggi serta multipotent, dapat digunakan untuk rekayasa jaringan, dapat digunakan untuk pengobatan regeneratif. MSCs dapat digunakan sebagai terapi kanker dikarenakan mensekresikan protein IFN–α, IFN-β, IL-2, IL-12 dan juga kemokin CX3CL1 dan MSCs dapat menginduksi apoptosis melalui tumor necrosis factor-related apoptosis inducing ligand (TRAIL) (Gjorgieva *et al*., 2013).

Hasil penelitian secara in vitro sel punca mesenkim yang diisolasi dari tali pusat tikus (rat-umbilical cord mesenchymal setem cells : rUCMSCs) memiliki aktivitas antikanker pada sel lini adenokarsinoma mammae (Mat B III) menunjukkan bahwa rUCMSCs yang dikulturkan menggunakan metode co cultur maupun *conditioned medium* dari rUCMSCs memiliki aktivitas antikanker berdasarkan parameter mengurangi ukuran sel, mengurangi jumlah sel, menstimulasi fosforilasi p38 dan menurunkan fosforilasi ERK1/2, menginduksi apoptosis dengan meningkatkan caspase-3 (Ganta *et al*., 2009).

Berdasarkan hasil penelitian in vivo pada ikus Fisher 344 yang diinduksi sel adenokarsinoma (Mat B III), kemudian 4 hari berikutnya diberikan $1x10^6$ rUCMSCs intratumoral dan $1,5x10^6$ rUCMSCs intravena dan diulang lagi pada hari ke 6, pada hari ke 18 setelah injeksi rUCMSCs menunjukkan ukuran tumor berkurang menjadi berukuran 2 cm dibanding ukuran sebelumnya 2 cm dan pada hari ke 34 setelah injeksi tumor tidak terdeteksi dan pada hari ke 100 tidak menunjukkan adanya metastasis. rUCMSCs dapat mengalami *homing* dikarenakan berbagai sitokin dan faktor pertumbuhan yang dihasilkan oleh sel tumor (Ganta *et al*., 2009).

Pemanfaatan limbah *Wharton's Jelly* bagian dari *human umbilical cord* (HUC) sebagai sumber *stem cells* yang memiliki karakteristik MSCs yang biasanya diisolasi dari human bone marrow (hBM) diharapkan dapat menghasilkan metode isolasi yang tepat serta meningkatkan karakteristik hMSCs pada lingkungan mikro hipoksia berdasrakan karakteristik fenotipe (mengekspresikan marker CD73, Cd90 dan CD105; CD34, CD14 dan CD45 serta HLA-DR), karakeristik isotip (19E1, PE, FITC, 192a PE), memiliki kemampuan berdiferensiasi menjadi *osteocyte, chondrocyte dan adipocyte*, serta menurunkan *doubling time*. *hMSCs-condition medium* yang dihasilkan MSCs dari *Wharton's Jelly* memiliki aktivitas antikanker pada *cell line* karsinoma mammae (naskah publikasi). Penelitian perlu dilanjutkan pada Cancer stem cells (CSCs) yang diisolasi dari sel kanker payuadara primer

sebagai penemuan baru, dari berbagai journal baru yang menyebutkan bahwa CSCs sebagi target terapi kanker yang lebih efektif sehingga kanker tidak mengalami kambuh.

BAB III

TUJUAN DAN MANFAAT PENELITIAN

3.1. Tujuan Penelitian tahun 2, 3

Penelitian secara umum bertujuan untuk meningkatkan aktivitas antikanker dari CM-hWJMSCs-CM dengan melakukan

1. Induksi IL-2, IL-15 pada sel NK (IL2-NK, IL15-NK) untuk menginduksi proliferasi sel NK
2. Induksi IL-2, IL-15 pada sel NK untuk meningkatkan reseptor aktivator (CD107a, NKG2D) dan menurunkan reseptor inhibitor (KIR2DL4)
3. Induksi IL-2, IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ
4. Induksi IL-2, IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK, hWJMSCs+IL5-NK
5. Induksi IL-2, IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL2-NK, MCF7+IL5-NK
6. CM-(hWJMSCs+IL2-NK), CM-(hWJMSCs+IL15-NK) untuk menginduksi apoptosis pada sel kanker MCF7
7. CM-(hWJMSCs+IL2-NK), CM-(hWJMSCs+IL15-NK) IL-15 untuk meningkatkan ekspresi gen apoptosis BAX, P53, mengurangi gen antiapoptosis BCL-2 pada sel kanker MCF7

3.2. Tujuan Penelitian Tahun 1

a) Menghasilkan sediaan h*Mesenchymal Stem Cells- Condition Medium* (hMSCs-CM) yang diisolasi *Wharton's Jelly* (WJ) untuk terapi kanker payudara
b) Meghasilkan sediaan hMSCs-CM dan hMSCs yang diaktivasi terlebih dahulu menggunakan TNFα, IFNγ
c) Menguji sitotoksik CM-hMSCs-TNFα, CM-hMSCs-IFNγ pada sel kanker T47D, MCF7
d) Menguji sitotoksik TNFα, IFNγ pada sel kanker T47D, MCF7
e) Menguji sitotoksik TNFα, IFNγ (hMSCs-TNFα, hMSCs-IFNγ)
f) Menguji sitotoksik, induksi apoptosis TNFα, IFNγ pada sel kanker T47D, MCF7 (BAX, BCL-2,P53)

3.3. Tujuan Penelitian Tahun 2

a) Induksi IL-2 pada sel NK (IL2-NK) untuk menginduksi proliferasi sel NK
b) Induksi IL-2 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ
c) Induksi IL-2 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK
d) Induksi IL-2 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL2-NK
e) CM-(hWJMSCs+IL2-NK) untuk menginduksi apoptosis pada sel kanker MCF7
f) CM-(hWJMSCs+IL2-NK) untuk meningkatkan ekspresi gen apoptosis BAX, P53, mengurangi gen antiapoptosis BCL-2 pada sel kanker MCF7

3.4. Tujuan Penelitian Tahun 3

a) Induksi IL-15 pada sel NK (IL15-NK) untuk menginduksi proliferasi sel NK
b) Induksi IL-15 pada sel NK untuk meningkatkan reseptor aktivator (CD107a, NKG2D) dan menurunkan reseptor inhibitor (KIR2DL4)
c) Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ
d) Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK
e) Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL15-NK
f) CM-(hWJMSCs+IL15-NK) untuk menginduksi apoptosis pada sel kanker MCF7
g) CM-(hWJMSCs+IL15-NK) untuk meningkatkan ekspresi gen apoptosis BAX, P53, mengurangi gen antiapoptosis BCL-2 pada sel kanker MCF7

3.5. Manfaat Penelitian

Hasil penelitian selain menyumbangkan dasar-dasar ilmu biomolekuler terutama tentang mekanisme *cancer stem cell* sebagai sel target terapi penyakit kanker yang diharapkan terapi kanker menjadi lebih efektif dan menghasilkan kesembuhan yang lebih baik, serta mekanimse sel punca mesenkim (hMSCs) sebagai bahan terapi kanker yang mampu membunuh sel kanker dan CSCs yang diawalai mekansime homing di dalam sel tubuh kemudian mampu meregenerasi sel yang rusak serta membunuh sel kanker. Pendekatan penelitian dilakukan secara menyeluruh diawali pengujian hMSCs-CM pada lini sel kanker payudara (T47D, MCF7) menunjukkan hasil bahwa hMSCs-CM bersifat sitotoksik pada sel

T47D dan MCF7 melalui mekanisme apoptosis yaitu ekspresi gen bax, bcl-2, p53. Penelitian perlu dilanjutkan, yaitu:

a. Induksi IL-15 pada sel NK (IL15-NK) untuk menginduksi proliferasi sel NK
b. Induksi IL-15 pada sel NK untuk meningkatkan reseptor aktivator (CD107a, NKG2D) dan menurunkan reseptor inhibitor (KIR2DL4)
c. Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ
d. Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK
e. Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL15-NK
f. CM-(hWJMSCs+IL15-NK) untuk menginduksi apoptosis pada sel kanker MCF7
g. CM-(hWJMSCs+IL15-NK) untuk meningkatkan ekspresi gen apoptosis BAX, P53, mengurangi gen antiapoptosis BCL-2 pada sel kanker MCF7

Pada sel target CSCs yang diisolasi dari sel primer kanker payudara dan mamosphere kanker payudara, untuk menemukan terapi kanker yang lebih efektif. Ditemukannya metode isolasi kanker payudara, sorting CSCs merupakan terobosan yang diharapkan dapat digunakan landasan untuk isolasi dan sorting CSCs sehingga metode isolasi dan sorting dapat digunakan untuk produksi cell line dan CSCs serta digunakan laboratorium untuk penelitian dan pengujian efektivitas terapi kanker, serta dapat diperluas untuk isolasi berbagai sel kanker maupun sorting CSCs dari berbagai sel kanker, sehingga terapi berbagai jenis kanker dapat lebih efektif dengan tingkat kesembuhan yang lebih tinggi.

Penerapan hasil penelitian selain bermanfaat bagi perkembangan ilmu pengetahuan, hasil penelitian dapat dipublikasikan pada Journal Internasional bereputasi, dapat digunakan untuk penyusunan buku teks yaitu tentang “Sel punca mesenkin sebagai bahan terapi” dan buku ke 2 “Sel NK Sebagai Imunomodulasi Penyakit Kanker”

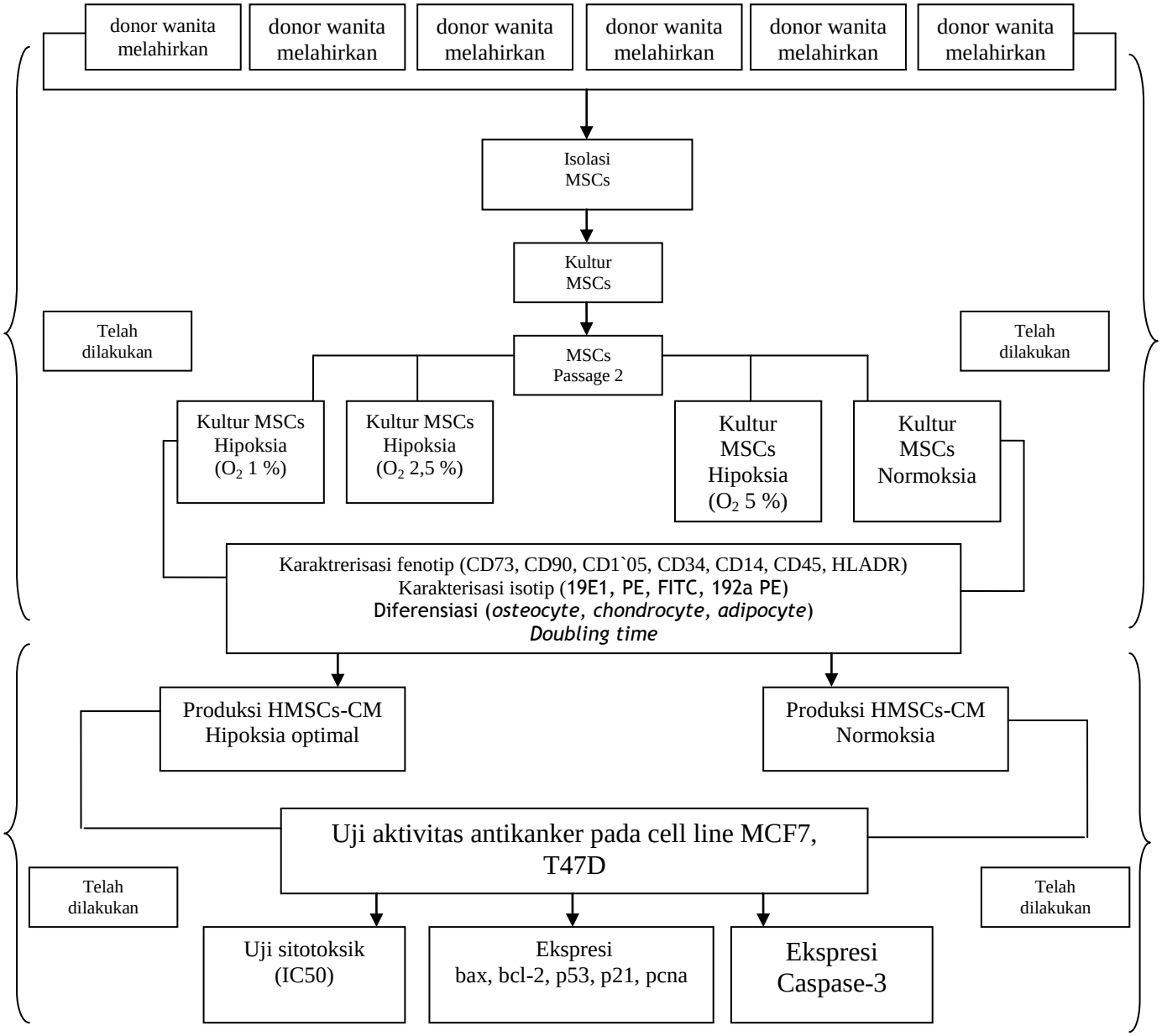
BAB IV

METODE PENELITIAN

4.1. Uraian kegiatan penelitian

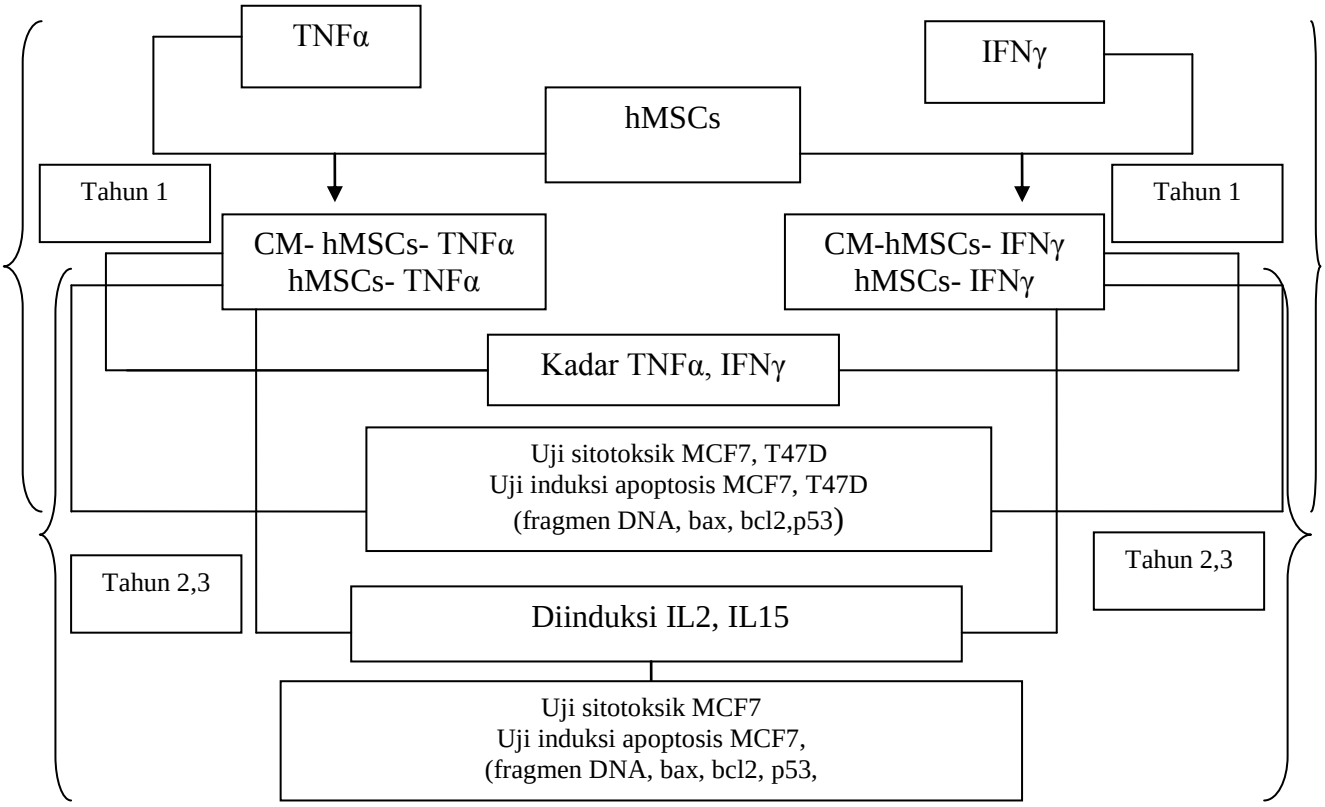
Tahun	Kegiatan
1	a). Produksi MSCs dari WJ, produksi hMSCs-CM b). Uji sitotoksik dari TNFα, IFNγ pada MSCs c). Uji kadar TNFα, IFNγ pada CM-MSCs-TNFα, CM-MSCs-IFNγ d). Uji antikanker CM-MSCs-TNFα, CM-MSCs-IFNγ pada sel lini MCF7, T47D e). Uji antikanker MSCs-TNFα, MSCs-IFNγ pada sel lini MCF7, T47D f). Uji induksi apoptosis CM-MSCs-TNFα, CM-MSCs-IFNγ pada sel lini MCF7, T47D g). Uji induksi apoptsosis MSCs-TNFα, MSCs-IFNγ pada sel lini MCF7, T47D
2	a). Induksi IL-2 pada sel NK (IL2-NK) untuk menginduksi proliferasi sel NK b). Induksi IL-2 pada sel NK untuk meningkatkan reseptor aktivator (CD107a, NKG2D) dan menurunkan reseptor inhibitor (KIR2DL4) c). Induksi IL-2 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ d). Induksi IL-2 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK e). Induksi IL-2 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL2-NK f). CM-(hWJMSCs+IL2-NK) untuk menginduksi apoptosis pada sel kanker MCF7 g). CM-(hWJMSCs+IL2-NK) untuk meningkatkan ekspresi gen apoptosis BAX, P53, mengurangi gen antiapoptosis BCL-2 pada sel kanker MCF7
3	a). Induksi IL-15 pada sel NK (IL15-NK) untuk menginduksi proliferasi sel NK b). Induksi IL-15 pada sel NK untuk meningkatkan reseptor aktivator (CD107a, NKG2D) dan menurunkan reseptor inhibitor (KIR2DL4) c). Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ d). Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK e). Induksi IL-15 pada sel NK untuk meningkatkan sekresi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL15-NK f). CM-(hWJMSCs+IL15-NK) untuk menginduksi apoptosis pada sel kanker MCF7 g). CM-(hWJMSCs+IL15-NK) untuk meningkatkan ekspresi gen apoptosis BAX, P53, mengurangi gen antiapoptosis BCL-2 pada sel kanker MCF7

Untuk mengetahui alur penelitian dapat yang telah dikerjakan dapat dilihat pada Gambar 4.1



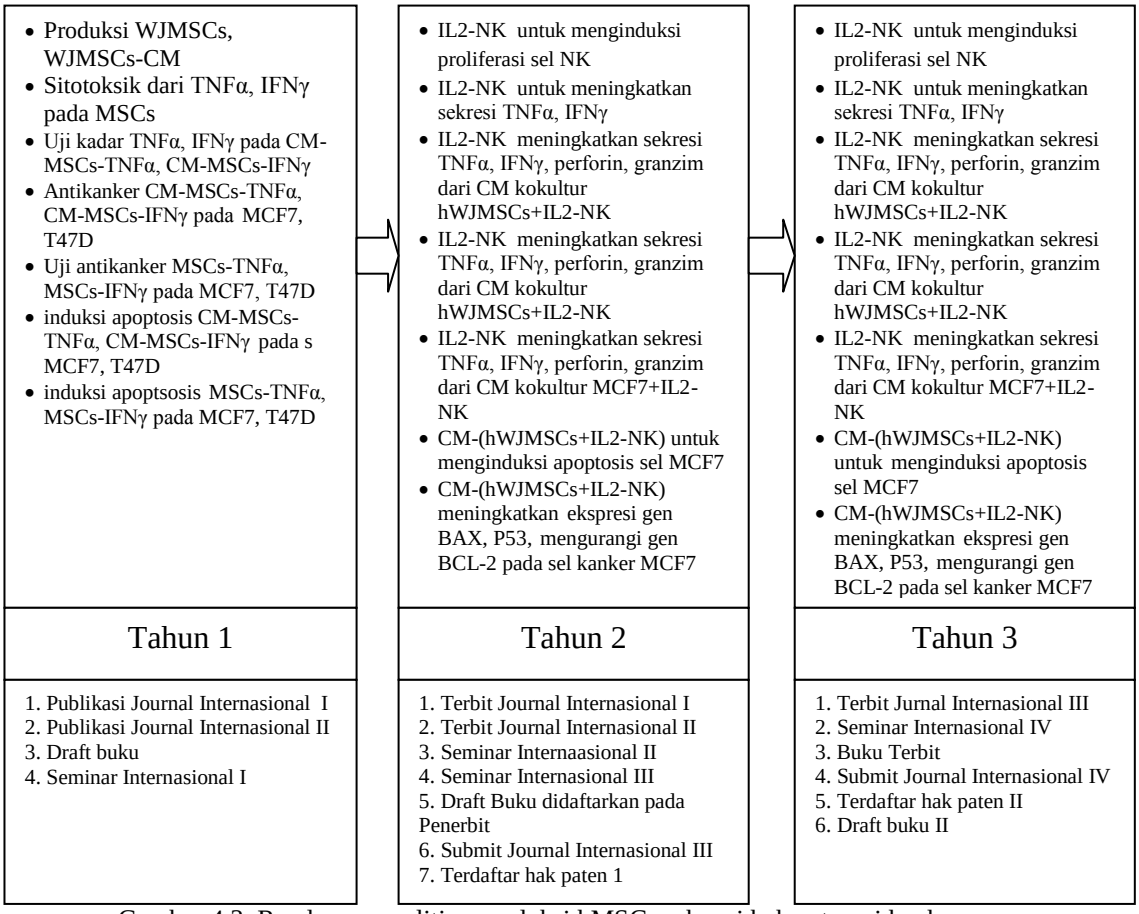
Gambar 4.1. Alur penelitian produksi hMSCs-CM yang telah dilakukan

Untuk mengetahui alur penelitian yang akan dikerjkana dapat dilihat pada Gambar 4.2.



Gambar 4.2. Alur penelitian hMSCs-CM sebagai bahan antikanker payudara pada target cancer stem cells (CSCs)

4.2. Roadmap penelitian



Gambar 4.3. Roadmap penelitian produksi hMSCs sebagai bahan terapi kanker

4.3. Prosedur kerja

4.3.1. Pengukuran viabilitas, proliferasi sel NK yang diinduksi interleukin

Sel NK ditanam ke dalam substrat 96 well plate dengan densitas 10.000 sel/well menggunakan medium free serum (Alpha Minimum Essensial Medium without ribonuclease and deoxirybonucleoside with 2 mM glutamine +1.5 g/L sodium bicarbonate + 0.2 mM inositol+ 0.1 mM 2-merchaptoethanol + 0.02 mM folic acid + 1% PenStrep). Sel selanjutnya diberikan perlakuan induksi IL-2, IL-15, dan IL-18 dengan konsentari perlakuan 5 ng/ml dan 10 ng/ml. Sel diinkubasi dengan empat variable waktu yaitu 24 jam, 48 jam, 72 jam dan 96 jam diinkubasi pada suhu 37^0 C, 5% CO_2, dimana setiap harinya diberikan penambahan perlakuan induksi. Selanjutnya ke dalam *well* di tambahkan 20 µl reagen cell titer 96 ® Aqueouse one solution assay dan sel diinkubasi selama 3 jam pada inkubator 37^0C, 5% CO_2. Absorbansi dibaca menggunakan Multiskan Go pada panjang gelombang 490 nm.

4.3.2. Kokultur hWJMSCs dan sel NK yang diinduksi interleukin

Sebanyak 10 x 10^5 sel hWJMSCs dikulturkan dalam 2 ml medium lengkap (α-MEM + 20% FBS + 1% ABAM) pada 24 *well plate* kemudian diinkubasi selama 24 jam pada suhu 37^0 C, 5% CO_2. Medium sel hWJMSCs kemudian diganti menggunakan 2 ml medium free serum kemudian diinkubasi selama 24 jam pada suhu 37^0 C, 5% CO_2. Sel kemudian dibilas menggunakan PBS sebanyak dua kali. Sebanyak 1 x 10^5 sel NK untuk perbandingan 1:1/hWJMSCs : NK (IL2-NK, IL15-NK ataupun normal) dan 5 x 10^5 sel NK untuk perbandingan 1:5/hWJMSCs : NK diresuspensikan ke dalam 24 well plate yang telah berisikan hWJMSCs dengan medium NK free serum dan α-MEM free serum dengan perbandingan 50%:50%. Sel diinkubasi selama 24 jam pada suhu 37^0 C, 5% CO_2. Sel dan medium dipanen dengan cara disentrifugasi pada kecepatan 500 g selam 4 menit untuk pengujian selanjutnya yaitu pengukuran kadar IFN-γ, TNF-α, perforin, granzim serta digunakan untuk uji sitotoksik pada sel kanker MCF7

4.3.3. Pemanenan medium terkondisi (CM), sel hasil kokultur hWJMSCs dan sel NK yang diinduksi interleukin pada sel kanker

Sebanyak 10 x 10^5 hWJMSCs dikulturkan dalam 2 ml medium lengkap (α-MEM + 20% FBS + 1% ABAM) pada 24 well plate kemudian diinkubasi selama 24 jam pada suhu 37^0 C, 5% CO_2. Medium hWJMSCs kemudian diganti menggunakan 2 ml medium free serum kemudian diinkubasi selama 24 jam pada suhu 37^0 C, 5% CO_2. Sel kemudian dibilas menggunakan PBS sebanyak dua kali. Sebanyak 10 x 10^5 sel NK untuk perbandingan 1 :

1/hWJMSCs : NK (IL2-NK, IL15-NK ataupun normal) dan 5 x 10^5 sel NK untuk perbandingan 1 : 5/hWJMSCs : NK diresuspensikan ke dalam 24 well plate yang telah berisikan hWJMSCs dengan medium NK *free serum* dan α-MEM free serum dengan perbandingan 50%:50%. Sebagai kontrol ditanam pula sel NK tanpa hWJMSCs dengan jumlah mewakili perbandingan kanker 1 : yaitu 1000.000 sel NK dan perbandingan 1 : 5 yaitu 5000.000 sel NK pada well terpisah. Sel diinkubasi selama 24 jam pada suhu 37^0C, 5% CO_2. Sel dan medium dipanen dengan cara disentrifugasi pada kecepatan 500 g selam 4 menit.

4.3.4. Pengujian sitotoksik CM kokultur hWJMSCs dan sel NK yang diinduksi interleukin pada sel kanker

Sel kanker MCF-7 ditanam ke dalam substrat 96 well plate dengan densitas 5x 10^3 sel/well menggunakan 100 µl medium lengkap (MCF-7 : DMEM + 10% FBS + 1% PenStrep). Sel kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Medium sel lama kemudian dibuang, sel dicuci menggunakan PBS. Medium sel kemudian diganti menggunakan medium *free serum* (MCF-7 : DMEM + 1% PenStrep) untuk kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam.

Sel selanjutnya diberikan perlakuan menggunakan medium terkondisi (CM) hasil kokultur hWJMSCs+NK (IL2-NK, IL15-NK, NIL18-NK ataupun normal) dengan konsentrasi 30% dan 60%. Sel kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Selanjutnya ke dalam sel di tambahkan 20 µl reagen *cell titer 96 ® Aqueouse one solution assay* dan sel diinkubasi selama 3 jam. Diinkubasikan pada 37^0 C, 5% CO_2. Absorbansi dibaca menggunakan Multiskan Go reader pada panjang gelombang 490 nm.

4.3.5. Pengukuran kadar TNF-α dan IFN-γ dari medium terkondisi (CM) hasil kokultur hWJMSCs+NK

Pada setiap *well* dimasukkan sebanyak 100 µl *capture antibody*. Selanjutnya *well* diinkubasi semalam pada suhu 4^0C. *Plate* dicuci sebanyak 4 kali menggunakan 200 µl *wash buffer.* Selanjutnya ditambahkan 200 *µl assay diluent* ke dalam setiap *well. Plate* kemudian disegel dan diinkubasi selama 1 jam dengan kecepatan 200 rpm pada suhu ruang. Plate kemudian dicuci 4 kali menggunakan 200 µl wash buffer. Setiap masing-masing plate ditambahkan 100 µl standar pada *well* standard an 100 µl sampel pada *well* sampel. *Plate* kemudian diinkubasi selama 2 jam dengan kecepatan 200 rpm pada suhu ruang. *Plate* dicuci sebanyak 4 kali menggunakan 200 µl *wash buffer.* Sebanyak 100 µl *detection antibody*

ditambahkan ke dalam setiap *well. Plate* kemudian disegel dan diinkubasi selama 1 jam dengan kecepatan 200 rpm pada suhu ruang. *Plate* dicuci sebanyak 4 kali menggunakan 200 µl *wash buffer*. Sebanyak 100 µl HRP-Avidin ditambahkan ke dalam setiap *well. Plate* kemudian disegel dan diinkubasi selama 30 menit dengan kecepatan 200 rpm pada suhu ruang. *Plate* dicuci sebanyak 5 kali menggunakan 200 µl *wash buffer.* Selanjutnya dimasukan 100 µl TMB sustrat ke dalam setiap *well* dan diinkubasi selama 15-30 menit dalam keadaan gelap. Pada tahap terakhir ditambahkan 100 µl *stop solution*. Absorbansi plate kemudian dibaca pada panjang gelombang 450 nm.

4.3.6. Pengukuran kadar perforin dan granzim dari medium terkondisi (CM), hasil kokultur hWJMSCs+NK

Setiap masing-masing plate ditambahkan 100 µl standar pada *well* standard dan 100 µl sampel pada *well* sampel. *Plate* kemudian disegel dan diinkubasi selama 90 menit pada suhu 37^0C. Segel *plate* kemudian dibuka, sampel dibuang dan tanpa proses pencucian langsung ditambahkan ke dalam masing-masing *well* 100 µl *Biotinylated Detection Ab*. *Plate* kemudian disegel dan diinkubasi selama 60 menit pada suhu 37^0C. *Plate* dicuci sebanyak 3 kali menggunakan 300 µl *wash buffer.* Sebanyak 100 µl HRP-Conjugate ditambahkan ke dalam setiap *well. Plate* kemudian disegel dan diinkubasi selama 30 menit pada suhu 37^0C. *Plate* dicuci sebanyak 3 kali menggunakan 300 µl *wash buffer.* Selanjutnya dimasukan 90 µl TMB substrat ke dalam setiap *well* dan diinkubasi selama 15-30 menit dalam keadaan gelap. Pada tahap terakhir ditambahkan 50 µl *stop solution.* Absorbansi plate kemudian dibaca pada panjang gelombang 450 nm.

4.3.7. Pengukuran induksi apoptosis kokultur CM-hWJMSCs+NK pada sel MCF7 metode flowcytometry

Sel kanker MCF-7 ditanam ke densitas 10 x 10^5 sel/ cm^2 pada 6 well plate menggunakan 1 mL medium lengkap (MCF-7 : DMEM + 10% FBS + 1% PenStrep). Sel kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Medium sel lama kemudian dibuang, sel dicuci menggunakan PBS. Medium sel kemudian diganti menggunakan medium *free serum* (MCF-7 : DMEM + 1% PenStrep) untuk kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam.

Sel selanjutnya diberikan perlakuan menggunakan medium terkondisi (CM) hasil kokultur hWJMSCs+NK (IL2-NK, IL15-NK ataupun normal) dengan konsentrasi 50% kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Setelah 24 jam sel dipanen

menggunakan tripsin-EDTA. Sel kemudian dihitung menggunakan hemositometer, sebanyak 10^6 sel dicuci menggunakan PBS + 2% FBS dingin. Sentrifugasi pada suhu 4^oC, 10 menit, 300g kemudian supernatan dibuang pelet sel diresuspensi dengan 1ml Binding buffer. Tambahkan 10 µl Annexin FITC, kemudian inkubasi 15 menit pada tempat gelap. Sel kemudian dicuci menggunakan 1ml Binding buffer, sentrifugasi pada suhu 4^oC, 10 menit, 300g. Resuspensi sel dengan 500 µl Binding buffer, dan tambahkan 5 µl PI. Sel dianalisa menggunakan MACSQuant® Analyzers (Miltenyi).

4.3.8. Pengukuran ekspresi gen apoptosis, gen antiapotosis CM-hWJMSCs+NK pada sel MCF7 metode RTPCR

Sel kanker MCF-7 ditanam ke densitas 10 x 10^5 sel/ cm^2 pada 6 well plate menggunakan 1 mL medium lengkap (MCF-7 : DMEM + 10% FBS + 1% PenStrep). Sel kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Medium sel lama kemudian dibuang, sel dicuci menggunakan PBS. Medium sel kemudian diganti menggunakan medium *free serum* (MCF-7 : DMEM + 1% PenStrep) untuk kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Sel selanjutnya diberikan perlakuan menggunakan medium terkondisi (CM) hasil kokultur hWJMSCs+NK (IL2-NK, IL15-NK ataupun normal) dengan konsentrasi 50% kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam, sel siap dipanen untuk dilkukan isolasi RNA.

Medium pertumbuhan sel dibuang, sel dicuci menggunakan PBS (4^0 C). Selanjutnya masing-masing dimasukan sebanyak 350 µl *RNA lysis buffer*. Sel selanjutnya dilisiskan menggunakan pipet secara ber-ulang. Suspensi sel dipindahkan ke dalam *microtube* 1.5 ml dan ditambahkan sebanyak 350 µl EtOh 70%. Seluruh suspensi sel selanjutnya dimasukan ke dalam *column spin* dan disentrifugasi pada kecepatan 13.000 g selama 1 menit pada suhu 4^0C. Selanjutnya filtrat dibuang, dan sebanyak 700 µl *Low stringency buffer* dimasukan ke dalam *column spin.* Selanjutnya sentrifugasi column pada kecepatan 13.000 g selama 1 menit pada suhu 4^0C. Selanjutnya filtrat dibuang, dan sebanyak 80 µl DNAase I (5 µl DNAase I + 75 µl 75 DNAase dilution) dimasukan ke dalam *column spin*. Inkubasi *column* pada suhu ruang selama 25 menit. Selanjutnya ditambahkan 700 µl *High stringency buffer* ke dalam *column spin*. Sentrifugasi *column* pada kecepatan 13.000 g selama 1 menit pada suhu 4^0C. Selanjutnya filtrat dibuang, dan sebanyak 700 µl *Low stringency buffer* dimasukan ke dalam *column spin*. Selanjutnya sentrifugasi *column* pada kecepatan 13.000 g selama 1 menit pada suhu 4^0C. Selanjutnya filtrat dibuang, *column* disentrifugasi kembali pada kecepatan 13.000 g selama 2 menit pada suhu 4^0C, untuk menghilangkan sisa *Low stringency*

buffer. Selanjutnya filtrat dibuang, dan sebanyak 45 µl RNA elution dimasukan ke dalam *column spin.* Inkubasi *column* pada suhu ruang selama 1 menit. Selanjutnya sentrifugasi *column* pada kecepatan 13.000 g selama 1 menit pada suhu 4^0C. Supernatan diambil dan disimpan pada suhu -80^0C.

Proses *reverse* RNA menjadi cDNA ini dilakukan menggunakan Kit I *Script cDNA Synthesis*. Sebanyak 16 µl sampel RNA dicampurkan dengan 4 µl I *Script supermix* dalam microplate PCR. Mix selanjutnya diinkubasi dengan suhu: Priming 25^0C 5 menit, Reverse 42^0C 30 menit, RT Inactivation 85^0C 5 menit, Hold 4^0C~. Hasil reverse berupa cDNA disimpan pada suhu -20^0C.

Proses analisis RT PCR ini dilakukan menggunakan Kit *Sso Fast Evagreen Supermix*. Berikut merupakan mix yang digunakan dalam analisis RT-PCR : Evagreen suoermix 5 µl, Primer forward 0.3 µl, Primer reverse 0.3 µl, Sampel cDNA 1 µl, Nuclease free water 3,4 µl

Kondisi RT-PCR dengan reference gene β-actin dan gen P53, BAX dan BCL2

Tahapan	Suhu (^{o}C)	Waktu
Denaturasi / Inaktivasi RT	95	30 detik
Jumlah siklus 40 :		
Denaturasi	95	5 detik
Penempelan primer (*Anealing*)	58	20 detik
Perpanjangan rantai (*Elongasi*)	72	40 detik

BAB V

HASIL DAN LUARAN YANG DICAPAI

5.1 Efek Pengaruh interleukin (IL2) terhadap viabilitas sel NK

Pengaruh induksi interleukin (IL-2) terhadap viablitas (persen sel hidup) sel NK pada berbagai lama inkubasi (hari 1,2,3,4) dapat dilihat pada tabel 5.1

Tabel 0.1. Pengaruh induksi interleukin (IL-) terhadap viabiliats (%) sel NK

Konsentrasi induksi IL pada sel NK	Viabilitas sel NK (%) Waktu inkubasi (hari 1)	Waktu inkubasi (hari 2)	Waktu inkubasi (hari 3)	Waktu inkubasi (hari 4)
Kontrol (NK)	100.00±13.16 ^{abA}	100.00±4.17 ^{abA}	100.00±0.46 ^{aA}	100.00±6.15 aA
IL2-NK (5 ng/ml)	104.14±12.49 ^{abA}	109.66±9.12 ^{abcAB}	134.50±16.67 ^{bcBC}	143.13±3.20 ^{bcC}
IL2-NK (10 ng/ml)	122.67±9.36 ^{bA}	129.12±0.39 ^{cA}	152.69±11.63 ^{cB}	153.16±4.21 ^{cB}

Data rata-rata dan standard deviasi hasil penelitian viabilitas sel NK (%) dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar konsentrasi dan jenis interleukin (IL-2)) pada setiap waktu inkubasi (hari 1, 2, 3, 4), perbedaan huruf besar pada baris yang sama (antar waktu inkubasi (hari 1,2,3,4)) menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

Berdasarkan hasil penelitian menunjukkan bahwa induksi interleukin (IL-2) dapat meningkatkan viabilitas sel NK. Semakin lama inkubasi sel NK semakin meningkatkan viabilitas. Viabilitas sel NK hari ke 2 paling tinggi adalah sel NK yng diinduksi IL-2 10 ng/ml (IL2-NK 10 ng./ml).

5.2. Pengaruh interleukin terhadap kadar TNF-α, IFN-γ, peforin, granzim CM kokultur hWJMSCs dan sel NK

Untuk mengetahui efek dari sel NK yang diindusk interleukin terhadap kadar TNF-α, IFN-γ, peforin, granzim *conditioned medium* kokultur hWJMSCs dan sel NK maka dilakukan uji sel tunggal NK yang tidak di kokultur bersama hWJMSCs. Hasil penelitian induksi interleukin sel NK terhadap kadar TNFα, IFN-γ pada berbagai jumlah sel NK dapat dilihat pada tabel 5.2

Tabel 5.2. Pengaruh induksi interleukin (IL-2) dan jumlah sl NK terhadap kadar TNFα, IFN-γ sel NK

Konsentrasi induksi IL pada sel NK	Kadar IFN-γ, TNF-α	
	IFN-γ (pg/ml)	TNF-α (pg/ml)
NK (1:1)	8,06±1,90 [a]	8,49±1,60 [a]
NK (1:5)	13,72±1,71 [b]	12,27±1,63 [ab]
NK-IL-2 10 ng/ml (1:1)	14,72±1,71 [b]	16,38±2,76 [b]
NK-IL-2 10 ng/ml (1:5)	21,39±1,68 [c]	22,88±1,51 [c]

Data rata-rata dan standard deviasi hasil penelitian ekspresi reseptor sel NK (%) dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar konsentrasi dan jenis interleukin (IL-2) menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

Berdasarkan hasil penelitian kadar IFN-γ (tabel 5.2) menunjukkan bahwa jumlah sel NK (1:5) atau berjumlah $6x10^5$ sel lebih besar dibanding sel NK berjumlah 1:1 atau berjumlah $2x10^5$ sel pada semua jenis sel baik sel NK kontrol tanpa induksi interleukin maupun sel NK yang diinduksi interleukin.

Untuk mengetahui kadar TNF-α, IFN-γ, peforin, granzim pada conditioned medium hasil kokultur hWJMSCs dan sel NK yang diinduksi induksi interleukin (IL-2) dapat dilihat pada tabel 5.3.

Tabel 5.3. Pengaruh induksi interleukin (IL-2) dan jumlah sel NK terhadap kadar TNFα, IFN-γ, perforin, granzim CM-(hJMSCs+NK)

Konsentrasi induksi IL pada sel NK, perbandingan jumlah sel NK	Kadar IFN-γ (pg/ml)	TNF-α (pg/ml)	Perforin (ng/ml)	Granzim (pg/ml)
CM-hWJMSCs	0,94±0,19 [a]	2,64±0,47 [a]	0,00±0,00 [a]	0,00±0,00 [a]
CM-hWJMSCs +NK(1:1)	7,72±2,52 [ab]	5,07±0,25 [ab]	0,05±0,02 [ab]	6,00±2,00 [a]
CM-hWJMSCs +NK(1:5)	22,61±1,95 [c]	8,58±0,07 [b]	0,09±0,03 [bc]	16,33±3,21 [ab]
CM-hWJMSCs +NK-IL2(1:1)	9,17±2,73 [ab]	14,34±0,72 [c]	0,11±0,03 [bcd]	30,67±6,66 [b]
CM-hWJMSCs +NK-IL2(1:5)	23,94±1,02 [c]	28,12± 3,02 [d]	0,18±0,01 [cde]	57,33±0,58 [c]

Data rata-rata dan standard deviasi hasil penelitian kadar IFN-γ, TNF-α, perforin, granzim dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar konsentrasi dan jenis interleukin (IL-2, IL-15, IL-18)) menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

Berdasarkan hasil penelitian kadar IFN-γ (tabel 5.3) menunjukkan bahwa sel tunggal hWJMSCs tanpa kokultur mensekresikan IFN-γ sangat rendah (0,94 pg/ml), kokultur hWJMSCS dan sel NK meningkatkan kadar IFN-γ. Perbandingan jumlah sel NK mempengaruhi kadar IFN-γ, jumlah sel NK 5 kali atau perbandingn sel NK 1:5 menunjukkan kadar IFN-γ lebih tinggi dibanding jumlah sel NK dengan perbandingan 1:1.

5.3. Pengaruh interleukin terhadap kadar TNF-α, IFN-γ, peforin, granzim conditioned medium kokultur sel MCF7 dan sel NK

Untuk mengetahui kadar TNF-α, IFN-γ, peforin, granzim pada conditioned medium hasil kokultur sel MCF7 dan sel NK yang diinduksi induksi interleukin (IL2) dapat dilihat pada tabel 5.4.

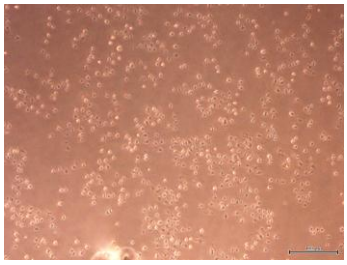
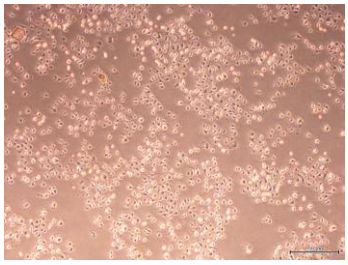
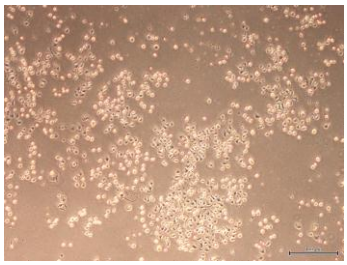
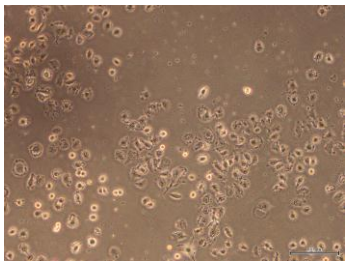
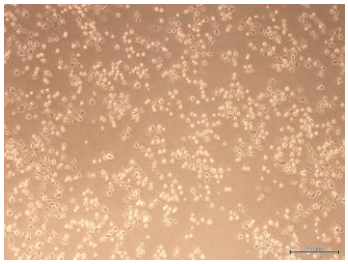
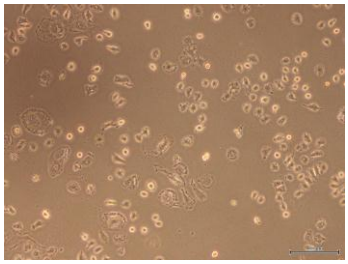
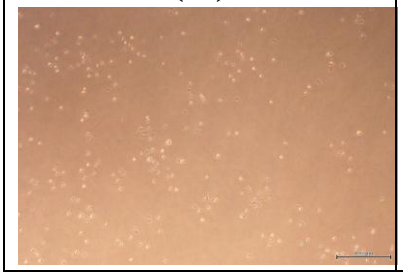
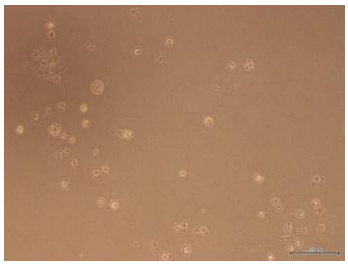
Tabel 5.4. Pengaruh induksi interleukin (IL-2) dan jumlah sel NK terhadap kadar TNFα, IFN-γ, perforin, granzim CM-(MCF7+NK)

Konsentrasi induksi IL pada sel NK, perbandingan jumlah sel NK	Kadar			
	IFN-γ (pg/ml)	TNF-α (pg/ml)	Perforin (ng/ml)	Granzim (pg/ml)
CM-MCF7	1,83±0,33 [a]	1,98±0,24 [a]	0,00±0,00 [a]	0,00±0,00 [a]
CM-MCF7+NK(1:1)	14,50±3,93 [b]	5,24±0,28 [a]	0,04±0,01 [ab]	29,00±4,36 [b]
CM-MCF7+NK(1:5)	32,28±1,35 [c]	13,85±2,79 [b]	0,11±0,01 [bc]	55,67±12,50 [b]
CM-MCF7+NKIL-2(1:1)	17,39±3,56 [b]	15,04±0,21 [bc]	0,16±0,04 [cde]	87,00±11,79 [c]
CM-MCF7+NKIL-2(1:5)	35,72±0,38 [c]	37,44±1,47 [d]	0,23±0,04 [def]	149,00±7,21 [d]

Data rata-rata dan standard deviasi hasil penelitian kadar IFN-γ, TNF-α, perforin, granzim dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar konsentrasi dan jenis interleukin (IL-2)) menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

Berdasarkan hasil penelitian kadar IFN-γ (tabel 5.4) menunjukkan bahwa sel tunggal sel kanker MCF7 mensekresikan IFN-γ (1,83 pg/ml), kokultur sel MCF7 dan sel NK meningkatkan kadar IFN-γ. Perbandingan jumlah sel NK mempengaruhi kadar IFN-γ, jumlah sel NK 5 kali atau perbandingn sel NK 1:5 menunjukkan kadar IFN-γ lebih tinggi dibanding jumlah sel NK dengan perbandingan 1:1.

Untuk mengetahui morfologi sel MCF7 yang diberi perlakuan CM-(hWJMSCs+IL2-NK) konsentrasi 50% dapat dilihat pada Gambar 5.1

4x	10x
Kontrol 	
CM-hWJMSCs (4x) 	CM-hWJMSCs (10x) 
CM-(hWJMSCs+NK) (4x) 	CM-(hWJMSCs+NK) (10x) 
CM-(hWJMSCs+IL2-NK) (4x) 	CM-(hWJMSCs+IL2-NK) (`10x) 

Gambar 5.1. Morfologi sel MCF7 yang diberi perlakuan
50 % CM-(hWJMSCs+IL2-NK)

5.4. Hasil sitotoksik CM dari kokulltur hWJMSCs dan sel NK yang diinduksi interleukin pada sel kanker

Berdasarkan hasil uji sitotoksik CM dari kokultur hWJMSCs dan sel NK yang diinduksi interleukin pada sel kanker MCF7 dapat dilihat pada Tabel 5.5.

Tabel 5.5. Pengaruh CM kokultur hWJMSCs dan sel NK terhadap viablitas dan penghambatan pertumbuhan sel MCF7

CM kokultur hWJMSCs dan sel NK diinduksi interleukin	MCF7			
	Viabilitas %		Penghambatan %	
	Konsentrasi CM 30% hWJMSCs+NK-IL	Konsentrasi CM 60% hWJMSCs+NK-IL	Konsentrasi CM 30% hWJMSCs+NK-IL	Konsentrasi CM 60% hWJMSCs+NK-IL
hWJMSCs-CM	67,45±6,57 ^{d}	52,00±8,70 ^{d}	32,55±6,57 ^{a}	48,00±8,70 ^{a}
hWJMSCs-CM+NK (1:1)	59,56±4,02 ^{cd}	48,14±3,17 ^{cd}	40,44±4,02 ^{a}	51,86±3,17 ^{ab}
hWJMSCs-CM +NK (1:5)	57,94±3,58 ^{cd}	45,37±8,00 ^{bcd}	42,06±3,58 ^{ab}	54,63±8,00 ^{abc}
hWJMSCs-CM+NK-IL-2 (1:1)	45,34±4,08 ^{bc}	39,76±0,71 ^{abcd}	54,66±4,08 ^{bc}	60,24±0,71 ^{abcd}
hWJMSCs-CM+NK-IL-2 (1:5)	38,52±1,26 ^{ab}	31,72±3,81 ^{ab}	61,48±1,26 ^{cd}	68,28±3,81 ^{cd}

Data rata-rata dan standard deviasi hasil penelitian viabilitas, penghambatan CM koklutur hWJMSCs+NK yang diinduksi interleukin (IL-2) pada jumlah perbandingan sel NK 1:1 dan 1:5 dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar jenis interleukin (IL-2, IL-15, IL-18) adn perbandingan sel NK) menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

Berdasarkan hasil penelitian uji sitotoksik CM koklutur hWJMSCs dan sel NK yang diinduksi interleukin (IL-2) pada perbandingan hWJMSCs dan NK 1:1 dan 1:5 menunjukkan bahwa konsentrasi CM 60 % lebih aktif dalam menghambat proliferasi sel MCF7 dibanding CM 30%.

5.5. Hasil induksi apoptosis CM dari kolkultur hWJMSCs dan sel NK yang diinduksi interleukin pada sel kanker

Berdasarkan hasil uji sitotoksisitas tabel 5.5 menunjukkan bahwa CM hasil kokultur hWJMSCs dan sel NK memiliki aktivitas antikanker pada sel kanker MCF7 dengan daya hambat 32,55 – 61,48 % pada konsentrasi CM 30% sedangkan konsentrasi CM 60% menunjukkan daya hambat lebih tinggi yaitu 48,00 – 68,28 %, sehingga dilanjutkan uji induksi apoptosis metode *flowcytometry* dilakukan pada konsentrasi CM 50 % dengan perbandingan hWJMSCs dan sel NK 1:5. Hasil uji induksi apoptosis hWJMSCs-CM+NK-IL pada sel MCF7 dapat dilihat pada tabel 5.6.

Tabel 5.6. Pengaruh CM kokultur hWJMSCs dan sel NK terhadap induksi apoptosis sel kanker MCF7

CM kokultur hWJMSCs dan sel NK diinduksi interleukin	Apoptosis (%)	Sel hidup (%)	Nekrosis (%)	Sel mati (%)
MCF7 medium FBS	4,51±0,78 ^{a}	87,10±1,56 ^{d}	6,74±0,29 ^{b}	1,65±0,78 ^{a}
MCF7 menium non FBS	11,40±2,69 ^{b}	61,82±3,03 ^{c}	4,17±0,36 ^{a}	22,51±0,61 ^{e}
CM-hWJMSCs	33,11±0,92 ^{c}	49,30±1,01 ^{b}	6,70±0,28 ^{b}	10,88±0,15 ^{c}
CM(hWJMSCs+NK)	34,66±1,93 ^{cd}	53,15±3,75 ^{b}	4,87±0,22 ^{a}	7,32±1,74 ^{b}
CM(hWJMSCs+IL2-NK)	36,50±2,65 ^{cd}	42,47±0,65 ^{a}	5,01±0,87 ^{a}	15,92±1,69 ^{d}

Data rata-rata dan standard deviasi hasil penelitian apoptosis, sel hidup, nekrosis, sel mati dari CM koklutur hWJMSCs+NK yang diinduksi interleukin 10 ng/ml (IL-2,) pada jumlah perbandingan sel 1:5 dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar jenis interleukin (IL-2, IL-15, IL-18) dan perbandingan sel NK) menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

5.6. Hasil ekspresi gen apoptosis, antiapoptosis CM dari kolkultur hWJMSCs dan sel NK yang diinduksi interleukin pada sel kanker

Setelah dilakukan uji induksi apoptosis menggunakan metode *flowcytometry*, menunjukkan bahwa CM hasil kokultur hWJMSCs dan sel NK bersifat antikanker (tabel 5.6.) dan menginduksi apoptosis (tabel 5.7.), maka perlu dilanjutkan uji ekspresi gen proapotosis, antiapoptosis untuk mengetahui gen yang terlibat dalam induksi apoptosis. Ekspresi gen proapoptosis yang diuji meliputi gen BAX dan BCL-2 sedangkan gen antiapotosis adalah gen BCL-2. Hasil uji ekspresi gen proapoptosis, antiapoptosis dari CM-hWJMSCs dan CM hasil kokultur hWJMSCs dan sel NK yang diinduksi IL-2, IL-15, IL-18 dapat dilihat pada tabel 5.7.

Tabel 5.7. Pengaruh CM kokultur hWJMSCs dan sel NK terhadap ekspresi gen apoptosis, antiapotosis pada sel kanker MCF7

CM kokultur hWJMSCs dan sel NK diinduksi interleukin	BAX	P53	BCL-2
MCF7 medium non FBS	1,00±0,00 ^{ab}	1,00±0,00 ^{a}	1,00±0,00 ^{a}
MCF7 medium FBS	0,80±0,11 ^{a}	1,11±0,24 ^{a}	1,08±0,02 ^{a}
CM-hWJMSCs	1,36±0,36 ^{ab}	1,23±0,25 ^{a}	0,93±0,09 ^{a}
CM-(hWJMSCs+NK)	1,52±0,17 ^{b}	1,47±0,15 ^{a}	0,96±0,19 ^{a}
CM-(hWJMSCs+IL2-NK)	1,46±0,16 ^{b}	1,94±0,44 ^{a}	0,88±0,19 ^{a}

Data rata-rata dan standard deviasi hasil penelitian ekspresi gen proapoptosis, antiapoptosis dari CM koklutur hWJMSCs+NK yang diinduksi interleukin 10 ng/ml (IL-2) pada jumlah perbandingan sel 1:5 dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar jenis interleukin (IL-2) dan perbandingan sel NK) menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

Berdasarkan hasil penelitian pengaruh CM hasil kokultur hWJMSCs dan sel NK dan sel NK yang diinduksi interleukin 10 ng/ml (IL-2) dengan perbandingan sel MCF7/NK sebesar 1:5 menunjukkan CM-hWJMSCs dan CM-(hWJMSCs+NK) secara signifikan dapat menginduksi ekspresi gen proapoptosis BAX dibanding kontrol (tanpa perlakuan).

5.7. Luaran yang dicapai

No	Kegiatan	Judul artikel/buku	Keterangan
1	Seminar dan Kongres Nasional ASPI ke-3 dengan tema "Indonesia Stem Cell Summit" Bogor 6-7 Februari 2016,	Conditioned Medium from Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) Has Lower Activity in Inhibiting Proliferation and Inducing Apoptosis on Breast Cancer Cells Line (T47D, MCF7) Compared to Normoxia (WJMSCs-norCM)	Invited Speaker
2	PCS Cell Science and Stem Cell Conference	The Cytotoxic Effect of Conditioned Medium from Interleukins-Induced	Oral Presentation

	(CSSC—2016), Barcelona, Spain 11-12 Juni 2016	human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs-ILs-CM), ILs Induced-Natutal Killer Cells (NK-ILs), Interleukins (ILs) toward Breast Cancer (BC) and Natural Killer (NK) Cells	
3	3rd International Conference of Advance Molecular Bioscience and Biomedical Engineering 2016 (ICAMBBE 2016)" 4-5 August, 2016	Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward TNF-α, IFN-γ, Perforin, Granzyme Concentration, Apoptotic Inducing Activity in Breast Cancer Cells	Oral Presentation
4	Draft manuscript telah submitted "Turkish Journal of Immunology" SCOPUS	Conditioned Medium from Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) Has Lower Activity in Inhibiting Proliferation and Inducing Apoptosis on Breast Cancer Cells Line (T47D, MCF7) Compared to Normoxia (WJMSCs-norCM)	Under review
5	Draft manuscript telah submitted "The Korean Association of Internal Medicine" SCOPUS	Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward Apoptotic Inducing Activity in Breast Cancer Cells	Under review
6	Telah terbit Chapter book	Hypoxia in Mesenchymal Stem Cells	Penerbit "In Tech"
7	Telah daftar hak paten 28 Sepetmber 2016	IL-2 meningkatkan sitotosik sel NK terhadap sel kanker payudara	Terdaftar SII 20160000016
8	Telah didaftarkan pada Penerbit Erlangga draft buku	Pemanfaatan Mesenchymal Stem Cell pada Terapi Kanker: Dasar Teori dan Strategi Aplikasi Klinis	Proses editing

BAB VI

KESIMPULAN DAN SARAN

6.1. Kesimpulan

1. IL2 meningkatkan proliferasi sel NK, semakin lama inkubasi semkain meningkat proliferasi
2. IL2 meningkatkan kadar TNFα, IFNγ sel NK,
3. Sel NK yang diinduksi IL2 (IL2-NK) dapat meningkatkan produksi TNFα, IFNγ, perforin, granzim dari CM kokultur hWJMSCs+IL2-NK
4. IL2-NK dapat menginduksi apoptosis pada sel MCF7,
5. Sel NK yang diinduksi IL2 (IL2-NK) dapat meningkatkan produksi TNFα, IFNγ, perforin, granzim dari CM kokultur MCF7+IL2-NK,
6. IL2-NK dapat menginduksi apoptosis dan meningkatkan ekspresi gen BAX, P53 dan menurunkan ekspresi gen BCL-2 pada sel MCF7.

6.2. Saran

1. Perlu dilakukan penelitian lebih lanjut untuk sorting sel NK dari penderita kanker payudara kemudian diaktifkan menggunkana interleukin (IL-2, IL-15)
2. Perlu dilakukan penelitian lebih lanjut untuk sorting cancer stem cell (CSCs) dari penderita kanker payudara kemudian sebagai target sel NK yang diaktifkan IL-2, IL-15

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Lampiran 1. Hasil uji efek induksi interleukin terhadap proliferasi sel NK

Lampiran 1.1. Absorbansi, viabilitas sel IL-NK hari 1

Perlakuan	Absorbansi			Blank	Viabilitas			Rata-rata	STD
	1	2	3		1	2	3		
Kontrol	1,6365	1,5825	1,6555	1,3371	104,05	85,29	110,66	100,00	13,16
IL-2 5 ng	1,6807	1,6172	1,6198	1,3396	118,55	96,48	97,38	104,14	12,49
IL-2 10 ng	1,7596	1,8075	1,8049	1,4377	111,87	128,52	127,62	122,67	9,36

Lampiran 1.2. Absorbansi, viabilitas sel IL-NK hari 2

Perlakuan	Absorbansi			Blank	Viabilitas			Rata-rata	STD
	1	2	3		1	2	3		
Kontrol	1,4611	1,4630	1,4213	0,8833	102,24	102,57	95,19	100,00	4,17
IL-2 5 ng	1,4040	1,5071	1,4556	0,8358	100,54	118,78	109,67	109,66	9,12
IL-2 10 ng	1,5620	1,5651	1,5662	0,8347	128,69	129,24	129,43	129,12	0,39

Lampiran 1.3. Absorbansi, viabilitas sel IL-NK hari 3

Perlakuan	Absorbansi			Blank	Viabilitas			Rata-rata	STD
	1	2	3		1	2	3		
Kontrol	1,4118	1,4064	1,4116	0,7497	100,28	99,46	100,25	100,00	0,46
IL-2 5 ng	1,5665	1,7227	1,5103	0,7118	129,45	153,11	120,94	134,50	16,67
IL-2 10 ng	1,6765	1,6952	1,8178	0,7217	144,62	147,45	166,02	152,69	11,63

Lampiran 1.4. Absorbansi, viabilitas sel IL-NK hari 4

Perlakuan	Absorbansi			Blank	Viabilitas			Rata-rata	STD
	1	2	3		1	2	3		
Kontrol	1,3104	1,3894	1,3952	0,5945	92,91	103,17	103,92	100,00	6,15
IL-2 5 ng	1,6478	1,6579	1,6110	0,5361	144,28	145,59	139,51	143,13	3,20
IL-2 10 ng	1,7314	1,7748	1,7113	0,5591	152,15	157,78	149,54	153,16	4,21

Lampiran 1.5. Viabiltas sel IL-NK hari 1 sd hari 4

Perlakuan	Viabilitas (%)											
	Hari 1			Hari 2			Hari 3			Hari 4		
Kontrol	104,05	85,29	110,66	102,24	102,57	95,19	100,28	99,46	100,25	92,91	103,17	103,92
IL-2 5 ng	118,55	96,48	97,38	100,54	118,78	109,67	129,45	153,11	120,94	144,28	145,59	139,51
IL-2 10 ng	111,87	128,52	127,62	128,69	129,24	129,43	144,62	147,45	166,02	152,15	157,78	149,54

Lampiran 2. Hasil uji kadar IFN-γ, TNF-α sel sel NK yang diinduksi interleukin

Lampiran 2.1. Kurva standard kadar IFN-γ pada IL-NK

Konsentrasi	Absorbansi			Absorbansi koreksi			Persamaan
(pg/ml)	1	2	Blank	1	2	Rata-rata	
500	0,1823	0,1769	0,0483	0,1341	0,1287	0,1314	Y =0,0003X+0,0038
250	0,1060	0,1143		0,0578	0,0661	0,0619	R2 =0,9925
125	0,0896	0,0829		0,0414	0,0347	0,0380	
62,5	0,0734	0,0728		0,0252	0,0246	0,0249	
31,25	0,0632	0,0647		0,0150	0,0165	0,0157	
15,625	0,0530	0,0534		0,0048	0,0052	0,0050	
7,8125	0,0539	0,0515		0,0057	0,0033	0,0045	
0	0,0485	0,0480		0,0003	-0,0003	0,0000	

Lampiran 2.2. Absorbansi, kadar IFN-γ pada IL-NK

	Absorbance			Blank	Kadar IFN-γ			Rata-rata	STD
Perlakuan	1	2	3	0,0483	1	2	3		
NK(1 : 1)	0,0540	0,0543	0,0551		6,50	7,50	10,17	8,06	1,90
NK(1 : 5)	0,0563	0,0556	0,0566		14,17	11,83	15,17	13,72	1,71
NKIL-2(1 : 1)	0,0559	0,0569	0,0566		12,83	16,17	15,17	14,72	1,71
NKIL-2(1 : 5)	0,0590	0,0584	0,0580		23,17	21,17	19,83	21,39	1,68

Lampiran 2.3. Kurva standard kadar TNF-α pada IL-NK

Konsentrasi	Absorbansi			Absorbansi koreksi			Persamaan
(pg/ml)	1	2	Blank	1	2	Rata-rata	
500	1,8296	1,8855	0,0568	1,7729	1,8288	1,8008	Y =0,0036X+0,0748
250	1,2117	1,2235		1,1550	1,1668	1,1609	R2 =0,983
125	0,6045	0,6096		0,5478	0,5529	0,5503	
62,5	0,3799	0,4037		0,3232	0,3470	0,3351	
31,25	0,2161	0,2638		0,1594	0,2071	0,1832	
15,625	0,1635	0,1684		0,1068	0,1117	0,1092	
7,8125	0,1405	0,1194		0,0838	0,0627	0,0732	
0	0,0565	0,0570		-0,0003	0,0003	0,0000	

Lampiran 2.4. Absorbansi, kadar TNF-α pada IL-NK

	Absorbance				Kadar TNF-α			Rata-rata	STD
Treatment	1	2	3	Blank					
				0,0568					
NK(1 : 1)	0,1581	0,1595	0,1687		7,38	7,76	10,32	8,49	1,60
NK(1 : 5)	0,1723	0,1825	0,1724		11,32	14,15	11,35	12,27	1,63
NKIL-2(1 : 1)	0,1805	0,1907	0,2004		13,60	16,43	19,13	16,38	2,76
NKIL-2(1 : 5)	0,2202	0,2108	0,2108		24,63	22,01	22,01	22,88	1,51

Lampiran 3. Hasil uji kadar IFN-γ, TNF-α, perforin, granzim pada CM-(hWJMSCs+IL2-NK)

Lampiran 3.1. Hasil uji kadar IFN-γ dari CM-(hWJMSCs+IL2-NK)

Perlakuan	Absorbansi 1	2	3	Blank 0,0483	Kadar IFN-γ 1	2	3	Rata-rata	STD
hWJMSCs	0,0523	0,0524	0,0523		0,83	1,17	0,83	0,94	0,19
MSCs+NK (1:1)	0,0535	0,0547	0,0549		4,83	8,83	9,50	7,72	2,52
MSCs +NK (1:5)	0,0586	0,0595	0,0584		21,83	24,83	21,17	22,61	1,95
MSCs +NKIL-2 (1:1)	0,0539	0,0550	0,0555		6,17	9,83	11,50	9,17	2,73
MSCs +NKIL-2 (1:5)	0,0589	0,0593	0,0595		22,83	24,17	24,83	23,94	1,02

Lampiran 3.2. Kadar TNF-α pada CM-(hWJMSCs+IL2-NK)

Perlakuan	Absorbansi 1	2	3	Blank 0,0570	Kadar IFN-γ 1	2	3	Rata-rata	STD
hWJMSCs	0,1390	0,1415	0,1422		2,11	2,81	3,00	2,64	0,47
MSCs+NK (1:1)	0,1493	0,1490	0,1507		4,97	4,89	5,36	5,07	0,25
MSCs +NK (1:5)	0,1622	0,1621	0,1626		8,56	8,53	8,67	8,58	0,07
MSCs +NKIL-2 (1:1)	0,1853	0,1802	0,1836		14,97	13,56	14,50	14,34	0,72
MSCs +NKIL-2 (1:5)	0,2252	0,2276	0,2451		26,06	26,72	31,58	28,12	3,02

Lampiran 3.3. Kurva standard kadar perforin pada CM-(MCF7+IL2-NK)

Konsentrasi (ng/ml)	Absorbance 1	2	Blank	Koreksi 1	2	Rata-rata	Persamaan
50	0,3015	0,3059	0,0500	0,2516	0,2560	0,2538	Y=0,0052X+0,0211
25	0,2402	0,2460		0,1903	0,1961	0,1932	R2 =0,9215
12,5	0,1785	0,1712		0,1286	0,1213	0,1249	
6,25	0,1131	0,1034		0,0632	0,0535	0,0583	
3,125	0,0854	0,0833		0,0355	0,0334	0,0344	
1,5625	0,0674	0,0650		0,0175	0,0151	0,0163	
0,78125	0,0580	0,0593		0,0081	0,0094	0,0087	
0	0,0502	0,0497		0,0003	-0,0003	0,0000	

Lampiran 3.4. Kadar perforin dari CM-(hWJMSCs+IL2-NK)

Perlakuan	Absorbansi 1	2	3	Blank 0,0500	Perforin 1	2	3	Rata-rata	STD
hWJMSCs	0,0489	0,0484	0,0486		-4,24	-4,34	-4,30	-4,29	0,05
MSCs+NK (1:1)	0,0711	0,0713	0,0712		0,03	0,07	0,05	0,05	0,02
MSCs +NK (1:5)	0,0714	0,0716	0,0713		0,09	0,12	0,07	0,09	0,03
MSCs +NKIL-2 (1:1)	0,0715	0,0717	0,0714		0,11	0,14	0,09	0,11	0,03
MSCs +NKIL-2 (1:5)	0,0719	0,0718	0,0719		0,18	0,16	0,18	0,18	0,01

Lampiran 3.5. Kurva standard kadar granzim pada CM-(MCF7+IL2-NK)

Konsentrasi (ng/ml)	Absorbance 1	Absorbance 2	Blank	Koreksi 1	Koreksi 2	Rata-rata	Persamaan
1000	0,1739	0,1714	0,0501	0,1238	0,1213	0,1226	Y=0,0001X+0,006
500	0,1364	0,1311		0,0863	0,0810	0,0837	R2 =0,9613
250	0,1059	0,0929		0,0558	0,0428	0,0493	
125	0,0717	0,0709		0,0216	0,0208	0,0212	
62,5	0,0650	0,0656		0,0149	0,0155	0,0152	
31,25	0,0554	0,0553		0,0053	0,0052	0,0053	
15,625	0,0517	0,0519		0,0016	0,0018	0,0017	
0	0,0496	0,0506		-0,0005	0,0005	0,0000	

Lampiran 3.6. Kadar granzim dari CM-(hWJMSCs+IL2-NK)

Perlakuan	Absorbansi 1	Absorbansi 2	Absorbansi 3	Blank 0,0501	Granzim 1	Granzim 2	Granzim 3	Rata-rata	STD
hWJMSCs	0,0552	0,0557	0,0554		-9,00	-4,00	-7,00	-6,67	2,52
MSCs+NK (1:1)	0,0565	0,0569	0,0567		4,00	8,00	6,00	6,00	2,00
MSCs +NK (1:5)	0,0576	0,0575	0,0581		15,00	14,00	20,00	16,33	3,21
MSCs +NKIL-2 (1:1)	0,0590	0,0586	0,0599		29,00	25,00	38,00	30,67	6,66
MSCs +NKIL-2 (1:5)	0,0618	0,0618	0,0619		57,00	57,00	58,00	57,33	0,58

Lampiran 4. Hasil uji kadar IFN-γ, TNF-α, perforin, granzim pada CM-(MCF7+IL2-NK)

Lampiran 4.1. Kurva standard IFN-γ pada CM-(MCF7+IL2-NK)

Konsentrasi (pg/ml)	Absorbansi 1	Absorbansi 2	Blank	Absorbansi koreksi 1	Absorbansi koreksi 2	Rata-rata	Persamaan
			0,0483				Y
500	0,1823	0,1769		0,1341	0,1287	0,1314	=0,0003X+0,0038
250	0,1060	0,1143		0,0578	0,0661	0,0619	R2 =0,9925
125	0,0896	0,0829		0,0414	0,0347	0,0380	
62,5	0,0734	0,0728		0,0252	0,0246	0,0249	
31,25	0,0632	0,0647		0,0150	0,0165	0,0157	
15,625	0,0530	0,0534		0,0048	0,0052	0,0050	
7,8125	0,0539	0,0515		0,0057	0,0033	0,0045	
0	0,0485	0,0480		0,0003	-0,0003	0,0000	

Lampiran 4.2. Hasil uji kadar IFN-γ pada CM-(MCF7+IL2-NK)

Treatment	Absorbance 1	Absorbance 2	Absorbance 3	Blank 0,0483	Kadar IFN-γ 1	Kadar IFN-γ 2	Kadar IFN-γ 3	Rata-rata	STD
MCF7	0,0526	0,0527	0,0525		1,83	2,17	1,50	1,83	0,33
MCF7+NK(1:1)	0,0551	0,0574	0,0567		10,17	17,83	15,50	14,50	3,93
MCF7+NK(1:5)	0,0613	0,0621	0,0618		30,83	33,50	32,50	32,28	1,35
MCF7+NKIL-2(1:1)	0,0561	0,0582	0,0575		13,50	20,50	18,17	17,39	3,56
MCF7+NKIL-2(1:5)	0,0627	0,0627	0,0629		35,50	35,50	36,17	35,72	0,38

Lampiran 4.3. Kurva standard TNF-α pada CM-(MCF7+IL2-NK)

Konsentrasi (pg/ml)	Absorbansi 1	Absorbansi 2	Blank 0,0570	Absorbansi koreksi 1	Absorbansi koreksi 2	Rata-rata	Persamaan
500	1,8296	1,8855		1,7726	1,8285	1,8006	Y
250	1,2117	1,2135		1,1547	1,1565	1,1556	=0,0036X+0,0744
125	0,6045	0,6096		0,5475	0,5526	0,5501	R2 =0,9835
62,5	0,3799	0,4037		0,3229	0,3467	0,3348	
31,25	0,2161	0,2638		0,1591	0,2068	0,1830	
15,625	0,1635	0,1684		0,1065	0,1114	0,1090	
7,8125	0,1405	0,1194		0,0835	0,0624	0,0730	
0	0,0570	0,0570		0,0000	0,0000	0,0000	

Lampiran 4.4. Hasil uji kadar TNF-α pada CM-(MCF7+IL2-NK)

Perlakuan	Absorbansi 1	Absorbansi 2	Absorbansi 3	Blank 0,0570	TNF-α 1	TNF-α 2	TNF-α 3	Rata-rata	STD
MCF7	0,1385	0,1377	0,1394		1,97	1,75	2,22	1,98	0,24
MCF7+NK(1:1)	0,1508	0,1509	0,1491		5,39	5,42	4,92	5,24	0,28
MCF7+NK(1:5)	0,1719	0,1800	0,1919		11,25	13,50	16,81	13,85	2,79
MCF7+NKIL-2(1:1)	0,1850	0,1864	0,1852		14,89	15,28	14,94	15,04	0,21
MCF7+NKIL-2(1:5)	0,2623	0,2722	0,2640		36,36	39,11	36,83	37,44	1,47

Lampiran 4.5. Kurva standard kadar perforin pada CM-(MCF7+IL2-NK)

Konsentrasi (ng/ml)	Absorbance 1	Absorbance 2	Blank	Koreksi 1	Koreksi 2	Rata-rata	Persamaan
50	0,3006	0,3016	0,0497	0,2510	0,2520	0,2515	Y=0,0051X+0,018
25	0,2181	0,2148		0,1685	0,1652	0,1668	R2 =0,9539
12,5	0,1573	0,1712		0,1077	0,1216	0,1146	
6,25	0,1032	0,1035		0,0536	0,0539	0,0537	
3,125	0,0850	0,0833		0,0354	0,0337	0,0345	
1,5625	0,0669	0,0653		0,0173	0,0157	0,0165	
0,78125	0,0578	0,0589		0,0082	0,0093	0,0087	
0	0,0499	0,0494		0,0003	-0,0003	0,0000	

Lampiran 4.6. Hasil uji kadar perforin pada CM-(MCF7+IL2-NK)

Perlakuan	Absorbansi 1	Absorbansi 2	Absorbansi 3	Blank 0,0497	Perforin 1	Perforin 2	Perforin 3	Rata-rata	STD
MCF7	0,0556	0,0558	0,0554		-2,36	-2,32	-2,40	-2,36	0,04
MCF7+NK(1:1)	0,0678	0,0679	0,0679		0,03	0,05	0,05	0,04	0,01
MCF7+NK(1:5)	0,0682	0,0683	0,0682		0,11	0,13	0,11	0,11	0,01
MCF7+NKIL-2(1:1)	0,0683	0,0687	0,0684		0,13	0,21	0,15	0,16	0,04
MCF7+NKIL-2(1:5)	0,0686	0,0690	0,0689		0,19	0,26	0,25	0,23	0,04

Lampiran 4.7. Kurva standard kadar granzim pada CM-(MCF7+IL2-NK)

Konsentrasi (ng/ml)	Absorbance 1	Absorbance 2	Blank	Koreksi 1	Koreksi 2	Rata-rata	Persamaan
1000	0,1760	0,1726	0,0499	0,1261	0,1227	0,1244	Y=0,0001X+0,0063
500	0,1360	0,1397		0,0861	0,0898	0,0880	R2 =0,9593
250	0,1037	0,0930		0,0538	0,0431	0,0485	
125	0,0713	0,0692		0,0214	0,0193	0,0204	
62,5	0,0645	0,0622		0,0146	0,0123	0,0135	
31,25	0,0549	0,0542		0,0050	0,0043	0,0047	
15,625	0,0612	0,0516		0,0113	0,0017	0,0065	
0	0,0496	0,0502		-0,0003	0,0003	0,0000	

Lampiran 4.8. Hasil uji kadar granzim pada CM-(MCF7+IL-NK)

Perlakuan	Absorbansi 1	Absorbansi 2	Absorbansi 3	Blank 0,0499	Granzim 1	Granzim 2	Granzim 3	Rata-rata	STD
MCF7	0,0593	0,0586	0,0594		-82,00	-112,00	-87,00	-93,67	16,07
MCF7+NK(1:1)	0,0630	0,0618	0,0605		31,00	24,00	32,00	29,00	4,36
MCF7+NK(1:5)	0,0636	0,0652	0,0659		68,00	56,00	43,00	55,67	12,50
MCF7+NKIL-2(1:1)	0,0705	0,0709	0,0719		74,00	90,00	97,00	87,00	11,79
MCF7+NKIL-2(1:5)	0,0661	0,0638	0,0653		143,00	147,00	157,00	149,00	7,21

Lampiran 5. Hasil uji efek toksik CM-(hWJMSCs+IL3-NK) pada sel kanker MCF7

Lampiran 5.1. Absorbansi CM-(hWJMSCs+IL3-NK) pada sel kanker MCF7

Treatment	Absorbansi			Blank	Koreksi			Rata-rata
	1	2	3		1	2	3	
Kontrol	1,7095	1,7349	1,7975	0,1928	1,5167	1,5421	1,6047	1,5279
Kontrol	1,6794	1,8131	1,6193	0,2027	1,4767	1,6104	1,4166	
MSCs+NK 1:1 30%	1,0476	1,1519	1,1560	0,2085	0,8391	0,9434	0,9475	0,9100
MSCs +NK 1:1 60%	0,9647	0,9021	0,8694	0,1766	0,7881	0,7255	0,6928	0,7355
MSCs +NK 1:5 30%	1,0066	1,1079	1,0929	0,1839	0,8227	0,9240	0,9090	0,8852
MSCs +NK 1:5 60%	0,9353	0,9596	0,7367	0,1840	0,7513	0,7756	0,5527	0,6932
MSCs +NKIL-2 1:1 30%	0,9145	0,9340	0,8175	0,1960	0,7185	0,7380	0,6215	0,6927
MSCs +NKIL-2 1:1 60%	0,7854	0,8021	0,8058	0,1903	0,5951	0,6118	0,6155	0,6075
MSCs +NKIL-2 1:5 30%	0,7857	0,8031	0,7646	0,1960	0,5897	0,6071	0,5686	0,5885
MSCs +NKIL-2 1:5 60%	0,7070	0,6059	0,7066	0,1886	0,5184	0,4173	0,5180	0,4846
MSCs 30%	1,2295	1,1271	1,3280	0,1976	1,0319	0,9295	1,1304	1,0306
MSCs 60%	1,1269	0,8612	0,9890	0,1978	0,9291	0,6634	0,7912	0,7946

Lampiran 5.2. Viabilitas, inhibisi CM-(hWJMSCs+IL2-NK) pada sel kanker MCF7

Treatment	Viabilitas (%)			Rata rata	STD	Inhibisi (%)			Rata-rata	STD
	1	2	3			1	2	3		
Kontrol	54,92	61,75	62,01	59,56	4,02	38,25	37,99	40,44	38,89	1,35
Kontrol	51,58	47,48	45,34	48,14	3,17	52,52	54,66	51,86	53,01	1,46
MSCs+NK 1:1 30%	53,85	60,48	59,49	57,94	3,58	39,52	40,51	42,06	40,70	1,28
MSCs +NK 1:1 60%	49,17	50,76	36,17	45,37	8,00	49,24	63,83	54,63	55,90	7,38
MSCs +NK 1:5 30%	47,03	48,30	40,68	45,34	4,08	51,70	59,32	54,66	55,23	3,84
MSCs +NK 1:5 60%	38,95	40,04	40,28	39,76	0,71	59,96	59,72	60,24	59,97	0,26
MSCs +NKIL-2 1:1 30%	38,60	39,74	37,22	38,52	1,26	60,26	62,78	61,48	61,51	1,26
MSCs +NKIL-2 1:1 60%	33,93	27,31	33,90	31,72	3,81	72,69	66,10	68,28	69,02	3,36
MSCs +NKIL-2 1:5 30%	40,19	37,47	51,88	43,18	7,66	62,53	48,12	56,82	55,82	7,26
MSCs +NKIL-2 1:5 60%	45,08	37,26	36,01	39,45	4,91	62,74	63,99	60,55	62,43	1,74
MSCs 30%	54,92	61,75	62,01	59,56	4,02	38,25	37,99	40,44	38,89	1,35
MSCs 60%	51,58	47,48	45,34	48,14	3,17	52,52	54,66	51,86	53,01	1,46

Lampiran 6. Hasil uji efek CM-(hWJMSCs+IL-NK) terhadap apoptosis pada sel kanker MCF7 metode flowcytometry

CM-(hWJMSCs+IL-NK)	Data flowcytometry (%)				
	Ulangan 1	Ulangan 2	Ulangan 3	Rata-rata	STD
Apoptosis					
MCF7 medium FBS	5,21	4,65	3,67	4,51	0,78
MCF7 medium non FBS	8,77	11,28	14,14	11,40	2,69
CM-hWJMSCs	32,40	34,15	32,79	33,11	0,92
CM-(hWJMSCs+NK)	32,45	35,95	35,59	34,66	1,93
CM-(hWJMSCs+IL2-NK)	33,57	37,21	38,72	36,50	2,65
Sel Hidup					
MCF7 medium FBS	85,93	86,50	88,87	87,10	1,56
MCF7 medium non FBS	64,69	62,20	58,57	61,82	3,08
CM-hWJMSCs	49,93	48,14	49,83	49,30	1,01
CM-(hWJMSCs+NK)	57,41	51,68	50,35	53,15	3,75
CM-(hWJMSCs+IL2-NK)	42,92	41,73	42,77	42,47	0,65
Nekrosis					
MCF7 medium FBS	6,51	7,06	6,65	6,74	0,29
MCF7 medium non FBS	4,22	4,50	3,79	4,17	0,36
CM-hWJMSCs	6,63	7,00	6,46	6,70	0,28
CM-(hWJMSCs+NK)	4,63	4,91	5,07	4,87	0,22
CM-(hWJMSCs+IL2-NK)	5,73	5,25	4,04	5,01	0,87
Sel mati					
MCF7 medium FBS	2,35	1,79	0,81	1,65	0,78
MCF7 medium non FBS	22,32	22,02	23,19	22,51	0,61
CM-hWJMSCs	11,01	10,72	10,92	10,88	0,15
CM-(hWJMSCs+NK)	5,51	7,46	8,98	7,32	1,74
CM-(hWJMSCs+IL2-NK)	17,78	15,51	14,47	15,92	1,69

Lampiran 7. Ekspresi gen proapoptosis, antiapoptosis CM kokultur hWJMSCs+IL-NK pada sel kanker MCF7

Lampiran 7.1. Efek CM-(hWJMSCs+IL2-NK) terhadap ekspresi gen BAX pada sel MCF7

Perlakuan	Cq bax			Rata2	Cq b-actin			Rata2	ΔCq			rata2 ΔCq
	1	2	3		1	2	3		1	2	3	
MCF7 medium NON FBS	22,56	22,38	22,99	22,64	26,04	24,64	25,77	25,48	-3,48	-2,26	-2,78	-2,84
MCF7 mesium FBS	21,95	23,69	23,02	22,89	25,20	25,37	25,59	25,39	-3,25	-1,68	-2,57	-2,50
CM-(hWJMSCs)	22,63	23,79	23,93	23,45	26,92	26,14	27,03	26,70	-4,29	-2,35	-3,10	-3,25
CM-(hWJMSCs+NK)	21,78	22,11	21,39	21,76	25,95	24,77	24,87	25,20	-4,17	-2,66	-3,48	-3,44
CM-(hWJMSCs+IL2-NK)	21,36	21,63	20,54	21,18	25,23	24,42	24,02	24,56	-3,87	-2,79	-3,48	-3,38

Perlakuan	ΔΔCq			Rata2	Ekspresi gen bax			Rata2	STD
	1	2	3		1	2	3		
MCF7 medium NON FBS					1,00	1,00	1,00	1,00	0,00
MCF7 mesium FBS	0,23	0,58	0,21	0,34	0,85	0,67	0,86	0,80	0,11
CM-(hWJMSCs)	-0,81	-0,09	-0,32	-0,41	1,75	1,06	1,25	1,36	0,36
CM-(hWJMSCs+NK)	-0,69	-0,4	-0,7	-0,60	1,61	1,32	1,62	1,52	0,17
CM-(hWJMSCs+IL2-NK)	-0,39	-0,53	-0,7	-0,54	1,31	1,44	1,62	1,46	0,16

Lampiran 7.2. Hasil uji efek CM-(hWJMSCs+IL2-NK) terhadap ekspresi gen P53 pada sel MCF7

Perlakuan	Cq bax			Rata2	Cq b-actin			Rata2	ΔCq			rata2 ΔCq
	1	2	3		1	2	3		1	2	3	
MCF7 medium NON FBS	35,7	35,89	35,44	35,68	26,04	24,64	25,77	25,48	9,66	11,25	9,67	10,19
MCF7 mesium FBS	34,53	36,89	34,95	35,46	25,2	25,37	25,59	25,39	9,33	11,52	9,36	10,07
CM-(hWJMSCs)	36,59	36,81	36,45	36,62	26,92	26,14	27,03	26,70	9,67	10,67	9,42	9,92
CM-(hWJMSCs+NK)	35,17	35,31	34,04	34,84	25,95	24,77	24,87	25,20	9,22	10,54	9,17	9,64
CM-(hWJMSCs+IL2-NK)	34,01	35,03	32,41	33,82	25,23	24,42	24,02	24,56	8,78	10,61	8,39	9,26

Perlakuan	ΔΔCq			Rata2	Ekspresi gen bax			Rata2	STD
	1	2	3		1	2	3		
MCF7 medium NON FBS					1,00	1,00	1,00	1,00	0,00
MCF7 mesium FBS	-0,33	0,27	-0,31	-0,12	1,26	0,83	1,24	1,11	0,24
CM-(hWJMSCs)	0,01	-0,58	-0,25	-0,27	0,99	1,49	1,19	1,23	0,25
CM-(hWJMSCs+NK)	-0,44	-0,71	-0,50	-0,55	1,36	1,64	1,41	1,47	0,15
CM-(hWJMSCs+IL2-NK)	-0,88	-0,64	-1,28	-0,93	1,84	1,56	2,43	1,94	0,44

Lampiran 7.3. Hasil uji efek CM-(hWJMSCs-IL2-NK) terhadap ekspresi gen BCL-2 pada sel MCF7

Perlakuan	Cq bax			Rata2	Cq b-actin			Rata2	ΔCq			rata2 ΔCq
	1	2	3		1	2	3		1	2	3	
MCF7 medium NON FBS	38,32	38,37	38,12	38,27	26,04	24,64	25,77	25,48	12,28	13,73	12,35	12,79
MCF7 mesium FBS	37,39	38,99	37,79	38,06	25,2	25,37	25,59	25,39	12,19	13,62	12,20	12,67
CM-(hWJMSCs)	39,16	40,09	39,51	39,59	26,92	26,14	27,03	26,70	12,24	13,95	12,48	12,89
CM-(hWJMSCs+NK)	38,47	38,74	36,99	38,07	25,95	24,77	24,87	25,20	12,52	13,97	12,12	12,87
CM-(hWJMSCs+IL2-NK)	37,98	38,02	36,64	37,55	25,23	24,42	24,02	24,56	12,75	13,60	12,62	12,99

Perlakuan	ΔΔCq			Rata2	Ekspresi gen bax			Rata2	STD
	1	2	3		1	2	3		
MCF7 medium NON FBS					1,00	1,00	1,00	1,00	0,00
MCF7 mesium FBS	-0,09	-0,11	-0,15	-0,12	1,06	1,08	1,11	1,08	0,02
CM-(hWJMSCs)	-0,04	0,22	0,13	0,10	1,03	0,86	0,91	0,93	0,09
CM-(hWJMSCs+NK)	0,24	0,24	-0,23	0,08	0,85	0,85	1,17	0,96	0,19
CM-(hWJMSCs+IL2-NK)	0,47	-0,13	0,27	0,20	0,72	1,09	0,83	0,88	0,19

Lampiran 8. Rencana dan Jadwal Bulan Agustus sampai Desember 2016

Tahun 3

No	Rencana Kegiatan	Bulan											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Penelusuran pustaka	x	x	x	x	x	x	x	x	x			
3	Induksi sel NK				x	x	x	x	x	x	x		
4	Proliferasi sel NK						x	x	x	x	x		
5	TNFα, IFN-γ (IL15-NK)						x	x					
6	TNFα, IFN-γ, Pr, Gzr CM-(MSCs+IL15-NK)						x	x	x				
7	TNFα, IFN-γ, Pr, Gzr CM-(MCF7+IL5-NK)						x	x	x				
8	Induksi apoptosis						x	x	x				
9	Ekspresi reseptor								x	x	x		
10	Seminar/publikasi								x	x			x
11	Pelaporan							x				x	

Lampiran 9. Laporan publikasi penelitian Hibah Kompetensi 2016

No	Kegiatan	Judul	Keterangan
1	Seminar dan Kongres Nasional ASPI ke-3 dengan tema "Indonesia Stem Cell Summit" Bogor 6-7 Februari 2016,	Conditioned Medium from Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) Has Lower Activity in Inhibiting Proliferation and Inducing Apoptosis on Breast Cancer Cells Line (T47D, MCF7) Compared to Normoxia (WJMSCs-norCM)	Invited Speaker
2	PCS Cell Science and Stem Cell Conference (CSSC—2016), Barcelona, Spain 11-12 Juni 2016	The Cytotoxic Effect of Conditioned Medium from Interleukins-Induced human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs-ILs-CM), ILs Induced-Natutal Killer Cells (NK-ILs), Interleukins (ILs) toward Breast Cancer (BC) and Natural Killer (NK) Cells	Oral Presentation
3	3rd International Conference of Advance Molecular Bioscience and Biomedical Engineering 2016 (ICAMBBE 2016)" 4-5 August, 2016	Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward TNF-α, IFN-γ, Perforin, Granzyme Concentration, Apoptotic Inducing Activity in Breast Cancer Cells	Oral Presentation
4	Draft manuscript telah submitted "Turkish Journal of Immunology" SCOPUS	Conditioned Medium from Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) Has Lower Activity in Inhibiting Proliferation and Inducing Apoptosis on Breast Cancer Cells Line (T47D, MCF7) Compared to Normoxia (WJMSCs-norCM)	Under review
5	Draft manuscript telah submitted "The Korean Association of Internal Medicine" SCOPUS	Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward Apoptotic Inducing Activity in Breast Cancer Cells	Under review
6	Telah terbit Chapter book	Hypoxia in Mesenchymal Stem Cells	Penerbit "In Tech"
7	Telah daftar hak paten 28 Sepetmber 2016	IL-2 meningkatkan sitotosik sel NK terhadap sel kanker payudara	Terdaftar SII 20160000016
8	Telah didaftarkan pada Penerbit Erlangga draft buku	Pemanfaatan Mesenchymal Stem Cell pada Terapi Kanker: Dasar Teori dan Strategi Aplikasi Klinis	Proses editing

Lampiran 9.1. Invited Speaker Seminar dan Kongres Nasional ASPI ke-3

Dengan Hormat,

Bersama surat ini, kami atas nama panitia mengajukan permohonan kesediaan Bapak / Ibu sebagai pembicara dalam rangkaian acara Seminar dan Kongres Nasional ke-3 Asosiasi Sel Punca Indonesia (ASPI) 2016 dengan tema: **"INDONESIA STEM CELL SUMMIT"**, yang akan diselenggarakan pada:

Hari/Tanggal : Sabtu & Minggu/ 6 & 7 Februari 2016

Tempat : IPB International Convention Center

Jl. Raya Pajajaran, Bogor 16151, Jawa Barat.

Mohon konfirmasi dapat dilakukan dengan mengisi formulir terlampir dan menyampaikan melalui email panitia: **aspiseminar@gmail.com** paling lambat Senin 16 November 2016.

Demikian surat permohonan ini kami ajukan, atas perhatian dan kesediaan Bapak/Ibu kami ucapkan terimakasih.

Ketua Panitia,
Prof. Drh. Arief Boediono, PhD, PAVet (K)

PANITIA SEMINAR DAN KONGRES NASIONAL KE-II
ASOSIASI SEL PUNCA INDONESIA (ASPI)
Sekretariat Panitia: Vet Stem IPB
Lab. Embriologi FKH IPB, Jl. Agatis Kampus IPB Dramaga, Bogor 16680
Telp./Faks: (0251) 8421823, HP: 081213912346; E-mail: aspiseminar@gmail.com

Bogor, 22 Desember 2015

No. : 21/XI/PSKN/ASPI/2016
Lamp. : 2 eksemplar
Hal : Permohonan pengisian formulir kesediaan, curriculum vitae, dan abstrak.

Kepada Yth.
Ibu. Dr. Wahyu Widowati, M.Si,
di tempat

Dengan hormat,

Sehubungan dengan adanya acara Seminar dan Kongres Nasional ke-2 Asosiasi Sel Punca Indonesia (ASPI) 2016 dengan tema: **"INDONESIA STEM CELL SUMMIT"**, yang akan diselenggarakan pada tanggal 6 dan 7 Februari 2016, IPB International Convention Center, kami mengundang Bapak/Ibu sebagai pembicara dengan keterangan sebagai berikut:

Topik: Stem Cell				
Sub Topik		**Pembicara**	**Tanggal**	**Alokasi waktu**
1	"Conditioned Medium from Hypoxia Treated WJMSCs (WJMSC,s-hypo CM) has Lower Activity in Inhibiting Proliferation and Inducing Apoptosis on Breast Cancer Cells Line (T470,MCF7) Compared to Normotia (WJMSCs-nosCM)"	Dr. Wahyu Widowati, M.Si,	Minggu, 7 Februari 2016	15 menit

Kami sangat mengharapkan kesediaan Bapak/Ibu untuk menjadi pembicara pada acara Seminar dan Kongres Nasional ke-2 Asosiasi Sel Punca Indonesia (ASPI) 2016 dan mengisi form kesediaan sebagai pembicara dan mengirimkan kembali form tersebut kepada panitia melalui telp/fax (0251-8421823), HP 081213912346, email: aspiseminar@gmail.com

Demikian surat permohonan ini kami ajukan, atas perhatian dan kesediaan Bapak/Ibu kami ucapkan terimakasih.

Ketua Panitia,

Prof. Drh. Arief Boediono, Ph.D, PAVet (K)

Catatan :

Lampiran 9.2. Oral Presentation PCS Cell Science and Stem Cell Conference (CSSC—2016), Barcelona, Spain

2016 PCS Cell Science & Stem Cell Conference

PCS Global Cell Science and Stem Cell Conference (CSSC-2016)

Theme: A Pathway from Basic Sciences to Industrialization
11-12 June; Barcelona, Spain
http://www.pcscongress.org/cssc2016/index.asp

Dear Dr. Wahyu Widowati,

You are cordially invited to participate in the PCS Global Cell Science and Stem Cell Conference (CSSC-2016), which will be held in Barcelona, Spain from 11 June to 12 June, 2016. The theme for this year's CSSC is "A Pathway from Basic Sciences to Industrialization".

We are very pleased to inform you to be a speaker in Session 1-5: Other Advanced Researches with the title *The Cytotoxic Effect of Conditioned Medium from Interleukins-Induced Human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs-ILs-CM), ILs Induced-Natural Killer Cells (NK-ILs), Interleukins (ILs) Toward Breast Cancer (BC) and Natural Killer (NK) Cells* in our conference.

As a high-end academic information exchange platform, CSSC-2016 has been planned more than 15 sessions on Novel Technology and Basic Research, Clinical Stem Cell Therapy and Industry Application. Furthermore, any new topics are expected to be initiated by participants.

We look forward to meeting you in Barcelona and hope you are taking this wonderful opportunity to integrate with all participants and seek cooperation worldwide. We also feel confident that you would enjoy both of our scientific program and the magnificent landscape of Barcelona with its unique architecture.

Please feel free to contact me if you need any assistance.

Yours Sincerely,

Ms. Mina Zhang
Coordinator of CSSC-2016
E-mail: mina@pcsconference.com
Pioneer Century Science
No. 134, Changjiang Road,
Huanggu District,
Shenyang Office, China
Tel: +86-24-31930319
Fax: +86-24-86085759
http://www.pcscongress.org/cssc2016/index.asp

On behalf of
Prof. Chiming Wei,
President of GOS-2016
Professor and Chief Scientist,
Chongqing Academy of Science and Technology, China

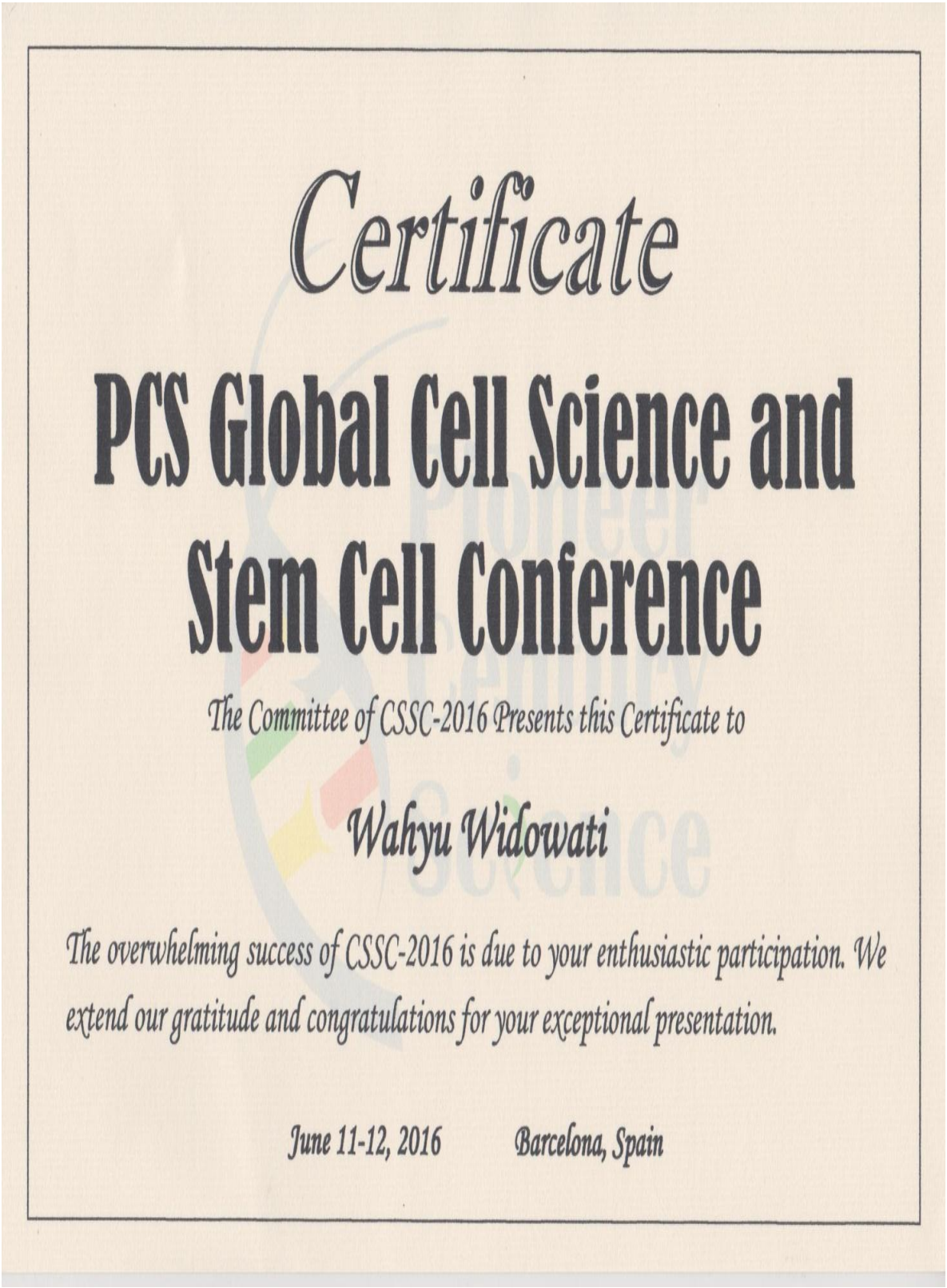
Program of Cell Science and Stem Cell Conference
Time: 11 June-12 June, 2016
Place: Barcelona, Spain
http://www.pcscongress.org/cssc2016/index.asp

Keynote Forum and Plenary

Section 1: Novel Technology and Basic Research
Session 1-1: Cell Mechanics, Genetics, Genomics and Bio-Banking
Session 1-2: Tissue Engineering
Session 1-3: Stem Cell Transplantation
Session 1-4: Advances in Adult & Pluripotent Stem Cells
Session 1-5: Other Advanced Researches

Section 2: Clinical Stem Cell Therapy
Session 2-1: Cancer (Oncology) Therapy
Session 2-2: Cardiovascular Therapy
Session 2-3: Diabetic Therapy
Session 2-4: Skin & Dermatological Application
Session 2-5: Regenerative Medicine
Session 2-6: Other Challenges in Cell Therapy

Session 3: Industry Application
Session 3-1: Stem Cell Product Development & Commercialization
Session 3-2: Ethic and Policy



Certificate

PCS Global Cell Science and Stem Cell Conference

The Committee of CSSC-2016 Presents this Certificate to

Wahyu Widowati

The overwhelming success of CSSC-2016 is due to your enthusiastic participation. We extend our gratitude and congratulations for your exceptional presentation.

June 11-12, 2016 Barcelona, Spain

Lampiran 9.3. Oral Presentation pada ICAMBBE 2016, Malang

3rd International Conference on Advance Molecular Bioscience & Biomedical Engineering 2016 (ICAMBBE 2016)
BioSains Institute, Brawijaya University Jalan Mayjen Panjaitan, Malang, 65145
Telp. +62 341- 578248, Fax. +62 341- 578248, icambbe@gmail.com

LETTER OF ACCEPTANCE

Dear **Wahyu Widowati**,

We are pleased that you will be with us at the "**3rd International Conference of Advance Molecular Bioscience and Biomedical Engineering 2016 (ICAMBBE 2016)**" on August 4th – 5th, 2016 at the Guest House UB Malang, Indonesia.

On behalf of the committee, I'm writing to confirm that your paper entitled:

Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward TNF-α, IFN-γ, Perforin, Granzyme Concentration, Cytotoxic and Apoptotic Inducing Activity in Breast Cancer Cells

Has been accepted for **Oral Presentation**, and will be scheduled for presentation at the seminar.

If you are interest to publish your article on the proceeding book, please submit your full paper before July 30th, 2016.

For registration please make a Bank transfer before August 1, 2016 to following bank account: **Bank Mandiri KCP Universitas Brawijaya, Account No: 144-00-1578170-8, Swift code: BMRIIDJA, Name: Perdana Finawati Putri**, and please directly send the receipt to icambbe@gmail.com.

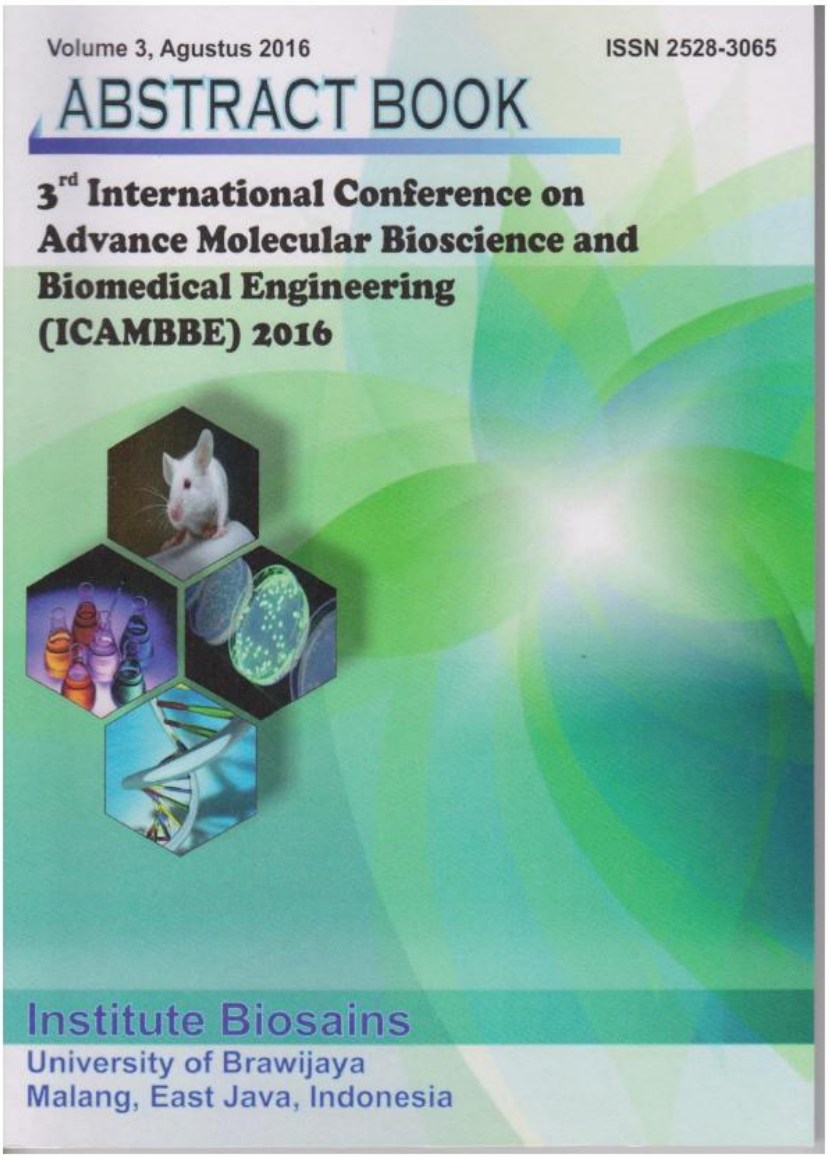
If you have any further questions, please feel free to contact us.

We are looking forward to meet you in Malang, Indonesia.

Sincerely yours,
President of 3rd ICAMBBE 2016

ICAMBBE
International Conference on Advance Molecular Bioscience and Biomedical Engineering

Dyah Kinasih Wuragil, S.Si., MP., M.Sc


Volume 3, Agustus 2016
ISSN 2528-3065
ABSTRACT BOOK
3rd International Conference on Advance Molecular Bioscience and Biomedical Engineering (ICAMBBE) 2016
Institute Biosains
University of Brawijaya
Malang, East Java, Indonesia

BMB-8

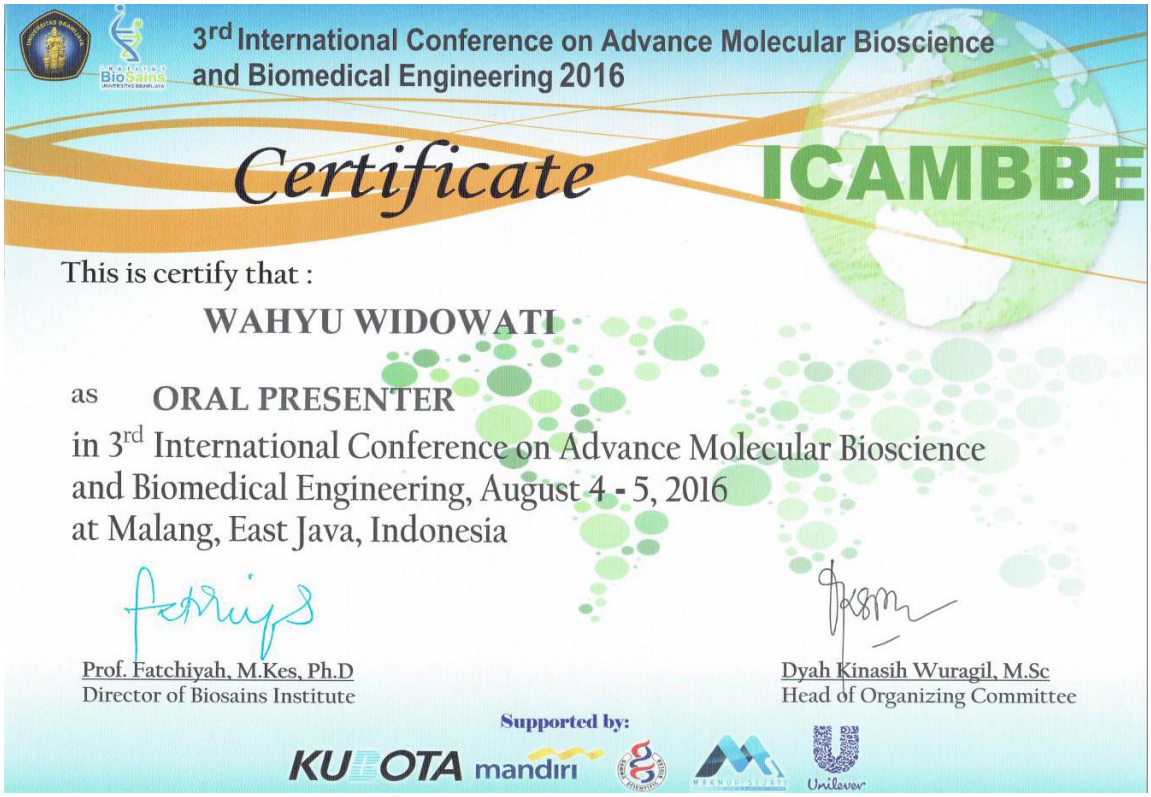
Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward TNF-α, IFN-γ, Perforin, Granzyme Concentration, Cytotoxic and Apoptotic Inducing Activity in Breast Cancer Cells

Wahyu Widowati[1], Diana Krisanti Jasaputra [1], Khie Khiong [1,4], Sutiman B. Sumitro [2], M. Aris Widodo [3], Merry Afni [4], Seila Arumwardana [4], Dwi Davidson Rihibiha [4] and Indra Bachtiar[5]

[1]Faculty of Medicine, Maranatha Christian University, Bandung, West Java, Indonesia
[2]Faculty of Science, Brawijaya University, Malang East Java, Indonesia
[3]Faculty of Medicine, Brawijaya University, Malang East Java, Indonesia
[4]Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung West Java, Indonesia
[5]Stem Cell and Cancer Institute, Jakarta, Indonesia

*Corresponding E-mail: wahyu_w06@yahoo.com

This study was performed to elucidate effect of conditioned medium (CM) of co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward breast cancer (BC), NK cells viability, TNF-α, IFN-γ, perforin, granzyme level, apoptosis and genes expression of Bax, Bcl-2, and p53. Human recombinant IL-2 was used to induce hWJMSCs and CM-IL2-hWJMSCs was collected to evaluate its effect toward BC cells (MCF7) and NK cells. The co-culture method of IL2-NK and MCF7, IL2-NK and hWJMSCs for evaluating the TNF-α, IFN-γ, perforin, granzyme level. The measurement of cytotoxic activity of CM-IL2-hWJMSCs toward MCF7 and NK cells, viability of IL2-NK in various incubation period, cytotoxic activity of IL-2 NK toward MCF7 cells in various ratio were conducted using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, concentration of TNF-α, IFN-γ, perforin, granzyme using ELISA assay. Apoptosis was measured using flow cytometry, and apoptotic genes were measured with RT-PCR. The CM-IL2-hWJMSCs and CM-hWJMSCs exhibited cytotoxic effect, higher concentration of CM (60%) showed higher inhibition in MCF7, NK cells. The higher number of NK cells increased the anticancer activity toward BC cells, the highest cytotoxic activity was obtained from co-culture of BC and NK cells in ratio of 1:5. The IL2-induced NK cells proliferation were higher compared to the


3rd International Conference on Advance Molecular Bioscience and Biomedical Engineering 2016
Certificate
ICAMBBE
This is certify that :
WAHYU WIDOWATI
as ORAL PRESENTER
in 3rd International Conference on Advance Molecular Bioscience and Biomedical Engineering, August 4 - 5, 2016
at Malang, East Java, Indonesia
Prof. Fatchiyah, M.Kes, Ph.D
Director of Biosains Institute
Dyah Kinasih Wuragil, M.Sc
Head of Organizing Committee
Supported by:
KUBOTA
mandiri
Unilever

Lampiran 9.4. Under review manuscript pada "Turkish Journal of Immunology"

Dear Wahyu Widowati,

You are subscribed Turkish Journal of Immunology online. Your username and password are as follows:

Username: wahyuw
Password: wahyuwidowati

You can change your personal profile after you login using your password and username. If you encounter any problem while sending a manuscript to our journal, please send an e-mail to info@turkimmunoloji.dergisi.org

With best regards,

Prof Gunnur Deniz
Turkish Journal of Immunology
info@turkimmunoloji.dergisi.org

Original article

Conditioned Medium from Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) Has Lower Activity in Inhibiting Proliferation and Inducing Apoptosis on Breast Cancer Cells Line (T47D, MCF7) Compared to Normoxia (WJMSCs-norCM)

Wahyu Widowati[a*], Harry Murti[b], Halida Widyastuti[b], Dwi Agustina[b], Dian Ratih Laksmitawati[c], Merry Afni[d], Ervi Afifah[d], Sutiman B. Sumitro[e], M. Aris Widodo[f], Indra Bachtiar[b]

[a] Faculty of Medicine, Maranatha Christian University, Jl. Prof drg. Suria Sumantri No.65, Bandung 40164, Indonesia
[b] Stem Cell and Cancer Institute, Jl. A Yani no 2 Pulo Mas, Jakarta 13210, Indonesia
[c] Faculty of Pharmacy, Pancasila University, Jakarta, Indonesia
[d] Biomolecular and Biomedical Research Center, Aretha Medika Utama,, Jl. Babakan Jeruk II no. 9, Bandung 40163, Indonesia
[e] Department of Biology, Faculty of Science, Brawijaya University, Malang, East Java, Indonesia
[f] Pharmacology Laboratories, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

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ABSTRACT

Mesenchymal stem cells (MSCs) are an appealing source of adult stem cells for cell therapy due to high rate of proliferation, multipotency, self-renewal capability and applicable therapy. Wharton's jelly (WJ), the main component of umbilical cord extracellular matrix, is multipotent stem cells with high proliferation rate, self-renewal capability, and has anticancer property. MSCs have been reported to secrete a variety of cytokines which have cytotoxic effect in various cancers. Oxygen tension affects MSCs proliferation, cytokines level but no in surface markers expression**,** MSCs' differentiation**.** We explored the cytotoxic effect and inducing apoptosis of WJMSCs secretions from normoxic WJMSCs (WJMSCs-norCM) and hypoxic WJMSCs (WJMSCs-hypoCM) in breast cancer cell lines (T47D, MCF7). The cytotoxic activity was determined with MTS assay. RT-PCR was performed to measure the expression of apoptosis inducing genes specifically p53, BAX, and capcase-9 and antiapoptosis gene BCL2. WJMSCs-norCM and WJMSCs-hypoCM were potent inhibitors of the proliferation in both cell lines. WJMSCs-norCM was more active as anticancer in T47D, MCF7. The IC_{50} value of WJMSCs-norCM on MCF7 was 42.34%, on T47D was 42.36%. WJMSCs-norCM induced significantly gene expression of apoptosis p53, BAX, CASP9 and insignificantly decreased the antiapoptotic gene BCL2 both MCF7 and T47D cells. WJMSCs-CM has anticancer activity by inducing p53, BAX, CASP9 apoptotic genes. WJMSCs-norCM has higher anticancer compared to WJMSCs-hypoCM.

Keywords: apoptosis, breast cancer, conditioned medium, hypoxia, Wharton's jelly derived-mesenchymal stem cells.

INTRODDUCTION

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23 % of the total cancer cases and 14% of the cancer deaths.[1] Mesenchymal stem cells (MSCs) emerge as potential cell source for clinical therapy for it have bioactive potential in secreting bioactive molecules, immunomodulatory[2], self-renewal and differentiation into various cell lineages[3]. Human bone marrow-derived mesenchymal stem cells (hBMMSCs) are widely used, but the cells isolation involves invasive procedures and lower

growth rate[4]. Wharton's Jelly (WJ) uninvasive collection procedure and have rapidly available donor pool, and ethically noncontroversial, WJ from umbilical cord can be used as an alternative source of MSCs. Wharton's Jelly-derived mesenchymal stem cells (WJMSCs) have multipotency, do not induce teratoma, and extensive *in vitro* expansion capabilities,[5] have a higher proliferation rate and self-renewal capacity than adult tissue-derived MSCs and have short doubling time[6-9]. WJMSCs have anticancer activity in rats mammary adenocarcinoma, the rats showed an improvement after WJMSCs treatment[10]. Human Wharton's Jelly-derived mesenchymal stem cells (hWJMSCs) significantly reduced breast cancer cell lines growth. Amount of 100.000 cells hWJMSCs can induced apoptosis on breast cancer cell lines MCF7.[11] Umbilical cord-derived mesenchymal stem cells (UCMSCs) and adipose tissue-derived mesenchymal stem cells (ADMSCs) can efficiently induced both apoptosis and differentiation in U251 human glioma cell line[12].

Oxygen concentration is an important for stem cells growth, most human embryonic stem cells (hESCs) exposed in hypoxia condition *in vivo*. Hypoxia condition can minimize spontaneous differentiation and maintain hESCs pluripotency.[13,14] Hypoxic microenvironment can increase proliferation rate and population doubling time (PDT) significantly of WJMSCs[9]. Tumor cell responses to hypoxia are important for tumor progression as well as tumor therapy[15].

MSCs or MSCs conditioned-medium (CM) containing micro particles mediates therapeutic effects.[16] CM from normoxia-treated WJMSCs (WJMSCs-norCM) and CM from hypoxia-treated WJMSCs (WJMSCs-hypoCM) have anticancer activity toward various cancer cell lines (HeLa, SKOV3, PC3, HSC3, HepG2) and have no cytotoxic effect toward mouse fibroblast (NIH3T3L1), human fibroblast and MSCs.[17] CM from human umbilical cord blood-derived MSCs (UCBMSCs-CM) significantly inhibited melanogenesis by suppressing melanin synthesis by regulating microphthalmia-associated transcription factor (MITF) expression. hUCBMSCs-CM induced extracellular signal-regulated kinases (ERK1/2) activation in melanocytes.[18]

MSCs have an intrinsic property for homing towards tumor sites and can be used as tumor therapy, but very poorly studies investigated the antitumor properties of MSCs secretion which incubated in normoxic and hypoxic condition, we investigated WJMSCs-hypoCM and WJMSCs-norCM in inhibiting proliferation and inducing apoptosis in MCF7, and T47D cell lines.

MATERIAL AND METHODS

Isolation and Cultivation of WJMSCs

WJMSCs were isolated as previously reported[9,17,19,20]. An approval after donor's written informed consent as guidelines Ethics Committee at the Stem Cell and Cancer Institute, Jakarta, Indonesia and from Ethics Committee collaboration between Maranatha Christian University, Bandung, Indonesia and Immanuel Hospital Bandung, Bandung, Indonesia. Fresh UC (n=3) were collected from women donor (aged 25-40) after full-term births (normal vaginal delivery). After UC was washed by phosphate buffer saline (PBS; 0.9% w/v sodium chloride) and was cut in small explants (1-2 mm) before placing them in a tissue culture plates. The explants were culture in minimum essential medium-α (MEM-α) with 2 mM GlutaMAX (Invitrogen, Carlsbad, CA, USA), supplemented with 20% fetal bovine serum (FBS, Invitrogen) and penicillin streptomycin amphotericin B (100 U/mL, 100 mg/mL, and 0.25 mg/mL; Invitrogen). Cultures were incubated in a humidified atmosphere with 5% CO_2 at 37^{o}C, with replacing medium every 5 days until 21 days. When cells reached 80-90% confluence, they were harvested and replated at a density 8 x 10^3 cells/cm^2. WJMSCs were cultured in 95% air (21% O_2) 5% CO_2 for normoxic and hypoxic (2.5%, 5% oxygen and 5% CO_2). Hypoxia was conducted using a tri-gas incubator (CO_2 incubators with additional process controls; BINDER GmbH, Tuttlingen, Germany) with internal O_2 and N_2 tank changer for connecting to separate gas tanks[9,19,20-22].

Detection of MSCs markers and multipotent differentiation

The WJMSCs were incubated in hypoxia 2.5%, 5% and normoxia until P4 and P8 were measured using surface marker detection for MSC characterization especially CD105, CD73, CD90, CD34, CD45, CD14, CD19 and HLA-II using a flow cytometer[9,17,19,20]. Multipotent differentiation include osteogenic, chondrogenic, adipogenic differentiation were used, WJMSCs (P4, P8) were seeded at density $1x10^4$ cells/cm^2 in culture dishes using StemPro Osteogenesis Differentiation Kit (Gibco A10072-01,Invitrogen) for 3 weeks, using StemPro Chondrogenesis Differentiation Kit (Gibco A10071-01, Invitrogen) for 2 weeks, using StemPro Adipogensis Differentiation Kit (Gibco A10070-01, Invitrogen) for 2 weeks. Calcium deposits were visualized using Alizarin red S (Amresco 9436; Amresco LLC, Solon, OH, USA), chondrocytes were visualized using Alcian blue (Amresco 0298, Amresco LLC), adipocytes were visualized using Oil Red O (Sigma-Aldrich-U0625)[12,17,20,22,23]. Harvest of WJMSCs-norCM or WJMSCs-hypoCM.

WJMSCs of P4 and P8 were used for this research. The WJMSCs were seeded at density 8 $x10^3$ cells/cm^2. CM was prepared by incubating the cells under normoxia (21% O_2, 5% CO_2), hypoxia 2.5% (2.5% O_2, 5% CO_2) and hypoxia 5% (5% O_2, 5% CO_2) for 72 h, when cells reached 80-90% confluence were harvested. The medium was collected and centrifuged at 2000 rpm for 4 min at room temperature, and the supernatant was filtered by a syringe filter 0.22-µm (Corning 431219) and used as WJMSCs-hypo2.5%CM, WJMSCs-hypo5%CM, WJMSCs-norCM conserved at -20^0 C until used [12,17,24,25].

Cell Viability Assay

MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell titter 96 ® Aqueous one solution (Promega G3581, Madison, WI, USA) assay was used to determine cell viability of MCF7 (ATCC® HTB22™) and T47D (ATCC®HB133™) cell line which were obtained from Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia. This assay used an optimized reagent containing tetrozolium converted to fluorescent formazen by viable cells that absorbs the light at 490 nm. Medium consist of Dulbecco's Modified Eagle's Medium (DMEM; Biowest L0104) for MCF7 and Roswell Park Memorial Institute medium **(**RPMI; Biowest L0495) for T47D, FBS 10% (Biowest S181H) and 1% Penicillin-Streptomycin (Biowest L0018) then incubated (21% O_2, CO_2 5%, 37°C), after cells reached 80% confluence, cells were seeded at density $5x10^3$ in 96 well plate for 24 h incubation[17,26,27], cells were supplemented by WJMSCs-hypo2.5%CM, WJMSCs-hypo5%CM, and WJMSCs-norCM in various concentrations (0%, 20%, 40%, 60%, 80%), then incubated for 72 h. MTS was added at 10 µl to each well, the plate was incubated at 21% O_2, 5% CO_2, 37ºC for 4 h. The absorbance of the cells was measured at 490 nm using a micro plate ELISA reader (Multiskan Go, Thermo Scientific Inc, USA). The data were presented as number of viable cells, the percentage of viable cells (%), the percentage of cells inhibition and the data of growth rate inhibition was calculated the median inhibitory concentration (IC_{50})[9,17,26,27].

Real-Time PCR Assay for Apoptotic Induction of T47D and MCF7

To determine the apoptosis inducer of WJMSCs-CM, we used WJMSCs-hypo5%CM-P4, WJMSCs-hypo2.5%CM-P4 and WJMSCs-norCM-P4 with concentration 50% both in MCF7 and T47D based on the cytotoxic results (Table 5). T47D, MCF-7 cancer cells were supplemented by WJMSCs-hypo5%CM-P4, WJMSCs-hypo2.5%CM-P4 and WJMSCs-norCM in 50% concentration for 72 h[17].

Total RNA was isolated from MCF7, T47D cells using Aurum total RNA mini kit reagent (Bio-Rad #732-6820) besed on manufacturer's instruction. Total RNA yield was estimated using spectrophotometer at 260, 280 and 320 nm. RNA quality was seen from the results of electrophoresis. RNA was then reverse-transcribed into cDNA synthesis using iScript cDNA

synthesis kit (Bio-Rad #170-8841), the mixture were incubated at 25°C for 5 min, 42°C for 30 min, and at 85°C for 5 min. PCR amplification was performed using *real time* PikoReal (Thermo Scientific Inc.). The qPCR conditions were as follow pre-denatured at 95˚C for 30 sec, and the cycle of qPCR is 40 cycle with condition denaturized at 95˚C for 5 sec, annealing at 58˚C for 20 sec. and the elongation at 72˚C for 30 sec. β-actin as house keeping gene was used as an internal control. Primers used for real-time PCR are summarized in Table 1.

Statistic analysis

Statistical test was performed using SPSS 20.0 program**.** Data were presented in means and standard deviation (M ± SD). The result was analyzed using ANOVA continued by Tukey HSD post-Hoc test 95 % confidence interval, p-value<0.05 was considered statistically significant. The Median Inhibitory Concentration (IC_{50}) value of cytotoxic effect was analyzed using probit analysis[17].

RESULT

MSCs markers and multipotent differentiation

WJMSCs markers were detected using flow cytometry analysis showed that cultured cells under normoxia and hypoxia (5% O_2, 2.5% O_2) for P4 and P8. WJMSCs were positive (more than 95%) for MSCs marker CD105, CD73, CD90 and negative (less than 2%) for CD34, CD45, CD14, CD19 and HLA-II. Surface marker expression of WJMSCs (P4, P8) on hypoxia and normoxia were not significantly different (data are not presented). WJMSCs cells to differentiate into osteocytes, chondrocytes, adipocytes (data are not presented).[17]

Cytotoxic activity

To determine anticancer activity of WJMSCs-norCM, WJMSCs-hypo2.5%CM, WJMSCs-hypo5%CM from P4 and P8 toward T47D and MCF7 cell lines, we counted the number, viability, inhibition of cells and median inhibitory concentration (IC_{50}) on T47D and MCF7 cell lines by MTS assay. The number of T47D and MCF7 cells decreased than control in a concentration dependent-manner (Table 2). Effect of WJMSCs-norCM and WJMSCs-hypoCM toward viability of cancer cells can be seen at Table 3. The cells viability decreased than control in a concentration dependent-manner. Effect of WJMSCs-norCM and WJMSCs-hypoCM toward inhibition of cancer cells can be seen at Table 4. The IC_{50} value of WJMSCs-norCM and WJMSCs-hypoCM (concentration of anticancer candidate which could inhibit 50% cell proliferation) was found to be 42.34%-62.84% (Table 5).

Based on Table 3 shows that viability of T47D and MCF7 cells were a significantly decreased than control in a concentration dependent-manner. Both normoxic and hypoxic CM and both MSCs of early passage (P4) and late passage (P8) could decrease viability of breast cancer cells. We determined the effect of WJMSCs-CM toward proliferation inhibition in breast cancer. WJMSCs-norCM and WJMSCs-hypoCM could inhibit proliferation of breast cancer cell significantly compared with control.

Based on the Table 5 and Figure 1 show that WJMSCs-norCM-P4 possessed the highest anticancer activity in MCF7 and T47D cell lines with IC_{50} = 42.34%, 42.36% respectively, and the lowest anticancer in MCF7 and T47D cell lines was WJMSCs-hypo2.5%CM-P8 with IC_{50} = 62.84%, 58.96% respectively. WJMSCs-CM which incubation in normoxia and hypoxia both early and late passage could inhibit breast cancer cell proliferation with IC_{50} range 42.34 - 62.84%. Figure 1 shows that control or untreated breast cancer cells (T47D, MCF7) exhibited the high density of living cells, this figure was confirmed with highest cells number and viablity (Table 2, 3). The breast cancer cells (T47D, MCF7) were treated by WJMSCs-norCM and WJMSCs-hypoCM exhibited lower density and much cells debris formation. The WJMSCs-norCM treatment was the most cytotoxic activity, it exhibited the lowest density of living cells and most cells debris formation.

Gene expression

To determine the apoptotic inducer of WJMSCs in T47D and MCF7, gene expressions were determined by RT-PCR. We continued the research for gene expression based on the anticancer activity that the average anticancer of WJMSCs-hypoCM and WJMSCs-norCM with range of IC_{50} 42.34 - 62.84%. Furthermore, we continued to measure the expression of proapoptotic gene especially p53, BCL2-Associated X Protein (BAX), cysteine-aspartic proteases 9 (CASP9) and B-Cell CLL/Lymphoma 2 (BCL2). Antiapoptotic genes were detected both in T47D and MCF7 cells using the concentrations 50% of WJMSCs-CM. The most detected p53, and BAX gene expressions in MCF7 and T47D cell lines were found at WJMSCs-norCM. WJMSCs-norCM and WJMSCs-hypoCM were insignificantly differences to decrease the antiapoptotic BCL2 gene expression (Table 6, Figure 2). Table 6, Figure 2 show that WJMSCs-CM at 50% concentration possessed the highest p53, BAX, CASP9 expression, but it was insignificant difference toward BCL2 gene expression both in MCF7 and T47D.

DISCUSSION

The data of surface markers and multipotent differentiation of WJMSCs under normoxia and hypoxia (2.5% O_2, 5% O_2) for P4 and P8 were not presented; the data has been presented in previous journal[17,20]. MSCs differentiated to three main mesenchymal lineages osteocytes, chondrocytes, adipocytes of WJMSCs normoxia and hypoxia (2.5% O_2, 5% O_2) for P4 and P8 to were not presented, the data has been presented in previous journal[17,20].

The result of this study suggest that WJMSCs-norCM and WJMSCs-hypoCM could inhibit proliferation of breast cancer cell lines both T47D and MCF7 with range of IC_{50} 42.34 - 62.84% %. This result was validated with my previous study that WJMSCs-norCM and WJMSCs-hypo5%CM could inhibit cancer cells proliferation included HeLa, SKOV3, PC3, HSC3, HepG2 with IC_{50} 51-74%[17]. MSCs-CM inhibited growth of several cancers including breast adenocarcinoma, ovarian carcinoma, osteosarcoma, benign neoplastic keloid cells, bladder tumor or lymphoma cells *in vitro*. By *in vivo* study, MSCs-CM inhibited mammary carcinoma, osteosarcoma, pancreatic and lung tumor growth[27]. CM contains a broad variety of cytokines, growth factors and putatively also micro vesicles containing (micro) RNA, which are held responsible for the beneficial effects in anticancer therapy[28]. WJMSCs-CM contained soluble factors such as interleukins, cell adhesion molecules, hyaluronic acid, growth factors and glycosaminoglycan's that probably posses the tumoricidal activity[29,30]. The soluble factors from MSCs also could inhibit tumor angiogenesis via down regulating of vascular endothelial growth factor (VEGF) expression in tumor cells.[31]

Based on Table 6, Figure 2 show that WJMSCs-CM upregulated p53, BAX, CASP9 gene expression in breast cancer cell lines, this data was validated with previous study that hUCMSCs can induce apoptosis in breast cancer MDA-MB 231 cell line[32]. MSCs-CM significantly inhibited proliferation, induced apoptosis, significantly upregulated the apoptotic genes of both CASP3 and CASP9, significantly downregulated the antiapoptotic genes such as SURVIVIN and XIAP, induced and completed differentiation in human U251 cell line[12]. The anticancer effect of WJMSCs-CM was via an apoptotic mechanism[33]. WJMSCs-CM (50%) showed the increases in the sub-G1 phase; G2/M cell population and annexin V-FITC positive culture[33]. WJMSCs-CM increased the pro-apoptotic BAX gene and decreased the anti-apoptotic BCL2 and SURVIVIN genes in three cancer cell lines including human ovarian cancer cells (TOV-112D), osteosarcoma cells (MG-63) and breast adenocarcinoma cells (MDA-MB-23)[33], MSCs-CM has potential for autoimmune, inflammatory, and malignant diseases and tissue regeneration. The most important factors present in MSCs-CM that can be considered protagonists of MSCs physiological effects including hepatocyte growth factor (HGF), transforming growth factor beta (TGF-β), VEGF, tumor suppressor gene (TSG-6), Prostaglandin E 2 (PGE2) and galectins 1, and 9[34]. Interferon beta (IFN-β) in CM induces the extrinsic and intrinsic apoptosis pathways in lung cancer, lowers the CASP8 and 9 expression and increase the cleaved CASP3[35]. WJMSCs-CM significant decreased in cell viability and cell proliferation via inducing apoptosis and cell cycle arrest in human Burkitt's lymphoma cell line (Rams and

CRL1596). The mechanism of WJMSCs-CM was activated by the secretory-product-mediated induction of cell cycle arrest at subG1 phase and reduction in entry to G2/M, increase oxidative stress or decrease in glutathione peroxidase (GPX)[36]. WJMSCs-CM induced apoptosis and autophagy in human foreskin fibroblast (CCD-112sk), significantly down-regulated the expression of TBP-associated factor (TAF) in keloid cells[37]. Micro vesicles of WJMSCs induced apoptosis in T24 bladder tumor cells with altered morphology including cell shrinkage, membrane damage, blebbing, cell debris formation.[38]

WJMSCs-norCM was more active as anticancer than WJMSCs-hypo5%CM and WJMSCs-hypo2.5%CM both in MCF7 and T47D cell lines. This result was not consistent with my previous study that WJMSCs-hypoCM was more active in servical (HeLa), prostate (PC3), human squamous carcinoma (HSC3), liver (HepG2) cancer cells but less active in ovarium (SKOV3) cells[17]. MSCs have been readily engineered to express anti-proliferative, pro-apoptotic, antiangiogenic agents that specifically target different cancer types[39]. This result was validated with previous finding that hypoxic preincubation of amniotic fluid MSCs (AFMSCs) inducing the secretome namely the upregulation of various secretable factors, such as VEGF, TGF-β1[40-42]. Hypoxia (2% O_2) up-regulated significantly the mRNA expression level of bFGF, IGF-1, VEGF-α, HGF, Indoleamine 2, 3-dioxygenase (IDO)[34,43], TGF β-2, TGF β-3, interleukin (IL-1β), IL-6 and IL-8 of BMMSCs compared with normoxia incubation[43], in agreement with RT-PCR data that hypoxia significantly increased level of bFGF, VEGF-A, IL-6, IL-8 using ELISA[43]. My previous research revealed that hypoxic incubation up-regulated the concentration level of VEGF, IL-1, IL-6, IL-8 compared to normoxia. Lower oxygen tension (2,5 % O_2) resulted higher VEGF, IL-1, IL-6, IL-8 than hypoxic 5% O_2.[44]

IL-1 network of cytokines and receptors control tumor cell subpopulation expression of other protumorigenic cytokines such as the angiogenic/growth factor, IL-8.[45] IL-1 family of cytokines may be important in regulating protumorigenic activities within the human breast cancer tumor microenvironment.[46] IL-1 promotes tumor growth, induces pro-metastatic genes such as matrix metaloproteinase (MMP) and through the stimulation of adjacent cells to produce angiogenic proteins and growth factors such as VEGF, IL-8, IL-6, tumor necrosis (TNF-α), and TGF-β.[47] IL-1 contributes in tumor progression and metastasis .[47] IL-6, and IL-8 can directly or indirectly promote tumor growth via induction of VEGF expression.[48]

IL-8 signalling increases proliferation and survival of endothelial and cancer cells, and potentiates the migration of cancer cells, endothelial cells, and infiltrating neutrophils at the tumor site. IL-8 expression correlates with the angiogenesis, tumorigenicity, and metastasis of tumors in numerous xenograft and orthotropic *in vivo* models.[49] IL-8 promotes breast cancer progression by increasing cell invasion, angiogenesis, and metastases and is upregulated in human epidermal growth factor receptor 2-positive (HER2-positive) cancers. IL-8 via its cognate receptors, CXC chemokine receptor type 1 (CXCR1) and CXCR2, is also involved in regulating cancer stem cells (CSCs) activity[50]. IL-8 can promote CSCs invasion, metastases, and treatment resistance.[50] IL-8 up-regulated the anti-apoptotic gene BCL2, down-regulated the pro-apoptotic gene CASP3 via PI3K/AKT signal pathway and significantly inhibited the apoptosis of MCF7 cells.[51] IL-8 binds CXCR2 receptors, on the surface of nearby cancer cells. Binding of IL-8 to CXCR2 produces signals within tumor cells that activate molecules transcription factors including NF-kB and AP-1, via the Akt and MAPK signalling pathways, ultimately causes the growth and survival of colon cancer cells.[52]

TGF-β contributes in the development of colorectal cancer.[53] TGF-β role plays in regulating cancer formation and progression, while acting cancer inhibitor in normal cells and early carcinomas. The development of novel anticancer therapies based on the use of TGF-β antagonists is very critical for the development anticancer therapies using TGF-β antagonists.[54] Therapeutic approaches for colorectal cancer based on an inhibition of TGF-beta-dependent IL-6 trans-signalling.[53] IL-6 enhances human skin carcinoma cell invasiveness by inducing the overexpression of MMP-1.[55] IL-6 contributes in mediating epithelial–mesenchymal transition (EMT) and metastasis neck squamous cell carcinoma (HNSCC).[56] Cytokines that are involved in inflammation-related carcinogenesis, such as hypoxia-inducible factor (HIF-1α), MMP-2 and -9,

BCLX, BCL2 and VEGF and IL-6.[57,58] IL-6 promotes prostate cancer cell proliferation and inhibit apoptosis via multiple signal pathways including Janus tyrosine family kinase (JAK), signal transducer and activator of transcription (STAT) pathway, the extracellular signal-regulated kinase 1 and 2 (ERK1/2), mitogen activated protein kinase (MAPK) pathway, and the phosphoinositide 3-kinase (PI3-K) pathway.[58]

VEGF-α is a member of the VEGF family induces expression of the anti-apoptotic proteins BCL2 and A1 in endothelial cells is mediated by the PI3K/Akt pathway,[59] VEGF-α influence the susceptibility and aggressiveness of breast cancer.[60] Many solid tumors with high levels of VEGF expression have been associated with poor clinical.[61] In conclusion, WJMSCs-hypoCM was less active to induce apoptosis for CM contained higher cytokines, chemokines, growth factors which increase breast cancer proliferation. In conclusion WJMSCs-norCM is more active as anticancer by inducing the apoptotic gene expression in breast cancer cell (T47D, MCF7) compared to WJMSCs-hypoCM.

CONFLICTS OF INTEREST

All contributing authors declare no conflicts of interest.

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Tabel 1. Primers for real-time PCR

Gene	Primer sequences	
	Forward	Reverse
β-actin	5'-TCT GGC ACC ACA CCT TCT ACA ATG-3'	5'-AGC ACA GCC TGG ATA GCA ACG-3'
p53	5' AGA GTC TAT AGG CCC ACC CC 3'	5'-GCT CGA CGC TAG GAT CTG AC 3'
CASP9	5'-CAT GCT CAG GAT GTA AGC CA -3'	5'-AGG TTC TCA GAC CGG AAA CA-3'
BAX	5'-TGC TTC AGG GTT TCA TCC AG 3'	5'-GGC GGC AAT CAT CCT CTG 3'
BCL2	5'- GGT CAT GTG TGT GGA GAG CG -3'	5'-GGT GCC GGT TCA GGT ACT CA-3'

Tabel 2. Effect of WJMSCs-norCM and WJMSCs-hypoCM toward number of T47D and MCF-7 cancer cells (data are expressed as means, standard deviation, Tukey HSD post hoc test)

Cell lines	Oxygen tension Passage	WJMSCs-CM 0%	WJMSCs-CM 20%	WJMSCs-CM 40%	WJMSCs-CM 60%	WJMSCs-CM 80%
MCF7	WJMSCs-norCM-P4	22,288±188 ^{d}	15,479±442 ^{c}	12,317±1,312 ^{b}	10,188±1,303 ^{b}	6,697±729 ^{a}
	WJMSCs-norCM-P8	22,288±188 ^{c}	14,636±1,197 ^{b}	12,433±1,397 ^{b}	9,544±1,216 ^{a}	6,864±631 ^{a}
	WJMSCs-hypo5% CM-P4	22,288±188 ^{e}	16,265±899 ^{d}	14,351±895 ^{c}	11,284±672 ^{b}	6,585±25 ^{a}
	WJMSCs-hypo5% CM-P8	22,288±188 ^{d}	16,399±1,543 ^{c}	14,592±1,015 ^{bc}	11,918±1,144 ^{b}	8,008±723 ^{a}
	WJMSCs-hypo2.5% CM-P4	22,288±188 ^{d}	16,143±697 ^{c}	13,465±1,013 ^{b}	11,688±594 ^{b}	8,103±630 ^{a}
	WJMSCs-hypo2.5% CM-P8	22,288±188 ^{d}	16,127±1,986 ^{c}	13,045±327 ^{b}	9,732±1,053 ^{a}	7,774±614 ^{a}
T47D	WJMSCs-norCM-P4	13,610±749 ^{c}	8,919±279 ^{b}	8,056±752 ^{b}	5,309±299 ^{a}	4,084±1,207 ^{a}
	WJMSCs-norCM-P8	13,610±749 ^{c}	9,700±210 ^{b}	8,487±966 ^{b}	5,936±543 ^{a}	4,862±1,299 ^{a}
	WJMSCs-hypo5% CM-P4	13,610±749 ^{d}	10,131±948 ^{c}	8,847±186 ^{c}	7,260±355 ^{b}	5,493±306 ^{a}

	WJMSCs-hypo5% CM-P8	13,610±749 ^{d}	9,736±843 ^{c}	8,296±249 ^{bc}	7,071±1,279 ^{ab}	5,951±272 ^{a}
	WJMSCs-hypo2.5% CM-P4	13,610±749 ^{c}	9,955±990 ^{b}	8,893±691 ^{b}	6,117±1,210 ^{a}	5,380±711 ^{a}
	WJMSCs-hypo2.5% CM-P8	13,610±749 ^{d}	9,701±933 ^{c}	8,304±1,127 ^{bc}	6,506±41 ^{ab}	5,293±680 ^{a}

The data are expressed as mean±standard deviation; different supercript small letters (a,b,c,d) in the same row (among concentrations of CM in T47D and MCF-7 cell) are siginificant differences at $p<0.05$ (Turkey HSD post hoc test)

Tabel 3. Effect of WJMSCs-norCM and WJMSCs-hypoCM toward viability of T47D and MCF-7 cancer cells (data are expressed as means, standard deviation, Tukey HSD post hoc test)

Cell lines	Oxygen tension Passage	WJMSCs-CM 0%	WJMSCs-CM 20%	WJMSCs-CM 40%	WJMSCs-CM 60%	WJMSCs-CM 80%
MCF7	WJMSCs-norCM-P4	100.00±0.85 ^{d}	69.45±1.98 ^{c}	55.26±5.89 ^{b}	45.71±5.85 ^{b}	30.04±3.27 ^{a}
	WJMSCs-norCM-P8	100.00±0.85 ^{c}	65.67±5.37 ^{b}	55.78±6.27 ^{b}	42.82±5.45 ^{a}	30.80±2.83 ^{a}
	WJMSCs-hypo5% CM-P4	100.00±0.85 ^{e}	72.98±4.03 ^{d}	64.39±4.02 ^{c}	50.63±3.02 ^{b}	29.54±0.11 ^{a}
	WJMSCs-hypo5% CM-P8	100.00±0.85 ^{d}	73.58±6.92 ^{c}	65.47±4.55 ^{bc}	53.47±5.13 ^{b}	35.93±3.24 ^{a}
	WJMSCs-hypo2.5% CM-P4	100.00±0.85 ^{d}	72.43±3.13 ^{c}	60.41±4.54 ^{b}	52.44±2.66 ^{b}	36.36±2.83 ^{a}
	WJMSCs-hypo2.5% CM-P8	100.00±0.85 ^{d}	72.35±8.91 ^{c}	58.53±1.47 ^{b}	43.67±4.73 ^{a}	34.88±2.76 ^{a}
T47D	WJMSCs-norCM-P4	100.00±5.51 ^{c}	65.53±2.05 ^{b}	59.19±5.52 ^{b}	39.01±2.19 ^{a}	30.00±8.86 ^{a}
	WJMSCs-norCM-P8	100.00±5.51 ^{c}	71.27±1.54 ^{b}	62.35±7.10 ^{b}	43.61±3.99 ^{a}	35.72±9.55 ^{a}
	WJMSCs-hypo5% CM-P4	100.00±5.51 ^{d}	74.43±6.96 ^{c}	65.00±1.37 ^{c}	53.34±2.61 ^{b}	40.36±2.25 ^{a}
	WJMSCs-hypo5% CM-P8	100.00±5.51 ^{d}	71.54±6.19 ^{c}	60.95±1.83 ^{bc}	51.95±9.40 ^{ab}	43.73±2.00 ^{a}
	WJMSCs-hypo2.5% CM-P4	100.00±5.51 ^{c}	73.14±7.27 ^{b}	65.34±5.08 ^{b}	44.94±8.89 ^{a}	39.53±5.23 ^{a}
	WJMSCs-hypo2.5% CM-P8	100.00±5.51 ^{d}	71.28±6.86 ^{c}	61.01±8.28 ^{bc}	47.80±0.30 ^{ab}	38.89±4.99 ^{a}

Data are expressed as mean±standard deviation; different supercript small letters (a,b,c,d) in the same row (among concentrations of CM in T47D and MCF-7 cell) are siginificant differences at $p<0.05$ (Turkey HSD post hoc test)

Tabel 4. Effect of WJMSCs-norCM and WJMSCs-hypoCM toward proliferation inhibition of T47D and MCF-7 cancer cells (data are expressed as means, standard deviation, Tukey HSD post hoc test)

Cell lines	Oxygen tension Passage	WJMSCs-CM 0%	WJMSCs-CM 20%	WJMSCs-CM 40%	WJMSCs-CM 60%	WJMSCs-CM 80%
MCF7	WJMSCs-norCM-P4	0.00±0.85 ^{a}	30.55±1.98 ^{b}	44.74±5.89 ^{c}	54.29±5.85 ^{c}	69.96±3.27 ^{d}
	WJMSCs-norCM-P8	0.00±0.85 ^{a}	34.33±5.37 ^{b}	44.22±6.27 ^{b}	57.18±5.45 ^{c}	69.20±2.83 ^{c}
	WJMSCs-hypo5% CM-P4	0.00±0.85 ^{a}	27.02±4.03 ^{b}	35.61±4.02 ^{c}	49.37±3.02 ^{d}	70.46±0.11 ^{e}
	WJMSCs-hypo5% CM-P8	0.00±0.85 ^{a}	26.42±6.92 ^{b}	34.53±4.55 ^{bc}	46.53±5.13 ^{c}	64.07±3.24 ^{d}
	WJMSCs-hypo2.5% CM-P4	0.00±0.85 ^{a}	27.57±3.13 ^{b}	39.59±4.54 ^{c}	47.56±2.66 ^{c}	63.64±2.83 ^{d}
	WJMSCs-hypo2.5% CM-P8	0.00±0.85 ^{a}	27.65±8.91 ^{b}	41.47±1.47 ^{c}	56.33±4.73 ^{d}	65.12±2.76 ^{d}
T47D	WJMSCs-norCM-P4	0.00±5.51 ^{a}	34.47±2.05 ^{b}	40.81±5.52 ^{b}	60.99±2.19 ^{c}	70.00±8.86 ^{c}
	WJMSCs-norCM-P8	0.00±5.51 ^{a}	28.73±1.54 ^{b}	37.65±7.10 ^{b}	56.39±3.99 ^{c}	64.28±9.55 ^{c}
	WJMSCs-hypo5% CM-P4	0.00±5.51 ^{a}	25.57±6.96 ^{b}	35.00±1.37 ^{b}	46.66±2.61 ^{c}	59.64±2.25 ^{d}
	WJMSCs-hypo5% CM-P8	0.00±5.51 ^{a}	28.46±6.19 ^{b}	39.05±1.83 ^{bc}	48.05±9.40 ^{cd}	56.27±2.00 ^{d}
	WJMSCs-hypo2.5% CM-P4	0.00±5.51 ^{a}	26.86±7.27 ^{b}	34.66±5.08 ^{b}	55.06±8.89 ^{c}	60.47±5.23 ^{c}
	WJMSCs-hypo2.5% CM-P8	0.00±5.51 ^{a}	28.72±6.86 ^{b}	38.99±8.28 ^{bc}	52.20±0.30 ^{cd}	61.11±4.99 ^{d}

Data are expressed as mean±standard deviation; different supercript small letters (a,b,c,d) in the same row (among concentrations of CM in T47D and MCF-7 cell) are siginificant differences at $p<0.05$ (Turkey HSD post hoc test)

Tabel 5. The IC_{50} of WJMSCs-norCM and WJMSCs-hypoCM in breast cancer cell lines for 72 hour incubation

Cell lines	IC_{50} (%)					
	WJMSCs-norCM		WJMSCs-hypo5% CM		WJMSCs-hypo2.5%CM	
	Passage 4	Passage 8	Passage 4	Passage 8	Passage 4	Passage 8
MCF7	42.34±4.94	49.51±2.13	52.10±10.16	54.1±3.32	57.93±15.49	62.84±7.95
T47D	44.87±3.50	42.36±2.49	51.98±2.16	48.06±4.08	56.27±5.42	58.96±7.22

Data are expressed as IC_{50} value; the anticancer activity of WJMSCs-CM effect toward breast cancer cell lines (MCF7, T47D). The experiment was conducted in triplicate for each treatment.

Tabel 6. Gene expression of 50% concentration of WJMSCs-hypo5%CM-P4 and WJMSCs-hypo5%CM-P8 in T47D, MCF7 cells for 72 h incubation

Cell line	**Treatment**	**Genes expression**			
		p53	BAX	CASP9	BCL2
MCF7	Control	1.00±0.00 [a]	1.00±0.00 [a]	1.00±0.00 [a]	1.00±0.00 [a]
	WJMSCs-norCM-P4	24.67±7.04 [b]	14.12±2.82 [b]	8.62±1.93 [b]	0.90±0.58 [a]
	WJMSCs-hypo5%CM-P4	6.08±2.71 [a]	2.39±0.56 [a]	5.98±4,18 [a]	0.37±0.20 [a]
	WJMSCs-hypo2.5%CM-P4	5.01±1.02 [a]	3.27±0.81 [a]	6.15±0.36 [a]	0.52±0.24 [a]
T47D	Control	1.00±0.00 [a]	1.00±0.00 [a]	1.00±0.00 [a]	1.00±0.00 [a]
	WJMSCs-norCM-P4	10.45±0.46 [b]	3.94±1.25 [b]	13.86±2.57 [b]	0.80±0.09 [a]
	WJMSCs-hypo5%CM-P4	1.74±0.30 [a]	1.61±0.56 [ab]	14.43±3.84 [b]	0.81±0.10 [a]
	WJMSCs-hypo2.5%CM-P4	1.79±0.20 [a]	2.01±0.05 [ab]	10.18±3.00 [ab]	0.11±0.05 [a]

Data are expressed as mean±standard deviation; different letters in the same column (among WJMSCs-norCM, WJMSCs-hypo5% and WJMSCs-hypo2.5% in T47D and MCF-7 cell lines) are significant differences at $p<0.05$ (Turkey HSD post hoc test)

Figure 1. Morphological appearance of T47D (A-D) and MCF 7 (E-H) cells treated with WJMSCs-CM using inverted Microscope. A & F : breast cancer untreated; B&F : breast cancer treated with 50% concentration of WJMSCs-norCM; C&G : breast cancer treated with 50% concentration of WJMSCs-hypo5%CM; D&H : breast cancer treated with 50% concentration of WJMSCs-hypo2.5%CM. Scale bar : 100 µm. Control or un treated cells both T47D and MCF7 showed high density and the highest cells number compared treatment cells. Treatment WJMSCs-norCM in T47D and MCF7 showed the lowest cells number and many cells debris formation compared to control, WJMSCs-hypo5%CM and WJMSCs-hypo2.5%CM. Both T47D and MCF7 cells treated with WJMSCs-hypo2.5%CM showed the lowest anticancer with less cells debris formation and less cell density.

Figure 2. Effect WJMSCs-norCM, WJMSCs-hypo5%CM, WJMSCs-hypo2.5%CM at 50 % concentration level toward apoptotic (p53, BAX, CASP9) and antiapoptotic genes expression in breast cancer cell lines (MCF7, T47D). (A) p53 gene expression; (B) BAX gene expression; (C) CASP9 gene expression; (D) BCL2 gene expression

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Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) on TNF-α, IFN-γ, Perforin, and Gra

Background/Aims: This study was performed to elucidate the effects of conditioned medium (CM) of co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) on breast cancer (BC), NK cells viability, TNF-α, IFN-γ, perforin, and granzyme level. **Methods:** The co-culture method of IL2-NK and MCF7, IL2-NK and hWJMSCs was conducted for evaluating the TNF-α, IFN-γ, perforin, and granzyme level. Cytotoxic activity of CM-IL2-hWJMSCs against MCF7 and NK cells, viability of IL2-NK in various incubation period, cytotoxic activity of IL2-NK toward MCF7 cells in various ratio, were measured with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay; the TNF-α, IFN-γ, perforin, and granzyme concentrations were determined using ELISA. **Results:** The CM-IL2-hWJMSCs and CM-hWJMSCs e! xhibited cytotoxic effects, MCF7 and NK cells has higher inhibition, the higher number of NK cells increased the anticancer activity in BC cells, the highest cytotoxic activity was obtained from co-culture of BC and NK cells in the ratio of 1:5. The IL2-induced NK cells proliferation was higher compared to the noninduced cells. Co-culture of NK cells and hWJMSCs, MCF7 cells produced TNF-α, IFN-γ, perforin, granzyme. IL2-induced NK cells produced higher level of TNF-α, IFN-γ, perforin, and granzyme compared to IL2-noninduced NK cells. The hWJMSCs and MCF7 cells did not secrete perforin and granzyme but secreted low level of TNF-α and IFN-γ. The highest level of TNF-α, IFN-γ, perforin, and granzyme were secreted by the co-culture of hWJMSCs, MCF7 cells and NK cells (1:5). **Conclusions:** The CM-IL2-hWJMSCs and CM-hWJMSCs had anticancer activities against BC cells and were toxic against NK cells.

Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward TNF-α, IFN-γ, Perforin, Granzyme Concentration in Breast Cancer Cells

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Running title: Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward TNF-α, IFN-γ, Perforin, Granzyme Concentration in Breast Cancer Cells.

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Abstract

Aims: This study aimed to elucidate effect of conditioned medium (CM) of co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward breast cancer (BC), NK cells viability, TNF-α, IFN-γ, perforin, and granzyme level.

Methods: human recombinant IL-2 was used to induce hWJMSCs and CM-IL2-hWJMSCs was collected to evaluate its effect toward BC cells (MCF7) and NK cells. Co-culture method of IL2-NK and MCF7, IL2-NK and hWJMSCs was conducted to evaluate TNF-α, IFN-γ, perforin, and granzyme level. CM-IL2-hWJMSCs cytotoxicity toward MCF7 and NK cells, IL2-NK viability, IL-2-NK cytotoxicity toward MCF7 cells were measured with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, whilst TNF-α, IFN-γ, perforin, and granzyme level with ELISA.

Results: The CM-IL2-hWJMSCs and CM-hWJMSCs were cytotoxic to MCF7 and NK cells in concentration-dependent manner. Higher NK cells increased anticancer activity toward MCF-7. Co-culture of MCF-7 and NK cells showed highest cytotoxic activity in ratio of 1:5. The IL2-NK proliferation were higher than noninduced cells. Co-culture of NK cells and hWJMSCs, MCF7 cells produced TNF-α, IFN-γ, perforin, granzyme. IL2-NK produced higher level of TNF-α, IFN-γ, perforin, granzyme than IL2-noninduced NK cells. hWJMSCs and MCF7 cells did not secrete perforin, granzyme, and secreted low TNF-α and IFN-γ level. Co-culture of hWJMSCs, MCF7 and NK cells (1:5) produced highest level of TNF-α, IFN-γ, perforin, granzyme.

Conclusions: The CM-IL2-hWJMSCs and CM-hWJMSCs have anticancer activity against BC cells and toxic against NK cells. The IL-2 increase NK cells proliferation. Higher NK and IL-2 increase secretion of TNF-α, IFN-γ, perforin, granzyme both in hWJMSCs and MCF7 cells.

Keywords: Human Wharton's Jelly Mesenchymal Stem Cells; Interleukin-2; Conditioned Medium; Natural Killer; Breast Cancer

1. Introduction

Breast cancer (BC) is one of the most leading causes of cancer deaths in women [1]. The mortality of most patients with solid tumors mainly due to metastatic spread to other organs [1]. An ideal therapeutic strategy is therefore needed to target tumor cells directly both in primary and metastatic sites, and possess the ability to act locally over a sustained period of time [2]. Metastasis occurs when tumor cells acquire invasive features [3] and the ability to escape from antitumor immunity [4]. These defects in antitumor immunity may also facilitate BC occurrence [5]. Metastasis in BC is caused by immunosurveillance deficiency including impairment of NK cell maturation, low NK cell counts in peripheral blood mononuclear cells (PBMCs), low NK activity [6], decreased cytotoxic functions [7, 8], NK abnormalities [10], poor tumor infiltrate [9], low NK cell numbers in tumors due to their inefficient homing into malignant tissues [7], defective expression of activating receptors: NKG2D, NKG2C, NKp30, NKp46, CD161, $CD56^{dim}$, CD16, DNAM-1, CD69, and high immunosuppression namely overexpression of inhibitory receptors CD158a, CD158b, NKG2A [7].

One of promising cancer therapies is to stimulate NK cell functions and combination with other agents to boost its anti-cancer activity [10]. NK cells respond to a variety of cytokines, such as interleukin-2 (IL-2) [11]. NK cells kill cancer cells directly by releasing cytoplasmic granules namely perforin and granzymes which lead to

apoptosis [12]. Activated NK cells secrete TNF-α, various effector molecules, such as IFN-γ which can induce cancer-cell apoptosis [12]. The number, purity, and state of NK cell proliferation and activation, are important aspects in the NK cell-based immunotherapy, as NK cell number should be sufficient which is critical in clinical protocols [13]. NK cells are known as necessary effectors in suppressing cancer proliferation [13]. Therefore, the focus of recent cancer therapy is to promote developed-NK cells as drugs [8], using NK effector such as cytokines [7].

The other promising anti-cancer agent is the secretome of mesenchymal stem cells (MSCs) namely MSCs conditioned-medium (CM), which contains micro particles that mediate therapeutic effects toward cancer [14]. It has been reported that CM from normoxia-treated WJMSCs (WJMSCs-norCM) and CM from hypoxia-treated WJMSCs (WJMSCs-hypoCM) posses anticancer activity toward various cancer cell lines, such as HeLa, SKOV3, PC3, HSC3, HepG2 cells [15]. Furthermore, CM-MSCs inhibited mammary carcinoma, osteosarcoma, pancreatic, and lung tumor growth [16].

To date, CM-hWJMSCs have been known to exhibit anticancer activities, yet further development in enhancing its potential is still encouraged. An approach suggested to increase the anticancer potential, is to utilize cytokines, such as IL-2 which may boost the anticancer of NK cells directly and CM-hWJMSCs indirectly. In this research, we aim to elucidate the properties of CM-IL2-hWJMSCs toward BC and NK cells viability, IL2-induced NK (IL2-NK) cells toward BC cells inhibition, effect of IL2 toward NK cells proliferation in various incubation, cytotoxic activity of IL2-NK toward BC cells in various ratio, effect of IL2-NK cells toward TNF-α, IFN-γ, perforin, granzyme level in CM-MCF7 and CM-hWJMSCs.

2. Materials and Methods

2.1. Interleukin 2-induced human Whartons Jelly Mesenchymal Stem Cells (hWJMSCs-IL2)

The human Wharton's Jelly mesenchymal stem cells (hWJMSCs) of passage 4 (P4) were obtained from our previous research, the cells have been characterized for the cell surface phenotype and multipotent differentiation [15]. This research has followed the approval protocol and informed consent of the Institutional Ethics Committee at the Stem Cell and Cancer Institute, Jakarta, Indonesia and the Institutional Ethics Committee collaboration between Maranatha Christian University, Bandung, Indonesia and Immanuel Hospital Bandung, Bandung, Indonesia. The hWJMSCs in density of $5x10^5$ were cultured in minimum essential medium-α (α-MEM) with low glutamine without ribonucleosides acid and deoxyribonucleosides (Biowest, L0475), supplemented with 20% fetal bovine serum (FBS, Biowest, S181H) and 1% penicillin-streptomycin (PenStrep) (Biowest, L0018). The cells were incubated in a humidified atmosphere with 5% CO_2 at 37^{o}C for 24 h. The medium was discarded, using PBS, the adherent hWJMSCs were supplemented with FBS-free medium (α-MEM + 1% PenStrep). The cells were then treated with 125 and 250 ng/ml of IL-2 (Genscript, Z00368-10). The IL2-hWJMSCs were incubated in a humidified atmosphere at 5% CO_2, 37^{o}C for 24 h. The IL2-hWJMSCs were harvested and medium supernatant was collected and centrifuged at 500g for 4 min at room temperature, then the supernatant was filtered using a 0.22 µm MillexeGV Filter Unit with Durapore (SLGV 033 RS, Millipore Corporation, Billerica, MA, USA). The filtered supernatant which was the conditioned medium of IL2-hWJMSCs (CM-IL2-hWJMSCs), was preserved in deep freezer at temperature of -80^{o}C [15].

2.2. Cytotoxic activity of CM-IL2-hWJMSCs toward breast cancer cells

The BC cell lines MCF-7 (ATCC® HTB22™) were obtained from Aretha Medika Utama Biomolecular and Biomedical Research Center, Bandung, Indonesia. The cancer cell lines ($5x10^3$/well) were cultured in complete medium which consist of Dulbecco's Modified Eagle's Medium (DMEM; Biowest L0104) supplemented with 10 % FBS and 1% PenStrep. The cells were incubated in a humidified atmosphere, 5% CO_2, 37°C for 24 h. The cell medium was then discarded and washed using phosphat buffer saline (PBS). The medium was replaced using FBS-free medium (DMEM + 1% PenStrep), incubated for 24h. Furthermore, cells were treated with CM-IL2-hWJMSCs in 30, 60% concentrations and were incubated for 24 h. The cells viability was assayed based on an optimized reagent containing resazurin converted to fluorescent resorufin by viable cells that absorbs the light at 490 nm using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Promega, Madison, WI, USA) [15, 17].

2.3. Cytotoxic activity of CM-IL2-hWJMSCs toward NK cells

The NK92MI cell (ATCC® CRL2408™) was obtained from Aretha Medika Utama Biomolecular and Biomedical Research Center, Bandung, Indonesia. The NK cells ($1x10^4$) were grown and maintained in FBS-free medium containing α-MEM without ribonuclease and deoxirybonucleoside with 2 mM glutamine (Gibco, 12000022), 1.5 g/l sodium bicarbonate (Gibco, 25080094), 0.2 mM inositol (Sigma Aldrich, 17508), 0.1 mM 2-merchaptoethanol (Gibco, 21985023), and 0.02 mM folic acid (Sigma Aldrich, F8758) at 96- well plate. On the same day, cells were treated with CM-IL2-hWJMSCs in 30, 60% concentrations then cells incubated for 24 h. The cells viability was tested by adding 20 µl of MTS into each well and incubated at 5% CO_2, 37°C for 3 h. The absorbance of the cells was measured at 490 nm using a microplate enzyme-linked immunosorbent assay reader (Multiskan Go, Thermo Scientific, USA) [15].

2.4. Cell viability of IL2-induced NK (IL2-NK)

The NK cells in density of $1x10^5$ were grown in 96-well plate in FBS-free NK medium. The cells were induced directly to 5, 10 ng/ml of IL-2 and incubated at 5% CO_2, 37°C, humidified atmosphere for 24, 48, 72, 96 h (11). MTS assay was used to determine the NK-IL2 viability per day [15].

2.5. Optimization medium for co-culture

The medium that will be used for co-culture between and MCF7-NK cells should be optimized to ensure the combination medium were not toxic or reduce the cancer and NK cells viability. This preliminary research was done to obtain the best ratio between NK medium and cancer (MCF7) medium (DMEM). Various ratios of medium for growing MCF7 using DMEM and NK medium were v/v 1:1, 7:3, 3:7; and for NK cells were DMEM : NK medium (v/v : 1:1, 7:3, 3:7). The best medium ratio was the combined medium with high viability of both MCF7 and NK cells. The MCF7 cells ($5x10^3$/well), and NK cells ($1x10^5$/well) were cultured in various combined medium at 96-well plate, incubated at 5% CO_2, 37°C for 24 h. The cells viability was measured using MTS assay with cell absorbance read at 490 nm [15].

2.6. Growth inhibition of IL2-NK cells on breast cancer cells

This research method were adopted and modified based on the previous research by Parihar *et al* [18] and Lu *et al*., [19]. The NK cells in density of $1x10^6$/well were grown and maintained in FBS-free NK medium (*α*-MEM, 1.5 g/L sodium bicarbonate, 0.2 mM inositol, 0.1 mM 2-merchaptoethanol, 0.02 mM folic acid), cells were treated with 5, 10 ng/ml of IL-2, per 24 h and incubated at 5% CO_2, 37°C for 96 h, resulted in IL2-induced NK (IL2-NK) cells. Meanwhile, breast cancer cell lines, MCF7 ($1x10^4$/well) were cultured into the 96-well plate in a 100 µl of complete medium and incubated at 5% CO_2, 37°C for 24 h. The following day, culture supernatant was aspirated, washed with PBS and replaced with FBS-free medium (DMEM + 1% PenStrep). The cells were incubated at 5% CO_2, 37°C for 24 h. The breast cancer cells treated with NK cell in number-dependent expriment, the ratios of cancer cells to NK cells were 1 : 5 (10.000 : 50.000), 1: 2 (10.000 : 20.000), 1 : 1 (10.000 : 10.000) and 2 : 1 (10.000 : 5.000). NK medium and cancer medium (1:1) was the best ratio from preliminary study, which was then used for cell co-culture. The single culture of NK92MI, and MCF7 were served as controls. The NK and breast cancer co-culture cells were incubated at 5% CO_2, 37°C for 24 h. MTS assay was performed to determine the growth inhibition or cytotoxic effect of NK cells toward cancer cell. Approximately, 20 µl of cell titer 96 ® Aqueous one solution reagent (Promega, Madison, WI, USA) were added into each well and incubated for 3 h. The cells absorbance was measured at 490 nm (Multiskan Go, Thermo Scientific, USA). The inhibitory effect of ILs-NK cells on cancer cell viability was calculated based on the following equation:

$$\text{Growth Inhibition}(\%) = \frac{[(\text{Absorbance}_{\text{cancer}} - \text{Absorbance}_{\text{cancer+NK}}) + \text{Absorbance}_{\text{NK}}]}{\text{Absorbance}_{\text{cancer}}} \times 100\%$$

2.7. Co-culture hWJMSCs, MCF7 and IL2-NK cells

The hWJMSCs in density of $10x10^5$ in complete medium (α-MEM + 20% FBS + 1% PenStrep), the MCF7 cells $10x10^5$ (DMEM+205 FBS+1% PenStrep) were cultured in 24 well plate with. The cells were incubated in a humidified atmosphere with 5% CO_2 at 37°C for 24 h. The medium of hWJMSCs or MCF7 cells were replaced with 2 ml FBS-free medium and incubated with 5% CO_2 at 37°C for 24 h, then cells were washed with PBS. NK cells $1x10^5$ for comparison cells 1:1 (hWJMSCs/MCF7 : IL2-NK = 1 : 1) and $5x10^5$ NK cells for comparison cells 1:5 (hWJMSCs/MCF7 : IL2-NK = 1 : 5). NK cells were resuspended to 24 well plate which consisted in hWJMSCs with medium NK (FBS free), α-MEM (serum free) in comparison 50% : 50%, MCF7 with medium NK (FBS free), DMEM (FBS free) in comparison 50% : 50%. Co-culture of IL2-NK and hWJMSCs or IL2-NK and MCF7 incubated in a humidified atmosphere with 5% CO_2 at 37°C for 24 h. Cells and medium were centrifuged at 500xg for 4 min, the medium were preserved in -80^0 C for continuing assay such as IFN-γ, TNF-α, perforin, and granzyme [18, 20].

3. RESULTS

3.1. MSCs markers and multipotent differentiation

The hWJMSCs of passage P4 highly expressed CD105, CD73, CD90 (>95%) and lowly expressed CD34, CD45, CD14, CD19, and HLA-II. The cells were able to

differentiate into osteocytes, chondrocytes, and adipocytes (the data are not shown). This hWJMSCs has been confirmed based on the surface marker and differentiation capability [15].

3.2. Cytotoxic activity of CM-IL2-hWJMSCs toward breast cancer cells

To determine the effect of conditioned medium from hWJMSCs treated with IL2 of concentration 125 ng/ml, 250 ng/ml (IL2-induced hWJMSCs : CM-IL2-hWJMSCs) toward breast cancer cells proliferation, we evaluated the inhibition of CM-IL2-hWJMSCs on MCF7 in concentration of CM (30%, 60%)cells (Figure 1).

Figure 1 shows that CM-hWJMSCs (non induced of CM-hWJMSCs) and CM-IL2-hWJMSCs inhibited cells proliferation and had anticancer activities toward MCF7 cells. Higher concentration (60%) of CM-hWJMSCs and CM-IL2-hWJMSCs increased growth inhibition of BC cells (MCF7) compared 30% of CM based on t-test ($p<0.05$).

3.3. Effect of CM-IL2-hWJMSCs toward NK cells inhibition

IL-2 can activate and improve NK cells, we evaluated the effect of CM-IL2-hWJMSCs toward the NK cells inhibition. The effect of IL2-induced CM-hWJMSCs (CM-IL2-hWJMSCs) with concentrations (125, 250 ng/ml) toward inhibition of NK cells can be seen at Figure 2.

Figure 2 shows both CM-hWJMSCs and CM-IL2-hWJMSCs were toxic against the NK cells. The CM-IL2-hWJMSCs were significantly more toxic in higher concentration (60% CM) than lower concentration (30% CM) of CM and there was no significant differences between concentration of IL-2

3.4. Effect of IL-2 toward NK cells viability

The IL-2 activate and improve the NK cells activity, therefore it was necessary to measure the potential of IL-2 to promote NK cells number with various periodic incubations.

The data in Figure 3 indicated that the longer incubation period increased the NK viability significantly. On the first day, induction by IL-2 (5, 10 ng/ml) resulted NK cell viability which were similar to control, but the viability increased over time and finally peak at the third and fourth day.

3.5. Effect combined-medium toward breast cancer cells viability

The potential of NK cells as immunomodulator to inhibit BC cells, was performed by co-culture method using MCF7 and NK cells. The co-culture method require a combined medium between cancer cells medium and NK cells medium, the MCF7 cells were cultured in NK medium and DMEM. This preliminary research was performed to obtain the best medium ratio for co-culture, in which the resulted combined medium should be not toxic on BC cells. Therefore, the results of co-culture method were not influenced by medium or other external factors.

Based on the data (Table 1), it shows that the NK medium either with or without FBS, were not toxic toward MCF7 cells. The FBS free-NK medium were more favorable for MCF7 cells compared to the FBS free-cancer cells medium (DMEM). Moreover, the FBS free-NK medium treatment was comparable with complete cancer cells medium treatment (DMEM+FBS).

3.6. Effect of combined-medium toward NK cells viability

The previous research (Table 1) shows that NK cells medium was not toxic toward BC cells. This preliminary research was done to know the cytotoxic effect of cancer and NK cells combined-medium toward NK cells viability, so that the combined-medium were confirmed not toxic toward both breast cancer and NK cells in co-culture method. The effect of cancer and NK cells combined-medium toward the NK cells viability (%) can be seen at the Table 2.

Based on the data (Table 2), it was revealed that DMEM was favorable for NK cells viability. For the following cytotoxic assay, we used the combined medium DMEM : NK medium in ratio of 5:5 for co-culture NK cells, MCF7 and NK cells.

3.7. Effect of IL2-NK cells toward breast cancer cells growth inhibition in co-culture method

The potential of NK cells as immunomodulator to kill and inhibit BC cells proliferation, was performed using co-culture method between MCF7 and NK cells in various ratios of NK and cancer cells. The effect of NK cells against the cancer cells growth inhibition can be seen at Figure 4.

Based on the data (Figure 4), the ratio of cancer and NK cells clearly determined the growth inhibition of cancer cells. It can be clearly seen that the number of NK cells affected its cytotoxicity toward the cancer cells, as the higher NK cells ratio resulted in higher cancer cell growth inhibition. The highest ratio (1:5) exhibited the highest growth inhibition value of cancer cells in all treatment (control, IL-2 5 ng/ml, IL-2 10 ng/ml), there were no significant differences between non-induced NK cells and induced NK cells

3.8. Effect of IL2-NK cells toward TNF-α, IFN-γ, perforin, granzyme level in breast cancer and NK cells

To determine cytotoxic effect of NK cells toward BC cells through many secretion various molecules such as TNF-α which induce tumor-cell apoptosis, IFN-γ which stimulate adaptive immunity, peforin and granzyme are released by cytoplasmic granules which lead apoptosis [12]. IL-2 increase the NK cells cytotoxic, we measured NK cells both activated-NK cells using IL-2 (10 ng/ml) and noninduced-NK cells, which contacted with BC cells, the cells comparison (MCF7 : NK cells = 1 : 1, 1 : 5) to secrete TNF-α, IFN-γ, perforin, granzyme. The effect of IL-2 induced NK cells which contacted with BC cells (MCF7) to release cytoplasmic granules (perforin, granzyme), death receptor (TNF-α), effector molecules (IFN-γ) can be seen at Figure 5.

Higher ratio NK cells and MCF7, IL-2 induction increased IFN-γ, TNF-α, perforin, and granzyme level in co-culture MCF7 and NK cells.

3.9. Effect of IL2-NK cells toward TNF-α, IFN-γ, perforin, granzyme level in co-culture hWJMSCs and NK cells

NK cells defense against pathogens, and also play in repairing and degenerating the damaged tisusse [18]. Several studies described that MSCs suppress the proliferation and function of immune system cells like [19], MSCs influence proliferation and cytolytic activities of NK cells [21-22], MSCs influence on IFN-γ production [12], we measured NK cells both activated-NK cells using IL-2 (10 ng/ml) and noninduced-NK cells, which contacted with hWJMSCs, the cells comparison (hWJMSCs : NK cells = 1

: 1, 1 : 5) to secrete TNF-α, IFN-γ, perforin, granzyme. The effect IL-2 induced NK (IL-2NK) cells which contacted with hWJMSCs to release cytoplasmic granules (perforin, granzyme), death receptor (TNF-α), effector molecules (IFN-γ) can be seen at Figure 6.

Higher ratio NK cells and hWJMSCs, IL-2 induction increased IFN-γ, TNF-α, perforin, and granzyme level in co-culture hWJMSCs and NK cells.

4. Discussion

The IL-2 did not increase the anticancer activity of hWJMSCs-CM against MCF7 cancer cells in either 30% or 60% of CM concentration (Figure 1). The CM-IL2-hWJMSCs anticancer activity were not significantly different compared to the uninduced-hWJMSCs-CM (CM-hWJMSCs). Nonetheless, the CM-hWJMSCs could inhibit MCF7 cells viability with inhibition value of 29-49%. These findings are consistent with previous research that WJMSCs-norCM and WJMSCs-hypo5%CM could inhibit cancer cells proliferation including HeLa, SKOV3, PC3, HSC3, HepG2 with IC_{50} 51-74% [15]. Previous study exhibits that concentration 50% CM of hWJMSCs inhibit breast adenocarcinoma (MDA-MB-231), ovarian carcinoma (TOV-112D), and osteosarcoma (MG-63) cells with 30-60% inhibition [23]. CM contains a broad variety of cytokines, growth factors, and putatively micro vesicles containing (micro) RNA, that generate beneficial effects in anticancer therapy [14]. The CM contains tumor growth factor-β 1 (TGF-β1); IL-6, IL-8, monocyte chemotractant protein-1 (MCP-1); regulated on activation normal T cell expressed and secreted (RANTES); collagen type I; fibronectin; secreted protein acidic and rich in cysteine (SPARC); and insulin-like growth factor-binding protein-7 (IGFBP-7) [24-25]. The recombinant of IL-2 is unable to improve the anticancer component in CM-hWJMSCs. This result is in accordance with previous research that the cytotoxicity of hWJMSCs toward cancer cells can be enhanced using engineering method such as transfection and transduction, therefore hWJMSCs expressed IL-2 which regulate inflammatory and possess anticancer activity via direct tumoricidal effects or positive modulation of the endogenous immune system [25-26].

On the other hand, CM-hWJMSCs and CM-IL2-hWJMSCs inhibited NK cells proliferation (Figure 2), higher CM concentration (60% CM) was more toxic on the NK cells. The cytotoxic activity of CM-hWJMSCs and CM-IL2-hWJMSCs on NK cells were not different significantly, that indicates IL-2 induction did not have major effect toward the CM-hWJMSCs cytotoxic activity. Previous study reveals that the hWJMSCs were toxic toward NK cells, as MSCs produce PGE2 and TGF-β that efficiently inhibit the activation and downstream cytotoxicity of resting NK cells [25]. Several studies investigated interaction between NK cells and MSCs, including NK cells kill MSCs, and the MSCs strongly inhibit IL-2 induced NK-cell proliferation [26], stem cells suppressed the NK cell cytotoxicity [26], and human adult stem cells (hASCs) along with hBM-MSCs impair the function of NK cells [27]. The lytic activity toward MSCs by NK cells also occurs in the presence of IL-2 or IL-15, or stimulation by combinations of IL-12/IL-15 and IL-12/1L-18. It was revealed that CD244 and NKG2D are downregulated under cell-to-cell contact in the interaction between NK and MSCs [28].

Based on the current research, IL-2 increased NK proliferation with longer periodic incubation significantly, which increased the NK cells viability (Figure 3). NK cell activity is regulated by cytokines such as interleukin (IL)-2, IL-12, IL-15, IL-18 and type I interferons (IFNs) [29]. Another study reveals that IL-2 induced the proliferation of injected NK cells in the lung, which resulted increase of the overall survival of mice

with osteosarcoma lung metastasis [30]. The NK cells expansion has been attempted using cytokines such as IL-2 [11]. Aerosol IL-2 increases lung NK cell numbers by stimulating local NK cell proliferation [31]. Treatment with aerosol IL-2 in mice with osteosarcoma lung metastasis induce the proliferation of injected NK cells in the lung, it did not increase the proliferation of NK cells in the spleen and liver [32]. IL-2 is known to activate NK cells that kill and inhibit cancer proliferation [12]. Several common ILs that activate the NK cells are IL-2, IL-12, IL-15, IL-18 [29]. The cytolytic, secretory, proliferative and anticancer effect of NK cells increased as response to various cytokines [12].

In the co-culture method, the NK cells were confirmed to possess anticancer property toward the BC (MCF7) cells, indicated by significantly higher cytotoxic activity in the higher NK cells number ratio (Figure 4). These are in accordance with previous studies that ratio of effector (NK cells) and target tumor cells (cervical carcinoma cell llines CaSki and SiHa) determines the NK cell cytotoxicity toward the tumor cells, as shown in low E:T ratio (1.25:1) that results high percentage of viable target cell, and high E:T ratio (20:1) results low viable target cells [33]. The NK cells are the major effector cells of the innate immunity and generally thought to play a fundamental role in the antitumor responses [7]. The NK cells can control tumor growth and metastasis diffusion *in vivo* [33]. In tumors, the NK cell numbers are low due to their inefficient homing into malignant tissues, and there is a decreased tumor infiltrate compared to normal tissue [7]. Thus, the activity and numbers of NK cells should be enhanced to generate better outcome [12] [33]. NK cells are further required to be expanded in sufficient numbers as effector cells [12].

Activated NK cells kill target cancer cells through at least four mechanisms : release of cytoplasmic granules (perforin, granzyme), death receptor-induced apoptosis (FasL, TRAIL, TNF-α), effector molecule production (IFN-γ) or antibody-dependent cellular cytotoxicity (ADCC) is a result of Fc-gamma receptor (FcγR) mediated interaction with NK cells [12]. The binding of FcγR to the Fc domain induces the release of granzyme and perforin from NK cells, leading to target tumor cell lysis and Fc-dependent tumor cell phagocytosis [34]. NK cells contribute to tumor death process by monoclonal antibodies (mAb) therapy by directing ADCC through Fcg RIIIA (CD16) [10].

Higher ratio of NK cells in co-culture NK+MCF7 cells increased TNF-α, IFN-γ, perforin, and granzyme production (Figure 5.). This data was supported by cytotoxic effect of IL2-NK and NK cells number toward BC cells (Figure 4.) that shows higher NK cells number increased cytotoxic effect. This result was supported by effect of IL-2 toward NK cells viability (Figure 3.), that shows IL-2 and longer contact IL-2 with NK cells increased NK viability. This result was in line with previous study that combining IL-2 with NK cell infusions, a potential new therapeutic approach for patients with osteosarcoma lung metastasis [31]. The IL-12 suppress tumor metastases via NKG2D ligand recognition and perforin-mediated cytotoxicity [4]. NK cells are able to kill transformed host cells by releasing cytotoxic granules containing granzyme and perforin. NK cells also secrete cytokines, such as IFN-γ and TNF [12][35]. Inactive perforin and granzyme B are safely packaged in cytotoxic granules in cytotoxic T cells (CTL), NK cells, and NKT cells [36]. Cytotoxic granules of NK cells fuse with cancer cell membrane which promote perforin and granzyme B release into the synapse, resulting in apoptosis of the cancer cell [37]. In present study, breast cancer cells without NK cells secreted low concentrations of IFN-γ and TNF-α. These results were validated

with previous finding that lung cancer cells (H1975) inhibit IFN-γ secretion from the NK cells [38]. Co-culture AML (K562) cells and primary NK cells produce the cytokines IFN-γ and TNF-α [35]. Primary NK cells and NK-92 cells have granules for secretion of cytolytic agents, such as perforin [34]. NK cells, a major source of IFN-γ and TNF-α induce target cancer cells cytolisis [39].

Higher ratio of NK cells in co-culture NK+hWJMSCs increase TNF-α, IFN-γ, perforin, granzyme production (Figure 6.) shows that higher NK cells increased cytotoxic effect of NK cells. This result was in line with previous study that MSCs can interact with cells of both the innate and adaptive immune systems, leading to the modulation of several effector functions [40]. Low NK-to-MSCs ratio, MSCs alter the phenotype of NK cells and suppress proliferation, cytokine secretion [24]. Interactions between MSCs and NK cells affect on innate immune responses and regulation of adaptive immunity [24]. Co-culture Adipocyte Stem Cells (hASCs), human bone marrow mesenchymal stem cells (hBM-MSCs) and NK cells at ratio 1:1 for 72 h release IFN-γ [30]. hASCs induce IFN-γ production in IL-2-expanded NK cells [28]. Co-culture NK cells and AML (K562) cancer cells secrete highest IFN-γ compared to co-culture NK+hASCs, NK+hBM-MSCs and the lowest IFN-γ is produced by NK control [28]. The MSCs inhibit activated NK cells and effector of inducing function such as cytotoxic activity and cytokines production [28].

5. Conclusions

The CM-IL2-hWJMSCs and CM-hWJMSCs had anticancer activity against BC cells, higher CM concentrations increase the inhibition potency. The CM-IL2-hWJMSCs and CM-hWJMSCs were toxic against NK cells, higher CM concentrations increase the inhibition potency. The IL-2 could directly stimulate NK cells proliferation, the longer contact IL-2 and NK cells increase NK viability. The IL2-NK inhibit BC cells proliferation in co-culture method, higher number of NK cells boosted its anticancer activity. Higher cells number of NK and IL-2 inducer increase secretion of TNF-α, IFN-γ, perforin, granzyme as potent anticancer. The further research, preclinical and clinical studies should be pursued prior to therapy application.

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Table

Table 1. Effect various combined medium in co-culture NK and MCF7 cells toward MCF7 cells viability

Various ratios breast cancer cell medium (NK medium compared to DMEM)	MCF7 cells viability (%)
DMEM free FBS	100.00±9.62
NK medium free FBS	158.01±20.12
NK : DMEM =50:50	131.93±12.62
NK : DMEM =70:30	165.26±39.52
NK : DMEM =30:70	139.88±12.09
DMEM+FBS	142.50±9.33
NK medium + FBS	163.45±7.28

Data of breast cancer cells (MCF7) viability are presented as mean ±standard deviation. MCF7 cells were cultured in various combined medium between NK medium and breast cancer mdeium (DMEM)

Table 2. Effect various combined medium in co-culture NK and MCF7 cells toward NK cells viability

Various ratios breast cancer cell medium (DMEM compared to NK medium)	NK cells viability (%)
DMEM, FBS free	77.89±11.71
NK medium, FBS free	100.00±5.63
DMEM : NK medium = 50:50	94.00±22.50
DMEM : NK medium = 30:70	84.61±8.80
DMEM : NK medium = 70:30	94.85±15.72

Data of NK cells viability are presented as mean ±standard deviation. NK cells were cultured in various combined medium between NK medium and MCF7 medium (DMEM).

Figure Legends

Figure 1. Effect of IL-2 induced-hWJMSCs (CM-IL2-hWJMSCs) toward BC cells inhibitory

The data are presented as histogram among treatment, this research was conducted intriplicate for each treatment

symbol * present significant difference between CM-hWJMSCs (non-induced, inducing IL-2 125 ng/ml, inducing IL-2 250 ng/ml) and non CM-hWJMSCs (100% medium) both in concentrations (30%, 60% of CM-hWJMSCs) based on Tukey HSD post hoc test ($p < 0.05$), symbol # present significant difference between concentrations 30% and 60% of CM-hWJMSCs based on t-test ($p<0.05$).

Figure 2. Effect of CM-IL2-hWJMSCs toward NK cells inhibitory

The data are presented as histogram among treatment, this research was conducted in triplicate for each treatment

symbol * present significant between CM-hWJMSCs (non-induced, inducing IL-2 125 ng/ml, inducing IL-2 250 ng/ml) and non CM-hWJMSCs (100% medium) both in concentrations (30%, 60% of CM-hWJMSCs) based on Tukey HSD post hoc test ($p < 0.05$)

symbol ** present very significant between CM-hWJMSCs (non-induced, inducing IL-2 125 ng/ml, inducing IL-2 250 ng/ml) and non CM-hWJMSCs (100% medium) both in concentrations (30%, 60% of CM-hWJMSCs) based on Tukey HSD post hoc test ($p < 0.01$)

symbol # present significant difference between concentrations 30% and 60% of CM-hWJMSCs based on t-test ($p<0.05$).

Figure 3. Effect of IL-2 inducer and periodic incubation toward NK cells viability

The data are presented as histogram among treatment, this research was conducted in triplicate for each treatment

* symbol present significant difference among inducer concentrations of IL-2 (0, 5 ng/ml, 10 ng/ml) in periodic incubations (day 1, day 2, day 3, day 4) compared with control (non induced),

symbol present significant difference among day 3 and day 4 incubation in inducer concentrations of IL-2 (5 ng/ml, 10 ng/ml) compared with periodic incubations (day 1, day 2) in inducer concentrations of IL-2 (5 ng/ml, 10 ng/ml) based on Tukey Post hoc test ($p<0.05$)

Figure 4. Effect of IL-2 inducer and NK cells number toward breast cancer inhibition

The data are presented as histogram among treatment, this research was conducted in triplicate for each treatment

symbol * present significant between NK (IL-2 induction 0, 5, 10 ng/ml) and non induction in comparison 2:1 (cancer : NK cells) based on Tukey HSD post hoc test ($p < 0.05$)

symbol # present significant difference among cancer and NK cells comparison (1:5, 1:2, 1:1) compared to 2:1 comparison in induction IL-2 (0, 5, 0 ng/ml)

Figure 5. Effect of IL-2 inducer and NK cells number toward IFN-γ, TNF-α, perforin, and granzyme level in co-culture MCF7 and NK cells

The data are presented as histogram among treatment, this research was conducted in triplicate for each treatment

symbol * present significant differences among inducer (IL-2) and NK cells number toward IFN-γ, TNF-α, perforin, and granzyme level compared with control (CM-MCF7)

Figure 6. Effect IL-2 inducer and NK cells number toward IFN-γ, TNF-α, perforin, and granzyme level in co-culture MCF7 and NK cells

The data are presented as histogram among treatment, this research was conducted in triplicate for each treatment

symbol * present significant differences among inducer (IL-2) and NK cells number toward IFN-γ, TNF-α, perforin, and granzyme level compared with control (CM-hWJMSCs)

9.6. Chapter book "Hypoxia in Mesenchymal Stem Cells" telah diterbitkan

V "Hypoxia in Mesenchymal Stem Cell"

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Abstract
Mesenchymal stem cells (MSC) are non-hematopoietic multipotent stem cells with self-renewal properties and ability to diffrentiate into a variety of mesenchymal tissue. This chapter overviews effects of hypoxia on MSCs, makes it promising therapy to various diseases. Cultivation of MSCs under hypoxic condition results variety of outcome that is important to be noted in clinical use. In most studies, hypoxic condition appears to increase proliferation, differentiation and immune regulatory performance of MSCs without affecting its characteristic. Those benefits are therefore utillized in clinicial appication. However, there are also studies that report on negative effects of hypoxia in MSCs such as chromosomal instability. Molecular mechanism of MSCs in hypoxic condition is provided for better understanding which is crucial for further development with better outcome.

Keywords: Mesenchymal Stem Cells, Hypoxia

1. Introduction

These days, stem cell therapy is becoming more believable in treating degenerative diseases compared to conventional medicine. Various diseases such as diabetes, myocardial infarction, spinal cord injury, stroke, and Parkinson's and Alzheimer's diseases have become more prevalent with increasing life expectancy. It has been estimated that in the United States alone, approximately 128 million individuals would benefit from regenerative stem cell therapy during their lifetime. Mesenchymal stem cells (MSCs) have been highly utilized to treat degenerative diseases among other stem cells. These cells are found in tissues such as bone marrow, adipose tissue, umbilical cord, and dental pulp. Self-renewal and multipotency are the key features of MSCs that makes it promising tool. These properties have raised interest on researchers for finding appropriate method to optimize the genetic and environmental factors, which later enchance the biological activities of MSCs.

Many researches have been conducted in the last two decades to study the complex processes in stem cell maintenance. The role of hypoxic conditions (usually 2–9% O_2 concentration) on stem cell biology is very interesting subject due to its beneficial effects. Thus, cultivation of MSCs under hypoxia is currently studied to obtain better understanding, as well as further development to generate better outcome.

2. Mesenchymal Stem Cell

About 130 years ago, German pathologist Conheim proposed the presence of non-hematopoietic stem cells in the bone marrow that contributes to wound healing in numerous peripheral tissues. Later in the early 1970's, Friedenstein and colleagues demonstrated that the rodent bone marrow had fibroblastoid cells with clonogenic potential *in vitro* [1, 2]. In the study, after the non-adherent cells were removed a few hours later, spindle-like cells which were morphologically heterogeneous, appeared to attach to the plastic, and capable to form colonies. These cells could also make bone and reconstitute a hematopoietic microenvironment in subcutaneous transplants. Moreover, they could regenerate heterotopic bone tissue in serial transplants, thus indicated their self-renewal potential. Over the years, many studies have investigated these findings, and found that these cells were also present in the human bone marrow, and could be sub-passaged and differentiated *in vitro* into a variety of the mesenchymal lineages such as osteoblasts, chondrocytes, adipocytes and myoblasts [3-7]. It has been further renamed as "mesenchymal stem cell" or MSC [4].

MSCs or MSC-like cells are also are found in fat, umbilical cord blood, amniotic fluid, placenta, dental pulp, tendons, synovial membrane and skeletal muscle, yet the complete equivalency of such populations remains unclear [8-16]. Characteristic of MSCs according to The International Society for Cell Therapy [17] consists of (1) adherence to plastic in standard culture conditions; (2) expression of the surface molecules CD73, CD90, and CD105 in the absence of CD34, CD45, HLA-DR, CD14 or CD11b, CD79a, or CD19 surface molecules; and (3) an ability for differentiation to osteoblasts, adipocytes, and chondroblasts in vitro. These criteria were established to standardize human MSC isolation but may not apply uniformly to other species. For instance, marker expression and behavior in murine MSCs were different compared to human MSCs [18]. Certain in vivo surface markers may no longer be expressed after explantation, although new markers are obtained during expansion. In study done by Jones et al (2002), MSC uniformly expressed HLA-DR (a marker that should not be expressed on MSCs by the above definition) while also expressing CD90 and CD105, adhering to plastic in culture, and differentiating into osteoblasts, adipocytes, and chondroblasts [19]. Indeed, clear definition of MSCs specific characteristics is difficult to apply in both human and animal models.

3. Hypoxia in Mesenchymal Stem Cell

Numerous *in vitro* studies have been conducted in the last two decades to observe the complex processes in stem cell maintenance. However, the role of physiologically

hypoxic conditions (usually 2–9% O_2 concentration) on stem cell biology received very little attention. O_2 concentration is an environmental factor that plays a vital role on stem cell fate and function [20]. Stem cells are typically cultured under the ambient O_2 concentration without paying attention to the metabolic milieu of the niche in which they normally grow [21]. The effects of different O_2 levels in MSC culture was first studied in 1958, when Cooper *et al*. and Zwartouw & West- wood observed that some cells proliferated more rapidly under low O_2 tension levels compared to normal atmospheric levels [22, 23]. MSCs are present in perivascular niches in close association with blood vessels in virtually all tissues [11, 16, 24] and have been compared to pericytes [25]. Even though MSCs are located close to vascular structures, the different tissues where these stem cells are found exhibit low oxygen tensions [26-29]. Therefore, it is possible that maintaining MSCs in an undifferentiated state may require a hypoxic environment, in addition to other factors.

The higher O_2 concentration might cause environmental stress to the *in vitro* cultured MSCs. Recent studies have presented significant evidences regarding the negative outcome under ambient O_2 concentration on MSCs, including early senescence, longer population doubling time, DNA damage [30, 31], and poor engraftment following transplantation [32, 33]. These have shown the influential effect of O_2 concentration on MSCs biology and raised serious concern over its therapeutic efficiency and biosafety. Thus, the effect of different O_2 concentration on MSCs biology is further discussed based on recent research outcomes.

4. Characteristic of MSCs in Hypoxic Condition

As desribed above, MSC immunophenotype is characterized by the expression of CD73, CD90, CD105, CD106, CD146, and MHC class I molecules, and the absence of markers such as CD45 and CD34 or MHC class II molecules [17]. Many studies suggests that hypoxia has no effect on MSCs characteristic, indicated by surface markers. According to one study by Holzwarth et al., there were no significant differences in the expression of cell surface markers after 14 days of culture at 1% when compared to 20% of O_2 [34]. Referring to study done by Nekanti et al (2010), WJ-MSCs cultured under both hypoxia and normoxia for ten passages, were positive for CD44, CD73, CD90, CD105 and CD166 and negative for CD34, CD45 and HLA-DR and no significant difference between the two populations [35]. These results are also supported by study done by Widowati et al (2015). The surface marker of WJMSCs of P4 and P8 both normoxic and hypoxic 5% O_2 were not significantly different. WJ-MSCs was positive for CD105, CD73, and CD90 and negative for CD34, CD45, CD14, CD19, and HLA-II [36].

Morphology changes are also documented in MSCs under hypoxia. Referring to Nekanti et al (2010), WJ-MSC cultured under hypoxia showed a higher amount of large, smooth cells both at early and late passages, compared to normoxic cultures. The dilation in cell size under hypoxia might be due to a natural response to low oxygen, in which increased surface area enables an increase in oxygen diffusion rate [35].

5. MSCs Proliferation in Hypoxic Condition

Capability for self-renewal is a key feature of stem cells. An increased proliferation rate is necessary for more efficient use of stem cells in regenerative

therapies. Fehrer et al. (2007) demonstrated that bone marrow-derived MSCs (BM-MSCs) cultured in 3% O_2 concentration showed significant increased *in vitro* proliferative lifespan, with approximately 10 additional population doublings (PDs) (28.5 ± 3.8 PD in 20% O_2 and 37.5 ± 3.4 PD in 3% O_2) before reaching senescence compared to cells cultured in the ambient O_2 environment [31]. In addition, early passaged MSCs cultured in hypoxic conditions also exhibit increased proliferative lifespan along with significant difference in population doubling [30]. Furthermore, it is possible to harvest more than 1×10^9 MSCs from the first five passages cultured in 3% O_2, whereas in ambient condition only 2×10^7 cells can be obtained (30). Higher *in vitro* expansion rate in hypoxic conditions has also been reported by other researchers [37-39]. Such *in vitro* culture environment also allows to maintain a higher proportion of rapidly self-renewing MSCs for a longer period of time [40]. Other study showed the increased hypoxic (O_2 2.5%) condition was the best microenvironment for stem cells proliferation compared to normoxic and hypoxic (O_2 5%) for cells at a high passage (P7, P8) [41].

However, various responses of stem cells under hypoxia has been reported [42]. Those differences in cellular responses on hypoxia might be associated with degrees and durations of hypoxia, as well as other cell conditions. Oxygen tension in the stem cell niche for MSCs are suggested to be various from 1 to 7% [43]. A study by Holzwarth *et al*. showed rates of MSCs proliferation was reduced after 7 days of culture under hypoxia at 21%, 5%, 3%, and 1% O_2. In their study, only 1.37% of the cells entered the G2/M phase in hypoxic cultures (1% O_2) after 7 days, compared to 2.50% at hyperoxic cullture (21% O_2). Reduced O_2 concentrations was therefore confirmed to inhibit cell proliferation as indicated by reduced number of cells in the G2/M phase [34].

6. Chromosomal Stability of MSCs in Hypoxic Conditions

Some recent studies have found that hMSCs retained chromosomal stability following long-term culture in vitro [44-46]. Hypoxic environments have shown to increase mutation frequencies in cancer cell lines, and trigger genomic rearrangements [47, 48]. It is suggested that oxygen concentration has a major impact on karyotypic aberration. Referring to study of Ueayama et al (2012), chromosomal instability is associated with repeated cell division. A high frequency of chromosomal abnormality breakpoints in common fragile sites (CFSs) was detected by karyotypic analysis (e.g. 2q33, 7q11, 7q36, 8q22.1, 8q24.1, 11p15.1, 19q13) [49]. Generally, chromosomes have fragile sites that are prone to exhibiting gaps and breaks during metaphase [50], in which chromosome rearrangement occurs in cultured cells. Fragile sites are categorized into two main classes, common and rare, according to their frequency in the population [51]. In Ueyama study, several genes involved in regulation of the cell cycle, transcription and cell adhesion, are located in that region with a frequency of 6%, 5% and 2%, respectively. In particular, the 11p15.5 domain known as an important tumour-suppressor gene region such as TSPAN32 (tetraspanin 32) and TSSC4 (tumour-suppressing subtransferable candidate 4), are present in this region. Alterations in this region have been associated with some neoplasia. It is suggested that the deletion of contiguous genes may induce a multisystem developmental disorder and that these alterations might influence normal functioning and cell survival.

Sex-chromosome aneuploidies was also one of the most observed aberrational karyotypes. Frequency of sex chromosome in cultured lymphocytes was significantly higher in females than in males, and that loss of Y chromosomes correlated with age in

human bone marrow cells [52, 53]. There are several factors influencing karyotypic stability such as hypoxic culture conditions, donor age and multiple passages. Karyotypic aberrations increased with passage number and hMSCs undergo spontaneous transformation with tumorigenic potential, especially in later passages under hypoxic culture conditions in hMSCs of elderly donors [49]. Shortly, monitoring of chromosomal stability in culture expanded hMSCs is required prior to exposure to human beings, in order to detect mutations and potentially immortalized clones and to prevent transplant-associated tumour formation.

7. MSCs Plasticity in Hypoxic Condition

The multilineage potential of MSCs is one of the reasons underlying their use in regenerative medicine [54]. Results of MSC differentiation into other lineages diverse according to several studies [34, 55-56]. Some *in vitro* studies have shown that cultures with low O_2 concentrations stimulated cells to differentiate into adipogenic, osteogenic, or chondrogenic cells. Previous study showed rMSCs cultured in 5% oxygen produced more bone than cells cultured in 20% O_2 throughout their cultivation time, as indicated by increased markers for osteogenesis, including alkaline phosphatase activity, calcium content, and von Kossa staining. These markers was usually elevated above basal levels when cells were switched from control to low oxygen at first passage and decreased for cells switched from low to control oxygen [57]. Hypoxia appears to exert a potent lipogenic effect independent of PPAR-γ2 maturation pathway [58]. The level of differentiated antigen H-2Dd and the number of G2/S/M phase cells increased evidently under 8% O_2 condition. Also, the proportion of wide, flattened, and epithelial-like cells, increased significantly in MSCs. When cultured in adipogenic medium, there was a 5- to 6-fold increase in the number of lipid droplets under hypoxic conditions compared with that in normoxic culture. Oct4 was down-regulated under 8% O_2 condition, but still expressed after adipocyte differentiation in normoxic culture and treated with hypoxia-mimicking agents, cobalt chloride ($CoCl_2$) and deferoxamine mesylate (DFX). These findings indicate hypoxia enhances MSC differentiation and hypoxia and hypoxia mimicking agents generate different effects on MSC differentiation [59].

Conversely, some others have reported suppressive effects of low O_2 tension levels on the plasticity of MSCs. Differentiation capacity into adipogenic progeny was diminished and no osteogenic differentiation was detected at 3% oxygen. In turn, MSCs that had previously been cultured at 3% oxygen could subsequently be stimulated to successfully differentiate at 20% oxygen [31]. Temporary exposure of MSCs to hypoxia resulted in (i) persistent (up to 14 days post exposure) down-regulation of cbfa-1/Runx2, osteocalcin and type I collagen and (ii) permanent (up to 28 days post exposure) up-regulation of osteopontin mRNA expressions [60]. Other study by Widowati *et al* (2015) showed both nor-WJMSCs and hypo-WJMSCs differentiated to osteocytes, chondrocytes, and adipocytes, although there was no significant difference among treatments [36]. Study conducted by Georgi *et al* (2015) showed molecular fingerprints of human MSCs, primary chondrocytes, and MSCs/primary chondrocytes coculture differ when cultured in either normoxic (21% O_2) or hypoxic (2.5% O_2) conditions [61]. In the study, cartilage formation increased in cocultures of MSCs and primary chondrocytes was lost when the cells were cultured under hypoxia which was associated with a decrease in the mRNA expression of the chondrogenic marker SOX9, and FGF-1.

This coincided with a significant decrease in lipids. Lipid profiles of normoxic and hypoxic cultures are different. The improved cartilage formation in co-cultures of MSCs and chondrocytes may employs soluble factors, including small molecules, lipids, or proteins [62]. Lipids such as phospholipids, cholesterol, and diacylglycerols play significant roles in cellular signaling, membrane integrity, and metabolism [63]. Recent study described that short-term changes in sphingolipid metabolism resulted in long-term effects on the chondrogenic phenotype and the stimulation of chondrocytes with acyl ceramidase improves cartilage repair and MSC differentiation [64].

8. Immunomodulatory Effects of MSCs in Hypoxic Condition

One of the key factors of MSC in therapeutics development is their known anti-inflammatory/immunomodulatory properties. Clinical studies showed efficacy of MSC at inhibiting lethal, immune-based condition of graft versus host disease [65-70]. It has been reported that MSCs derived from adipose, bone marrow, and placenta have the capability to recover ischemia injury by increasing vascularization and reducing inflammation in ischemia injured hind limb, lung, heart, and brain [71-73]. Thus, these cells have been used in clinical trials to treat ischemic disease [74]. MSCs produce a broad variety of cytokines, chemokines and growth factors that may potentially be involved in tissue repair. Hypoxia increases the production of several of these factors, altough different response are also noted in few studies. Referring to Chang et al (2013), hypoxic preconditioning enhances the capacity of the secretome obtained from cultured human MSCs to release several of these factors and the therapeutic potential of the cultured MSC secretome in experimental TBI [75].

Treatment with interferon gamma (IFN-γ) is one of the most common mechanisms observed in inflammation-induced MSC activity. This cytokine is usually secreted during inflammatory Th1 immune responses that are associated with autoimmunity mediated by cellular means, such as CD8 T cells and NK cells, which commonly occur in multiple sclerosis, diabetes type 1, and rheumatoid arthritis [76]. Treatment of IFN-γ in MSC has been reported to enhance the immune suppressive activity through stimulation of the enzyme IDO [77-80]. MSC expression of the tryptophan catabolizing enzyme indolamine 2,3 deoxygenase (IDO) was markedly up-regulated under hypoxia [81]. IDO is critical in immune regulation by MSC through induction of T cell anergy [82], and stimulation of T regulatory cells (T-regs) [83, 84].

Moreover, IFN-γ induced secretion of other inhibitors of inflammation by MSCs, including the complement inhibitor Factor H [85], as well as the immune modulatory molecules TGF-β and HGF [86]. At a functional level, Noone *et al*. demonstrated that IFN-γ pre-treatment of MSC resulted in protection of MSCs from NK-mediated killing via upregulation of prostaglandin E (PGE)-2 synthesis [87]. IFN-γ, along with necrosis factor-alpha (TNF-α), IL-1α, and IL-1β, induce Gal-9 in MSC [88]

Another inflammatory mediator known to induce regenerative activities in MSC, is the macrophage-derived cytokine TNF-α. Pre-treatment of TNF-α in MSCs provided superior angiogenic activity *in vitro* indicated by expression of VEGF, and also *in vivo* in an animal model of critical limb ischemia compared to untreated MSCs [89]. In other study, TNF-α pre-conditioning increased proliferation, mobilization, and osteogenic differentiation of MSCs and up-regulated bone morphogenetic protein-2 (BMP-2). Osteogenic differentiation of MSC induced by TNF-α, was partially inhibited after

BMP-2 knockdown by siRNA [90]. Lipopolysaccharide, and toll like receptor (TLR) agonists, as activators of innate immunity, are also responsible in regenerative activity of MSCs by inducing paracrine factors secretion such as VEGF [91]. IFN-γ and TLR also up-regulate the glucocorticoids production which decreases T-cells stimulated by radiotherapy in colonic mucosa [92].

Akiyama et al. (2012) reported that MSCs induced T-cell apoptosis via the Fas-FasL pathway [93]. Telomerase improved immunomodulatory properties of MSCs by upregulating FasL expression [94]. Dental follicle cells and cementoblasts has been reported to trigger apoptosis of ameloblast-lineage cells, as well as Hertwig's epithelial root sheath (HERS)/epithelial rests of Malassez (ERM) cells, via the Fas- FasL pathway during tooth development [95]. FasL regulated the immunomodulatory properties of hGMSCs, which is promoted by hypoxia. However, the underying pathways of such event remains unclear. Further studies regarding he pathways involved in hGMSC-mediated immunomodulation are encouraged.

9. Molecular Mechanism of MSCs in Hypoxic Condition

O_2 concentration in the stem cell niche (usually 2–9% O_2) is considered a driver of cell function (20). Hypoxia plays a vital role in maintaining homeostasis within the body from the early stage of embryonic development. It facilitates proper embryonic development, maintain stem cell pluripotency, induce differentiation, and regulate the signalling of multiple cascades, including angiogenesis [96]. In hypoxic conditions, these functions are regulated by several transcription factors such as hypoxia-inducible factors (HIFs), prolyl-hydroxylases (PHDs), factor-inhibiting HIF-1 (FIH-1), activator protein 1 (AP-1), nuclear factor (NF)- κB, p53, and c-Myc [97]. Although interaction among all of the transcription factors is required for cellular response, HIFs (especially HIF-1) are the key regulators of cellular response to hypoxia [98]. The discovery of HIF-1a by Greg Semenza provided profound insight into the cellular mechanisms that control hypoxic adaptation [99-101].

Generally, under hypoxic conditions, low O_2 level supress the prolylhydroxylation, that leads to HIF-1α accumulation and nuclear translocation [102]. After nuclear translocation, it binds with HIF-1β to form the heterodimer. Then the HIF-1 heterodimer binds to a hypoxia-response element (HRE) in the target genes, associated with coactivators such as CBP/p300, and regulates the transcription **(Figure 1)** of as many as 70 genes involved in metabolism, angiogenesis, invasion/metastasis, and cell fate [103].

In 2007, iPSCs was discovered by Shinya Yamanaka and colleagues and the subsequent identification of the necessary transcriptional programs required to maintain stem cells in a pluripotent state [104, 105]. The measurement of low partial pressures of oxygen in various stem cell niches raise question whether HIF-1a and iPSCs pathways converged. It was described initially in ESCs [106], HSCs [107], NSCs [108], and CSCs [109], that now further expanded to include iPSCs [110]. Remarkably, Yamanaka first reprogrammed fibroblasts to iPSCs using only four transcription factors (Oct4, Sox2, c-Myc, and Klf4) [105] in the same year that Oct4 was shown to be a specific target gene of HIF-2a [111]. The correlation between HIF-2a and Oct4 has been proposed as underlying mechanism of stem cells response to hypoxic conditions in their niche and direct modification of stem cell function by low O_2. HIF-2a expression has recently been investigated in several stem cell lineages, and Oct4 expression is tightly regulated throughout embryogenesis. Loss or even decrease of Oct4 expression leads to differentiation [112]. Oct4 works in concert with Nanog and Sox2 to maintain stem cell

identity and repress genes that promote differentiation [113]. The recent identification of HIF-2a upregulation by Oct4 in CSCs and ESCs underscores the importance of this axis in maintaining stemness in both development and disease.

It is known that phosphorylation of protein kinase B (Akt), a downstream gene of phosphatidylinositol 3-kinase (PI3K) signalling pathway, is an important step in signalling pathways that mediate cell proliferation [114, 115]. In PI3K/Akt pathway, a large number of substrates is phosphorylated, including HIF-1 [116]. Referring to study done by Rosová et al. (2008), the preculture of MSCs in hypoxia prior to injection activated the PI3K/Akt signaling pathway while maintaining their viability and cell cycle rates [117].

Hypoxia mediated MSC differentiation by reducing apoptosis via activating the PI3K/Akt/FoxO pathway. Referring to Wang et al (2010), MSCs underwent apoptosis upon induction for chondrogenic differentiation [118]. Apoptosis has been demonstrated as a general phenomenon that occurs during endochondral differentiation of chondrocytes [119]. One study demonstrated that chondrocytes progression to endochondral ossification employed higher Fas receptor and caspase protein as indicators of apoptosis [120]. Other studies showed that both the Wnt/beta-catenin and Indian hedgehog (Ihh) signalling pathways play important roles in endochondral ossification. Beta-catenin is needed at upstream of Ihh signalling for chondrocyte survival and inhibition of apoptosis [121]. The expression of Sox9, col2a1 and aggrecan in prechondrogenic cells30 and chondrocytes14, are regulated by PI3K/Akt pathway. It has also been demonstrated that PI3K/Akt regulated col2a1 and aggrecan by modulating Sox9 expression and transcriptional activity in nucleus pulposus cells31.

Lee et al (2015) showed novel pathway for hypoxia-induced proliferation and migration in human mesenchymal stem cells that employs HIF-1α, FASN, and mTORC1O. Hypoxia treatment stimulates UCB-hMSC proliferation, along with expression of two lipogenic enzymes: fatty acid synthase (FASN) and stearoyl-CoA desaturase-1 (SCD1). FASN is a key enzyme in UCB-hMSC proliferation and migration. Hypoxia-induced FASN expression was regulated by the HIF-1α/SCAP/SREBP1 pathway. Mammalian target of rapamycin (mTOR) was phosphorylated by hypoxia, whereas inhibition of FASN by cerulenin suppressed hypoxia-induced mTOR phosphorylation, as well as UCB-hMSC proliferation and migration. Hypoxia-induced proliferation and migration are significantly inhibited by RAPTOR small interfering RNA. Hypoxia-induced mTOR also regulate CDK2, CDK4, cyclin D1, cyclin E, and F-actin expression as well as c-myc, p-cofilin, profilin, and Rho GTPase. Moreover, hypoxia-induced FASN stimulates FFA production as well as proliferation and migration. Several studies reported that FAs and FA derivatives inhibited and uncoupled oxidative phosphorylation of various cells [122-124]. Palmitic acid treatment rescues inhibition of mTOR phosphorylation as well as restriction of UCB-hMSC proliferation and migration. Change of cellular metabolite ratios may be another pathway, in addition to the HIF1a/SCAP/SREBP1 pathway, involved in the regulation of lipid metabolism in UCB-hMSCs. Some studies reported that alteration of cellular metabolites ratios, such as NADP/NADPH, by hypoxia has also an important role in the regulation of various stem cell functions such as cell cycle and self-renewal activities [125, 126].

10. Hypoxic MSCs in Clinical Application

MSCs possess anti-inflammatory/immunomodulatory properties which is utilized in therapeutics development. Clinical studies on efficacy of MSCs have been shown to inhibite lethal, immune-based condition of graft versus host disease [65-70]. Morevover, MSCs derived from adipose, bone marrow, and placenta have the capability to recover ischemia injury by increasing vascularization and reducing inflammation in ischemia injured hind limb, lung, heart, and brain [71-73, 127]. These cells have been used in clinical trials to treat ischemic disease [74] and the safety of MSCs has been evaluated [128, 129]. There are several modified approaches which has been proposed to improve the effect of MSCs on ischemia-related disease, such as over expression of angiogenesis-related genes such as bFGF on MSCs (130), combination with other cells such as endothelial cells [84], antioxidants such as melatonin [131], serum deprivation [72], and cell spheroids [132].

From isolation to engraftment, the MSCs usually pass through two different phases, consisting of *in vitro* culture condition (from isolation to transplantation), and *in vivo* or physiological condition (before isolation and after transplantation). At present, most of the expansion procedures of MSCs are performed under ambient O_2 concentration, where cells are exposed to 20% O_2, which is approximately 4–10 times more than the concentration of O_2 in their natural niches [133, 134]. Maintaining genetic stability has been a challenge during *in vitro* expansion of MSCs. Increased rates of aneuploidy, double-stranded DNA breakdown, and faster telomere shortening have been reported for MSCs cultured in ambient condition (30). According to recent review, major causes behind aneuploidy were defective spindle assembly checkpoint, centrosome amplification, and merotelic attachments [135], which is caused by ROS [136]. ROS also acts in acceleration of telomere shortening and DNA breakdown [137, 138]. Correlation between telomere shortening and aneuploidy in embryonic and hepatocellular carcinoma cells has also been reported in recent studies [139, 140]. The higher ROS production due to the increased mitochondrial respiration during expansion of MSCs in ambient O_2 concentration, might be the cause behind genetic instability in them. However, cells undergo anaerobic respiration during hypoxia, which lower the ROS concentration within the cells. This might reduces the DNA damage, telomere shortening, and aneuploidy which in return may increase the biosafety of stem cell-based therapy.

The ability of stem or progenitor cells to home and engraft into target tissues after transplantation is the key to succeed in clinical application. The degree of homing and engraftment of MSCs adult recipients is very low [141-143]. Hypoxic culture conditions may also provide a solution for more efficient engraftment. Recently, early passaged mouse BM-MSCs showed better engraftment than late passaged mouse BM-MSCs in *in vivo* model [144]. In other study, hypoxic preconditioned murine MSCs also enhanced skeletal muscle regeneration and improved blood flow and vascular formation compared to normoxic condition [145]. Hypoxic conditions also cause rapid MSCs growth [30] while maintaining a higher number of rapidly self-renewing cells [40]. Hypoxic environment also upregulated chemokine receptors CXCR4, CXCR7, and CX3CR1 [146, 147], that may facilitate tissue-specific trafficking of MSCs. Thus, sufficient numbers of MSCs with a higher fraction of rapidly self-renewing cells are suggested, and highly expressed chemokine receptors on their surface can be obtained from the early passages of hypoxic cultures, which could increase the efficiency of damaged-tissue-specific migration and engraftment following transplantation.

MSCs cultured under hypoxic conditions also increased in vascular endothelial growth factor receptor 1 (VEGFR1) expression and VEGF- or placental growth factor (PLGF)-dependent migration (Okuyama et al, 2006). Preconditioning with oxygen and combined glucose depletion also increased the survival of stem cell antigen (Sca)-1þ cells via PI3K/Akt-dependent caspase-3 downregulation, and thereby increased the engraftment rate [148]. In addition to the increase in migration and survival, MSCs with hypoxic preconditioning have also been shown to enhance revascularization after transplantation for hind limb ischemia [117]. Therefore, culturing MSCs in hypoxic conditions can also be considered as a solution for tissue-specific engraftment.

Hypoxia-stimulated immune regulation of MSCs has been observed in the situation of allogeneic use of BM-MSCs for stimulation of therapeutic angiogenesis. Recent study showed hypoxia-conditioned BM-MSCs from B6 mice repair limb ischemia of Balb/c mice compared to normoxic MSCs. Engraftment in allogeneic recipients increased by decreasing NK cells cytotoxicity, and the accumulation of host-derived NK cells when transplanted *in vivo*. These allogeneic hypoxia-treated BM-MSCs increased CD31+ endothelial cells and αSMA + and desmin + muscle cells, thereby enhancing angiogenesis and recovering muscle structure. Moreover, anti-NK antibodies along with normoxic MSCs, enhanced angiogenesis and prevented limb amputation in allogeneic recipients with limb ischemia [149].

Some studies have shown that MSCs transplantation contributes to tumour formation *in vivo* [24, 150-151], whereas Furlani et al.(2009) reported that cultured MSCs with spontaneous transformations had no functional effects after intracardiac transplantation [152]. Further studies regarding tumorigenicity and safety of the stem-cell-based products are encouraged. However, complexity of cell therapy requires more standards for advanced medicinal products [153]. Thus, especially in the field of regenerative medicine, concrete and specific standards, and governmental support systems, are necessary to promote their production [153].

11. Perspective

Hypoxic condition has been confirmed to enhance MSCs proliferation, differentiation and immune regulatory performance. However, some studies have also reported opposite and negative effects. Different outcomes in each study raises interest in availability of more appropriate methods for cell cultures which require further study in standardizing the culture of MSCs for use in cell therapy. Optimal conditions for the culture of MSCs have not yet been clearly defined and it is very crucial to precisely determine the effects of hypoxia on MSCs differentiation, proliferation, and morphology, among other aspects. Moreover, hypoxic MSCs-based therapies require a complete understanding of stem cell molecular mechanism. The clarity in stem cell regulation is important for further development such as periodic monitoring of chromosomal stability in culture prior to exposure to human to detect mutations and to prevent transplant-associated tumour formation, and also genetic engineering of physiology of MSCs to acquire better outcome.

Conclusion

The growing interest in the potential application of MSCs in regenerative medicine were followed by the several studies measuring the effects of low O_2 levels on the behavior

and function of MSCs. Hypoxic condition appears to enhance MSCs proliferation, differentiation and immune regulatory performance in damaged tissues without affecting its characteristic. However, there are also studies that report on negative effects of hypoxia in MSCs.

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Lampiran 9.7. Telah terbit terdaftar hak paten "IL-2 meningkatkan sitotosik sel NK terhadap sel kanker payudara"

KEMENTERIAN HUKUM DAN HAK ASASI MANUSIA R.I
DIREKTORAT JENDERAL HAK KEKAYAAN INTELEKTUAL

Global Tangkap 4

Formulir Permohonan Paten

Diisi oleh petugas
Tanggal Pengajuan : 28 SEP 2016
Nomor permohonan : S11 2016 000.006

Dengan ini saya/kami [1] : (71) Nama : Wahyu Widowati Alamat [2] : Fakultas Kedokteran, Universitas Kristen Maranatha, Jl. Surya Sumantri No. 63, Sukawarna, Sukajadi, Kota Bandung, Jawa Barat 40164 Warga Negara : Indonesia (WNI) Telepon : 081910040010 NPWP : 25.468.653.8-428000	
Mengajukan permohonan paten / ~~paten sederhana~~	[✓]
Yang merupakan permohonan paten Internasional/PCT dengan nomor :	
(74) melalui/tidak melalui *) Konsultan Paten Nama Badan Hukum [3] : Alamat Badan Hukum [2] : Nama Konsultan Paten : Alamat [2] : Nomor Konsultan Paten : Telepon / fax :	[✓]
(54) dengan judul invensi : IL-2 meningkatkan sitotoksik sel NK terhadap sel kanker payudara	[✓]
Permohonan Paten ini merupakan pecahan dari permohonan paten nomor :	[✓]

Deskripsi

IL-2 menginduksi sitotoksik sel NK terhadap sel kanker payudara

Bidang Teknik Invensi

Invensi ini berhubungan dengan suatu proses peningkatan proliferasi dan sitotoksitas NK terhadap sel kanker payudara, MCF-7. Lebih khusus lagi, proses tersebut diinduksi IL2 (IL2-NK).

Latar Belakang Invensi

Kanker muncul sebagai penyakit kronik penyebab kematian nomor 2 setelah penyakit kardiovaskuler (WHO, 2015). Berdasarkan hasil estimasi GLOBOCAN *International Agency for Research on Cancer* tahun 2012, terjadi 14,1 juta kasus baru dan 8.2 juta kematian yang disebabkan oleh kanker. Kanker yang paling sering terjadi adalah kanker paru-paru (1.82 juta), kanker payudara (1.67 juta), dan kanker kolorektal (1.36 juta), sedangkan kematian yang terjadi paling tinggi disebabkan oleh kanker paru-paru (1.6 juta kematian), kanker hati (745,000 kematian), dan kanker perut (723,000 kematian) (Ferlay *et al*., 2015).

Berdasarkan data Riset Kesehatan Dasar (Riskesdas) tahun 2013, prevalensi tumor/kanker di Indonesia adalah 1,4 per 1000 penduduk, atau sekitar 330.000 orang menderita kanker. Kanker tertinggi di Indonesia pada perempuan adalah kanker payudara dan kanker leher rahim. Berdasarkan data Sistem Informasi Rumah Sakit 2010, kasus rawat inap kanker payudara 12.014 kasus (28,7%), kanker leher rahim 5.349 kasus (12,8%) (Kemenkes RI, 2014).

Hingga saat ini, terapi kanker payudara dilakukan dengan pembedahan, radioterapi, terapi hormon, kemoterapi, imunoterapi dan hipertermia. (Singletary, 2008; Lippman, 2008). Berbagai usaha terapi baik kuratif maupun paliatif termasuk pencarian dari berbagai senyawa yang berasal dari bahan alam sebagai bahan antikanker, belum menemukan hasil yang memuaskan. Sistem imun dapat memberikan respon terhadap pertumbuhan kanker, namun banyak kanker yang tetap dapat tumbuh dikarenakan *immune surveillance* terhadap kanker tidak efektif, untuk itu diperlukan meningkatkan *surveillance* terhadap kanker, menginduksi resistensi terhadap sisa sel ganas dan kekambuhan kanker sebagai langkah terapi untuk kanker. Sel NK sebagai imunomodulator dapat diaktivasi menggunakan interleukin menjadi *lymphokine-activated killer cells* (LAK). Aktivitas sel NK sebagai imunomodulator dan antikanker dapat ditingkatkan dengan induksi menggunakan interleukin (IL-2, IL-12, IL15, IL-18) (Cheng *et al*., 2013).

Terdapat beberapa metode propagasi sel NK yang telah ada sebelumnya. Frias et al. (Exp Hematol 2008; 36: 61-68) menumbuhkan progenitor NK (CD7.sup.+CD34.sup.-Lin.sup.-CD56.sup.-) yang dipilih dari cord blood pada lapisan stroma dengan serum-free medium, menginduksi diferensiasi NK dengan SCF, IL-7, IL-15, FL dan IL-2, serta meningkatkan proliferasi dan sitotoksis sel NK. Harada et al. (Exp Hematol. 2004; 32:614-21) menumbuhkan sel NK pada sel tumor Wilm's. Penelusuran yang dilakukan melalui http://www.uspto.gov/patft/index.html menunjukkan Waldmann et al. (US20070160578) meningkatkan proliferasi sel NK dan CD8-T dari darah blood, bone marrow atau spleen cells pada kultur menggunakan kompleks IL-15/R-ligand

activator, untuk menurunkan produksi sitokin yang tidak diinginkan. Campana et al. (US20090011498) melakukan kultur ex-vivo dan aktivasi sel NK, untuk transplantasi, dengan sel leukemia yang mengekspresikan IL-15 dan 4-1BB, dan sedikit mengekspresikan atau bahkan tidak mengekspresikan MHC-I atau II. Childs et al. (US20090104170) juga melakukan kultur ex-vivo untuk melihat proliferasi, dan aktivasi sel NK dengan melakukan ko-kultur dengan lymphoblastoid yang ditransform irradiated-EBV, dengan induksi IL-2. Tsai menggunakan pendekatan lain (US20070048290) dan menghasilkan continuous sel NK dari stem sel hematopoietik dengan kultur ex-vivo progenitor immortalized NK dengan irradiated 3T3-derived OP-9S sel, untuk aplikasi terapi. Invensi terbaru oleh Peled et al (US 20140023626 A1)menunjukkan metode kutur ex-vivo sel NK, khususnya metode untuk meningkatkan propagasi dan fungsi sel NK dengan aryl hydrocarbon antagonist dalam kombinasi dengan sitokin yang mengatur proliferasi sel NK.

Invensi ini melakukan ko-kultur NK yang diinduksi IL-2 (NK-IL2) dan sel kanker payudara, MCF-7. Dari invensi ini diketahui bahwa induksi IL-2 pada sel NK meningkatkan proliferasi an sitotoksitas sel NK terhadap sel MCF-7. *Conditioned Medium* (CM) hasil kokultur sel NK yang telah diinduksi IL-2 mengandung TNF-α, IFN-γ, peforin, granzim lebih tinggi dibandingkan NK yang tidak diinduksi. Induksi IL-2 sel NK dapat meningkatkan produksi TNF-α, IFN-γ, perforin, granzim pada CM kokultur sel NK dan MCF-7.

Uraian Singkat Invensi

Invensi ini bertujuan memperbaiki aktivitas antitumor sel NK melalui aktivasi sel NK dan mempromosikan proliferasi sel NK dengan induksi IL-2. Tujuan akhir dari invensi tersebut telah dicapai dengan diperolehnya sel NK yang memiliki proliferasi sel dan sitotoksik yang tinggi pada sel kanker.

Invensi dilakukan dalam beberapa tahap yaitu kultur sel NK diinduksi IL-2, pengukuran proliferasi sel NK, dilanjutkan kokultur sel NK yang telah diinduksi IL-2 dan sel kanker payudara (MCF7) untuk selanjutnya diukur kandungan IFN-γ, TNF-α, peforin, granzim.

Uraian Singkat Gambar

Gambar-gambar yang menyertai invensi ini, dicakup untuk memberikan pemahaman lebih lanjut dari invensi ini dan digabungkan disini dan merupakan bagian dari aplikasi ini, perwujudan-perwujudan ilustrasi dari invensi ini dan bersama dengan penjelasan ini menerangkan prinsip dari invensi ini. Dalam gambar-gambar:

Gambar 1 mengilustrasikan pengaruh induksi IL-2 terhadap viabilitas (%) sel NK;

Gambar 2 mengilustrasikan pengaruh NK-IL-2 terhadap inhibisi sel kanker payudara sekali-tulis dari Gambar 1 menurut invensi ini;

Gambar 3 mengilustrasikan pengaruh induksi IL-2 dan jumlah sel NK terhadap kadar TNFα, IFN-γ, perforin, granzim CM-(MCF7+NK)sekali-tulis dari Gambar 1 menurut invensi ini;

Uraian Lengkap Invensi

Invensi ini bertujuan memperbaiki aktivitas antitumor sel NK melalui aktivasi sel NK dan mempromosikan proliferasi sel NK dalam tubuh pasien dengan induksi IL-2. Tujuan akhir dari invensi tersebut telah dicapai dengan diperolehnya sel NK yang

memiliki proliferasi sel dan sitotoksik yang tinggi, dan dapat menginduksi apoptosis pada sel kanker.

Penelitian didahului dengan kultur sel NK diinduksi IL-2 dan diuji terhadap proliferasi sel NK, dilanjutkan kokultur sel NK yang telah diinduksi IL-2 dan sel kanker payudara (MCF7) untuk selanjutnya diukur kandungan IFN-γ, TNF-α, peforin, dan granzim.

Kultur sel NK diinduksi IL-2 diakukan dengan menanam sel NK (10.000sel/*well*) ke dalam substrat 96 well plate menggunakan medium *free serum* (*Alpha Minimum Essensial Medium without ribonuclease and deoxirybonucleoside with 2 mM glutamine* +1.5 g/L *sodium bicarbonate* + 0.2 mM inositol+ 0.1 mM 2-merchaptoethanol + 0.02 mM *folic acid* + 1% PenStrep). Sel selanjutnya diberikan perlakuan induksi IL-2 dengan konsentrasi 5 ng/ml dan 10 ng/ml, kemudian diinkubasi selama 24 jam, 48 jam, 72 jam dan 96 jam pada suhu 37^0 C, 5% CO_2, dimana setiap harinya diberikan penambahan perlakuan. Selanjutnya ke dalam sel di tambahkan 20 µl *reagen cell titer 96 ® Aqueouse one solution assay* dan sel diinkubasi selama 3 jam. Absorbansi dibaca pada panjang gelombang 490 nm.

Kemudian dilakukan pengujian sitotoksik medium terkondisi ko-kultur sel NK-IL2 pada sel MCF-7. Sel ditanam ke dalam substrat 96-*well plate* dengan densitas 5x 10^3 sel/*well* menggunakan 100 µl medium lengkap (MCF-7 : DMEM + 10% FBS + 1% PenStrep). Sel kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Medium sel lama kemudian dibuang, sel dicuci menggunakan PBS. Medium sel kemudian diganti menggunakan medium *free serum* (MCF-7 : DMEM + 1% PenStrep) untuk kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Sel MCF-7 selanjutnya diberikan perlakuan menggunakan medium terkondisi (CM) hasil ko kultur NK-IL2 dengan MCF-7 dengan konsentrasi 30% dan 60%. Sel kemudian diinkubasi pada suhu 37^0 C, 5% CO_2 selama 24 jam. Selanjutnya ke dalam sel di tambahkan 20 µl reagen *cell titer 96 ® Aqueouse one solution assay* dan sel diinkubasi selama 3 jam. Absorbansi dibaca pada panjang gelombang 490 nm.

Pengukuran kadar TNF-α dan IFN-γ dari medium terkondisi (CM) hasil kokultur sel NK-IL2 pada sel MCF-7. Pada setiap *well* dimasukkan sebanyak 100 µl *capture antibody*. Selanjutnya well diinkubasi semalam pada suhu 4^0C. Plate dicuci sebanyak 4 kali menggunakan 200 µl *wash buffer.* Selanjutnya ditambahkan 200 *µl assay diluent* ke dalam setiap *well. Plate* kemudian disegel dan diinkubasi selama 1 jam dengan kecepatan 200 rpm pada suhu ruang. Plate kemudian dicuci 4 kali menggunakan 200 µl wash buffer. Setiap masing-masing plate ditambahkan 100 µl standar pada *well* standard an 100 µl sampel pada *well* sampel. *Plate* kemudian diinkubasi selama 2 jam dengan kecepatan 200 rpm pada suhu ruang. *Plate* dicuci sebanyak 4 kali menggunakan 200 µl *wash buffer.* Sebanyak 100 µl *detection antibody* ditambahkan ke dalam setiap *well. Plate* kemudian disegel dan diinkubasi selama 1 jam dengan kecepatan 200 rpm pada suhu ruang. *Plate* dicuci sebanyak 4 kali menggunakan 200 µl *wash buffer.* Sebanyak 100 µl HRP-Avidin ditambahkan ke dalam setiap *well. Plate* kemudian disegel dan diinkubasi selama 30 menit dengan kecepatan 200 rpm pada suhu ruang. *Plate* dicuci sebanyak 5 kali menggunakan 200 µl *wash buffer.* Selanjutnya dimasukan 100 µl TMB sustrat ke dalam setiap *well* dan diinkubasi selama 15-30 menit dalam keadaan gelap. Pada tahap terakhir ditambahkan 100 µl *stop solution.* Absorbansi plate kemudian dibaca pada panjang gelombang 450 nm.

Untuk pengukuran kadar perforin dan granzim dari medium terkondisi (CM), setiap masing-masing plate ditambahkan 100 µl standar pada *well* standard dan 100 µl

sampel pada *well* sampel. *Plate* kemudian disegel dan diinkubasi selama 90 menit pada suhu 37^0C. Segel *plate* kemudian dibuka, sampel dibuang dan tanpa proses pencucian langsung ditambahkan ke dalam masing-masing *well* 100 µl *Biotinylated Detection Ab*. *Plate* kemudian disegel dan diinkubasi selama 60 menit pada suhu 37^0C. *Plate* dicuci sebanyak 3 kali menggunakan 300 µl *wash buffer*. Sebanyak 100 µl HRP-Conjugate ditambahkan ke dalam setiap *well*. *Plate* kemudian disegel dan diinkubasi selama 30 menit pada suhu 37^0C. *Plate* dicuci sebanyak 3 kali menggunakan 300 µl *wash buffer*. Selanjutnya dimasukan 90 µl TMB substrat ke dalam setiap *well* dan diinkubasi selama 15-30 menit dalam keadaan gelap. Pada tahap terakhir ditambahkan 50 µl *stop solution*. Absorbansi plate kemudian dibaca pada panjang gelombang 450 nm.

Berdasarkan Gambar 1, induksi IL-2 meningkatkan viabilitas sel NK. Semakin lama inkubasi sel NK semakin meningkatkan viabilitas. Sel NK yng diinduksi IL-2 10 ng/ml (IL2-NK 10 ng./ml) menunjukka viabilitas sel NK paling tinggi dari hari 1-hari 4.

Dapat dilihat di Gambar 2, sel NK yang diinduksi IL-2 memiliki inhibisi terhadap sel kanker MCF-7. Inhibisi terbesar diperoleh pada rasio ko-kultur MCF-7:NK (1:5) pada konsentrasi 5 ng/mL sebesar 101,42 %.

Berdasarkan hasil penelitian kadar IFN-γ (Gambar 3) menunjukkan bahwa sel tunggal sel kanker MCF-7 mensekresikan IFN-γ (1,83 pg/ml), kokultur sel MCF-7 dan sel NK meningkatkan kadar IFN-γ. Perbandingan jumlah sel NK mempengaruhi kadar IFN-γ, jumlah sel NK 5 kali atau perbandingan sel NK 1:5 menunjukkan kadar IFN-γ lebih tinggi dibanding jumlah sel NK dengan perbandingan 1:1. Kadar IFN-γ tertinggi adalah kokultur sel MCF-7 dan sel NK yang diinduksi IL-2, adalah MCF-7 : NK (1:5) sebesar 35,72 pg/ml.

Berdasarkan hasil penelitian kadar TNF-α (Gambar 3) menunjukkan bahwa sel tunggal sel kanker MCF-7 mensekresikan TNF-α (1,98 pg/ml), kokultur sel MCF-7 dan sel NK meningkatkan kadar TNF-α. Perbandingan jumlah sel NK mempengaruhi kadar TNF-α, jumlah sel NK 5 kali atau perbandingn sel NK 1:5 menunjukkan kadar TNF-α lebih tinggi dibanding jumlah sel NK dengan perbandingan 1:1. Kadar TNF-α tertinggi adalah kokultur sel MCF-7 dan sel NK yang diinduksi IL-2, adalah MCF-7 : NK (1:5) sebesar 37,44 pg/ml.

Berdasarkan hasil penelitian kadar perforin (Gambar 3) menunjukkan bahwa sel tunggal sel kanker MCF-7 tidak mensekresikan perforin, kokultur sel MCF-7 dan sel NK meningkatkan kadar perforin. Perbandingan jumlah sel NK mempengaruhi kadar perforin, jumlah sel NK 5 kali atau perbandingn sel NK 1:5 menunjukkan kadar perforin lebih tinggi dibanding jumlah sel NK dengan perbandingan 1:1. Kadar perforin tertinggi adalah kokultur sel MCF-7 dan sel NK yang diinduksi IL-2, adalah MCF-7 : NK (1:5) sebesar 0,23 pg/ml.

Berdasarkan hasil penelitian kadar granzim (Gambar 3) menunjukkan bahwa sel tunggal sel kanker MCF-7 tidak mensekresikan granzim, kokultur sel MCF-7 dan sel NK meningkatkan kadar granzim. Perbandingan jumlah sel NK mempengaruhi kadar granzim, jumlah sel NK 5 kali atau perbandingn sel NK 1:5 menunjukkan kadar granzim lebih tinggi dibanding jumlah sel NK dengan perbandingan 1:1. Kadar perforin tertinggi adalah kokultur sel MCF-7 dan sel NK yang diinduksi IL-2, adalah MCF-7 : NK (1:5) sebesar 149,00 pg/ml.

Kegunaan dalam Industri

Dalam terapi kanker payudara, pemilihan jenis terapi sangat tergantung pada stadium kanker payudara sehingga pemilihan terapi setiap stadium kanker berbeda-beda (Lippman, 2008). Terapi untuk kanker yang diterapkan di Indonesia meliputi radioterapi (70%), bedah (20-25%) dan kemoterapi (5-10%) (Didit & Rukmini, 2012)

Jenis terapi kanker antara lain pembedahan memberikan hasil cukup efektif dan kemoterapi merupakan obat-obatan yang memiliki kemampuan sebagai antikanker (Fulda, 2011). Beberapa kekurangan pada terapi pembedahan diantaranya biaya yang relatif mahal, risiko infeksi saat operasi pembedahan, juga keluhan pasien sehubungan dengan estetika (Ruppel & Cole, 2007). Kemoterapi dapat meningkatkan harapan hidup secara signifikan kepada pasien kanker payudara. Kemoterapi bersifat sitotoksik terhadap sel kanker. Obat kemoterapi diberikan secara oral dan parenteral sehingga agen kemoterapi dapat mencapai lokasi kanker yang sulit dicapai dengan pembedahan serta mampu mencapai lokasi metastasis kanker payudara (Findlay *et al.*, 2008). Kemoterapeutik yang digunakan saat ini untuk kanker adalah sitostatika yang bekerja memusnahkan dan merusak sel kanker, yang bekerja kurang spesifik yang akan menimbulkan efek samping antara lain: 1). supresi sumsum tulang yang mengarah pada terjadinya neutropenia, anemia, trombositopenia; 2). kerusakan folikel rambut yang mengarah pada kerontokan rambut (*alopecia*); 3). induksi apoptosis pada sel kripta traktus gastrointestinal yang mengarah pada diare dan ulkus pada mukosa mulut (*mucositis*) (Tjay & Rahadja, 2003; Sikic, 2008). Efek kemoterapi berupa gejala gastrointestinal seperti mual, muntah, kehilangan berat badan, perubahan rasa, konstipasi, diare, *fatigue*, perubahan emosi, dan perubahan pada sistem saraf (Azim, 2011). Selain hal tersebut, terdapat juga toksisitas yang berkaitan dengan golongan obat tertentu, misalnya *anthracycline* yang dapat menyebabkan kerusakan kumulatif pada jantung, cisplatin dapat menyebabkan kerusakan ginjal, dan organ yang lainnya (Tjay and Rahadja, 2003). Frekuensi pemberian kemoterapi dapat menimbulkan beberapa efek yang dapat memperburuk status fungsional pasien.

Sistem imun dapat memberikan respon terhadap pertumbuhan kanker, namun banyak kanker yang tetap dapat tumbuh dikarenakan *immune surveillance* terhadap kanker tidak efektif, untuk itu diperlukan meningkatkan *surveillance* terhadap kanker, menginduksi resistensi terhadap sisa sel ganas dan kekambuhan kanker sebagai langkah terapi untuk kanker. Sel NK sebagai imunomodulator dapat diaktivasi menggunakan interleukin menjadi *lymphokine-activated killer cells* (LAK). Pada invensi ini, aktivitas sel NK sebagai imunomodulator dan antikanker dapat ditingkatkan dengan induksi IL-2 yang diharapkan dapat digunakan dalam pengembangan terapi kanker, khususnya terapi kanker payudara.

Klaim

1. Metode ko-kultur NK yang diinduksi IL-2 (NK-IL2)dan MCF-7 dalam meningkatkan proliferasi sel NK, dan juga sitoksitas sel NK terhadap sel MCF7. Metode ini terdiri dari: (a) induksi IL-2 pada sel NK, (b) efek IL-2 terhadap viabilitas sel NK (c) ko-kultur MCF-7 dan NK-IL2, (d) efek ko-kultur MCF7 dan NK-IL-2 terhadap sitotoksik MCF7,(e)efek ko-kultur MCF7 dan NK-IL-2 terhadap kadar TNF-a, IFN, peforin, dan granzim

2. Metode ko-kultur NK yang diinduksi IL-2 (NK-IL2)dan MCF-7 seperti pada klaim 1 dimana menggunakan variasi rasio medium untuk MCF7 (DMEM:NK medium) 1:1, 7:3, 3:7 (v/v), dan untuk sel NK (DMEM:NK medium) 1:1, 7:3, 3:7 (v/v).
3. Metode ko-kultur NK yang diinduksi IL-2 (NK-IL2)dan MCF-7 seperti pada klaim 1 dimana sel NK ($1x10^6$/well) diinduksi IL-2 dengan konsentrasi 5, 10 ng/ml
4. Metode ko-kultur NK yang diinduksi IL-2 (NK-IL2)dan MCF-7 seperti pada klaim 1 dimana MCF-7 diberi sel NK dengan rasio MCF7:NK 1 : 5 (10.000 : 50.000), 1: 2 (10.000 : 20.000), 1 : 1 (10.000 : 10.000) and 2 : 1 (10.000 : 5.000).
5. Metode ko-kultur NK yang diinduksi IL-2 (NK-IL2)dan MCF-7 seperti pada klaim 1 dimana sel MCF-7 diberikan perlakuan menggunakan medium terkondisi (CM) hasil ko kultur NK-IL2 dengan MCF-7 dengan konsentrasi 30% dan 60%.
6. Metode ko-kultur NK yang diinduksi IL-2 (NK-IL2)dan MCF-7 seperti pada klaim 1 dimana sel NK yang diinduksi IL-2 memiliki viabilitas yang tinggi
7. Metode ko-kultur NK yang diinduksi IL-2 (NK-IL2)dan MCF-7 seperti pada klaim 1 dimana sel NK memiliki sitotoksik terhadap MCF-7 dengan aktivitas tertinggi pada ratio MCF7:NK 1:5.
8. Metode ko-kultur NK yang diinduksi IL-2 (NK-IL2)dan MCF-7 seperti pada klaim 1 dimana NK yang diinduksi IL-2 menghasilkan konsentrasi TNF-α, IFN-γ, perforin, an granzim yang tinggi

Abstrak

IL-2 menginduksi sitotoksik sel NK terhadap sel kanker payudara

Invensi ini melakukan ko-kultur NK yang diinduksi IL-2 (NK-IL2) dan sel kanker payudara, MCF-7. Invensi dilakukan dalam beberapa tahap antara lain kultur sel NK yang diinduksi IL-2, pengukuran proliferasi sel NK yang diinduksi IL-2, dilanjutkan kokultur sel NK yang telah diinduksi IL-2 dan sel kanker payudara (MCF7) untuk selanjutnya diukur kandungan IFN-γ, TNF-α, peforin, granzim. Dari invensi ini diketahui bahwa induksi IL-2 pada sel NK meningkatkan viabilitas dan sitotoksitas sel NK terhadap sel MCF-7. *Conditioned Medium* (CM) hasil kokultur sel NK yang telah diinduksi IL-2 mengandung TNF-α, IFN-γ, peforin, granzim lebih tinggi dibandingkan NK yang tidak diinduksi IL-2. Induksi IL-2 pada sel NK dapat meningkatkan produksi TNF-α, IFN-γ, perforin, granzim pada CM kokultur sel NK dan MCF-7.

Invensi ini diharapkan dapat memperbaiki aktivitas antitumor sel NK melalui aktivasi sel NK dengan induksi IL-2 sehingga diperoleh sel NK yang memiliki viabilitas sel dan sitotoksik yang tinggi pada sel kanker.

Gambar

Gambar 1. Pengaruh induksi IL-2 terhadap viabiliats (%) sel NK

Konsentrasi induksi IL pada sel NK	Viabilitas sel NK (%)			
	Waktu inkubasi (hari 1)	Waktu inkubasi (hari 2)	Waktu inkubasi (hari 3)	Waktu inkubasi (hari 4)
Kontrol (NK)	100.00±13.16 ^{abA}	100.00±4.17 ^{abA}	100.00±0.46 ^{aA}	100.00±6.15 aA
IL2-NK (5 ng/ml)	104.14±12.49 ^{abA}	109.66±9.12 ^{abcAB}	134.50±16.67 ^{bcBC}	143.13±3.20 ^{bcC}
IL2-NK (10 ng/ml)	122.67±9.36 ^{bA}	129.12±0.39 ^{cA}	152.69±11.63 ^{cB}	153.16±4.21 ^{cB}

Data rata-rata dan standard deviasi hasil penelitian viabilitas sel NK (%) dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar konsentrasi dan jenis interleukin (IL-2) pada setiap waktu inkubasi (hari 1, 2, 3, 4), perbedaan huruf besar pada baris yang sama (antar waktu inkubasi (hari 1,2,3,4)) menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

Gambar 2. Efek NK-IL2 terhadap inhibisi sel kanker

Perlakuan	Rasio sel kanker dan NK			
	(1:5)	(1:2)	(1:1)	(2:1)
MCF7				
Untreated	85.01±2.65 aD	65.16±4.21 aC	42.19±5.60 aB	16.79±1.08 aA
IL-2 (5 ng/ml)	101.42±1.26 aD	78.22±1.91 aC	65.02±2.23 aB	35.71±4.85 bcA
IL-2 (10 ng/ml)	98.79±4.20 aD	77.74±9.39 aC	58.72±1.86 aB	37.64±1.70 cA

Data inihibisi sel (%) ditampilkan dalam rata-rata ±standard deviasi. Perbedaan huruf kecil pada kolum yang sama (antar rasio sel kanker dan NK pada MCF-7), dan perbedaan huruf besar pada

kolum yang sama (antar rasio sel kanker dan NK terhadap konsentrasi IL-2 pada MCF-7) merupakan indikator perbedaan signifikan ($p < 0.05$) dengan uji Tukey.

Gambar 3. Pengaruh induksi IL-2 dan jumlah sel NK terhadap kadar TNFα, IFN-γ, perforin, granzim CM-(MCF7+NK)

Konsentrasi induksi IL pada sel NK, perbandingan jumlah sel NK	Kadar			
	IFN-γ (pg/ml)	TNF-α (pg/ml)	Perforin (ng/ml)	Granzim (pg/ml)
CM-MCF7	1,83±0,33 [a]	1,98±0,24 [a]	0,00±0,00 [a]	0,00±0,00 [a]
CM-MCF7 + NK(1:1)	14,50±3,93 [b]	5,24±0,28 [a]	0,04±0,01 [ab]	29,00±4,36 [b]
CM-MCF7 + NK(1:5)	32,28±1,35 [c]	13,85±2,79 [b]	0,11±0,01 [bc]	55,67±12,50 [b]
CM-MCF7 + IL2-NK(1:1)	17,39±3,56 [b]	15,04±0,21 [bc]	0,16±0,04 [cde]	87,00±11,79 [c]
CM-MCF7 + IL2-NK (1:5)	35,72±0,38 [c]	37,44±1,47 [d]	0,23±0,04 [def]	149,00±7,21 [d]

Data rata-rata dan standard deviasi hasil penelitian kadar IFN-γ, TNF-α, perforin, granzim dilakukan sebanyak 3 kali untuk masing-masing perlakuan. Perbedaan huruf kecil pada kolom yang sama (antar konsentrasi dan jenis interleukin (IL-2)menunjukkan berbeda nyata dengan nilai $p < 0.05$ berdasarkan hasil uji Tukey HSD post hoc test.

Lampiran 9.8. Draft buku "Pemanfaatan Mesenchymal Stem Cell pada Terapi Kanker: dasar teori dan strategi aplikasi klinis" telah didaftarkan pada Penerbit Erlangga

Pada kesempatan kali ini saya mewakili tim penulis yang baru kembali menyiapkan draft naskah buku dengan judul tentative: **"Pemanfaatan Mesenchymal Stem Cell pada Terapi Kanker: dasar teori dan strategi aplikasi klinis"**. Topik ini masih sangat baru sehingga buku ini nantinya dapat menjadi rujukan bagi peneliti, mahasiswa maupun klinisi yang tertarik untuk mempelajari potensi MSC untuk terapi kanker.

Buku ini kami susun dalam 7 bab, dengan gambaran daftar isi sebagai berikut:

Buku ini disusun oleh peneliti dan klinisi yang berpengalaman dalam penelitian MSC baik in vitro maupun in vivo berdasarkan referensi publikasi ilmiah terbaru, sehingga dapat menghadirkan informasi teraktual. Topik ini juga mash sangat baru dan sedang menjadi trending topic di luar negeri, pada sesi seminar internasional tentang stem cell biasanya ada topik khusus tentang MSC & Cancer Therapy.

Demikian informasi yang bisa kami sampaikan, apabila ada yang kurang jelas dapat kita diskusikan kembali. Draft naskah buku akan kami kirimkan atau berikan langsung setelah ada kesepakatan kerjasama dengan penerbit Erlangga.
Terima kasih atas kerjasamanya.
Salam,

Lampiran 10. Biodata Peneliti

Lampiran 10.1. Ketua Peneliti

A. Identitas Diri

1	Nama Lengkap (dengan gelar)	:	Dr. Wahyu Widowati, M.Si L/**P**√
2	Jabatan Fungsional	:	Lektor
3	NIK/NIP	:	111122
4	Tempat dan Tanggal Lahir	:	Malang, 17 April 1960
5	Alamat Rumah	:	Istana Regensi 2/E 10 Bandung 40173
6	Nomor Telp	:	0226078392
7	Nomor HP	:	081910040010 082118466433
8	Alamat Kantor	:	Jl. Prof. drg. Suria Sumantri MPH No. 65 Bandung 40164
9	No. Telepon/Fax	;	022-2012186/022-2017621
10	Alamat surel	:	wahyu_w60@yahoo.com
11	Bidang Keilmuan	:	1. Toksikologi 2. Biomedik

B. Riwayat Pendidikan

	S1	S2	S3	S3
Nama Perguruan Tinggi	Universitas Brawijaya	Universitas Gadjah Mada	Universitas Padjadjaran	Fakultas Kedokteran Universitas Brawijaya
Bidang Ilmu	Toksikologi	Toksikologi	Toksikologi Antioksidan	Biomedik
Tahun masuk-lulus	1979-1983	1992-1994	2001-2004	2013

C. PENGALAMAN PENELITIAN dalam 5 Tahun Terakhir

No	Tahun	Judul Penelitian	Pendanaan	
			Sumber	Jumlah (Juta Rp)
1	2009	Ekstrak dan Infusa Ramuan *Curcuma mangga* Val, *Piper betle* L., *Catharanthus roseus* [L] G.Don , *Dendrophtoe petandra* L. Dalam Mencegah dan Menghambat Keganasan Kanker Payudara (*Breast Cancer*)	(Hibah Bersaing DIKTI 2009)	17,5
2	2009	Potensi Ekstrak Berbagai Tanaman Rempah Terhadap Proliferasi dan Diferensiasi Sel Punca Yang Dikultur Menjadi Sel Progenitor Endotelial	(Hibah Bersaing DIKTI 2009)	47,5
3	2009	Peningkatan proliferasi dan diferensiasi sel punca dalam kultur progenitor endotelial dengan penambahan antioksidan SOD (Super Oxide Dismutase) ekstrak melon	(Program Insentif Ristek Kapasitas Produksi 2010)	490

4	2010	Peningkatan proliferasi dan diferensiasi sel punca dalam kultur progenitor endotelial dengan penambahan antioksidan SOD (Super Oxide Dismutase) ekstrak melon	(Program Insentif Ristek Kapasitas Produksi 2010)	475
5	2010	Ekstrak dan Infusa Ramuan *Curcuma mangga* Val, *Piper betle* L., *Catharanthus roseus* [L] G.Don , *Dendrophtoe petandra* L. Dalam Mencegah dan Menghambat Keganasan Kanker Payudara (*Breast Cancer*)	(Hibah Bersaing DIKTI 2010)	46,65
6	2011	Potensi Senyawa Golongan Flavonoid Dalam Meningkatkan Diferensiasi Sel Punca Darah Tepi Menjadi Sel Progenitor Endotelial dan Kemampuan Perbaikan Fungsi Kardia Pada Hewan Model Infark Miokardial	(Program Insentif Ristek Kapasitas Produksi 2011)	340
7	2011	Potensi Ekstrak Teh Hijau Dalam Meningkatkan Diferensiasi Sel Punca Darah Tepi Menjadi Sel Progenitor Endotelial	LPPM-Universitas Kristen Maranatha	30
8	2011	Ekstrak dan Infusa Ramuan *Curcuma mangga* Val, *Piper betle* L., *Catharanthus roseus* [L] G.Don , *Dendrophtoe petandra* L. Dalam Mencegah dan Menghambat Keganasan Kanker Payudara (*Breast Cancer*)	(Hibah Bersaing DIKTI 2010)	45
9	2012	Kondisi Hipoksia dan Normoksia Untuk Produksi Condition Medium Mesenchymal Stem Cells dari Human Umbilical Cord (HUC) Sebagai Bahan Antikanker	Hibah Unggulan Perguruan Tinggi (DIKTI 2012)	96
10	2012	Potensi Kulit Manggis (*Garcinia Mangostana*) Sebagai Obat Antimalaria Dan Antioksidan Serta Meningkatkan Daya Antimalaria Artemisinin	Hibah Unggulan Perguruan Tinggi (DIKTI 2012)	90
11	2013	Produksi Condition Medium Sel Punca Mesenkim (Human Mesenchymal Stem Cells-Condition Medium) dari Wharton's Jelly Sebagai Bahan Terapi Kanker	Insentif Riset Peningkatan Kapasitas Iptek Sistem Produksi (RISTEK 2013)	250
12	2013	Kondisi Hipoksia dan Normoksia Untuk Produksi Condition Medium Mesenchymal Stem Cells dari Human Umbilical Cord (HUC) Sebagai Bahan Antikanker	Hibah Unggulan Perguruan Tinggi (DIKTI 2013)	75
13	2013	Potensi Kulit Manggis (*Garcinia Mangostana*) Sebagai Obat Antimalaria Dan Antioksidan Serta Meningkatkan Daya Antimalaria Artemisinin	Hibah Unggulan Perguruan Tinggi (DIKTI 2012)	67,5
14	2013	Pengembangan Fitofarmaka Ekstrak Kulit Manggis Terstandar Sebagai Bahan Antiinflamasi, Antiagregasi, Antikolesterol Untuk Menghambat Penyakit Kardiovaskuler	(Hibah Bersaing DIKTI 2013)	60
15	2014	Produksi Condition Medium Sel Punca Mesenkim (Human Mesenchymal Stem Cells-Condition Medium) dari Wharton's Jelly Sebagai Bahan Terapi Kanker	Insentif Riset Peningkatan Kapasitas Iptek Sistem Produksi	350

			(RISTEK 2013)	
16	2014	Ekstrak Kulit Manggis (*Garcinia mangostana* L) Dan Senyawa Xanthone Sebagai Antiadipogenesis Pada Model Sel Lini HepG2	LPPM-Universitas Kristen Maranatha	30
17	2015	Human Mesenchymal Stem Cells-Condition Medium (hMSCs-CM) Dari Wharton's Jelly Sebagai Induksi Apoptosis Pada Cancer Stem Cells (CSCs)	(Hibah Kompetensi RISTEK-DIKTI 2015)	125
18	2015	Pengembangan Fitofarmaka Ekstrak Kulit Manggis Terstandar Sebagai Bahan Antiinflamasi, Antiagregasi, Antikolesterol Untuk Menghambat Penyakit Kardiovaskuler	(Hibah Bersaing RISTEK-DIKTI 2015)	75
19	2016	Human Mesenchymal Stem Cells-Condition Medium (hMSCs-CM) Dari Wharton's Jelly Sebagai Induksi Apoptosis Pada Cancer Stem Cells (CSCs)	(Hibah Kompetensi RISTEK-DIKTI 2016)	125
20	2016	Pengembangan Fitofarmaka Ekstrak Kulit Manggis Terstandar Sebagai Bahan Antiinflamasi, Antiagregasi, Antikolesterol Untuk Menghambat Penyakit Kardiovaskuler	(Hibah Bersaing RISTEK-DIKTI 2016)	75
21	2016	Rekayasa Sel Punca Mesenkim Yang Diisolasi dari Wharton's Jelly (hWJMSCs) Untuk Meningkatkan Potensi Sel Punca (hWJMSCs) dan Conditioned Medium (CM-WJMSCs) Sebagai Bahan Terapi Kanker Payudara	(Insentif Riset Sinas RISTEK-DIKTI 2016)	155
22	2016	Potensi Homing dan Regeneratif Sel Punca Mesenkim Yang Diisolasi dari Wharton's Jelly (WJ-MSCs) dalam Menghambat Osteo Arthritis	(Insentif Riset Sinas RISTEK-DIKTI 2016)	155

D. PENGALAMAN PENULISAN ARTIKEL ILMIAH DALAM JURNAL dalam 5 tahun Terakhir

No	Tahun	Judul Artikel Ilmiah	Nama Jurnal Volume/ Nomor/ Tahun	Penulis
1.	2012	Extract of Curcuma longa L. and (-)-Epigallo Catechin-3-Gallate Enhanced Proliferation of Adipose Tissue–derived Mesenchymal Stem Cells (AD-MSCs) and Differentiation of AD-MSCs into Endothelial Progenitor Cells	Journal of US-China Medical Science; 2012; 9(1)(86):22-29	**Wahyu Widowati**, Caroline Tan Sardjono, Laura Wijaya, Dian Ratih Laksmitawati, Ferry Sandra
2.	2013	Anticancer and free radical scavenging potency of *Catharanthus roseus, Dendrophthoe petandra, Piper betle* and *Curcuma mangga* extracts in breast cancer cell lines	*Oxidant and Antioxidants in Medical Science* 2013; 2(2):137-142	**Wahyu Widowati**, Tjandrawati Mozef, Chandra Risdian, Yellianty Yellianty

3.	2013	Antioxidant, anticancer, and apoptosis-inducing effects of Piper extracts in HeLa cells	*Journal of Experimental and Integrative Medicine* 2013; 3(3):225-230	**Wahyu Widowati**, Laura Wijajya, Teresa L Warga Setia, Yelliantty
4	2013	Potency Xanthones as Antioxidant and Antimalarial and Synergism with Artemesinin	Majalah Kedokteran Indonesia (Terakreditasi No 51/DIKTI/Kep/ 2010)63/3/2013 (p95-99)	Susy Tjahjani, **Wahyu Widowati**
5	2013	Stem Cell as Alternative Therapy	Zenit, J Ilmiah Universitas Kristen Maranatha 2013; 2(1):1-5	**Wahyu Widowati** Rachma Micho Widiyanto
6	2013	Hypolipidemic And Antioxidant Effects of Black Tea Extract and Quercetin in Atherosclerotic Rats	*Int Journal of Medical Science and Engineering* 2013; 7(10):153-160	**Wahyu Widowati** Hana Ratnawati, Tjandrawati Mozef, Dwiyati Pujimulyani, Yelliantty Yelliantty
7	2013	Mangosteen Peel (Garcinia mangostana L.) Extract for Effervescent Tablet	*World Academy of Science, Engineering and Technology* 82 2013	**Wahyu Widowati,** Djaja Rusmana, Heddy Herdiman, Hartini Tiono, Teresa Liliana Wargasetia, Dwiyati Pujimulyani, Yelliantty Yelliantty
8	2014	Antioxidative and Antibacterial Activities of Indonesian Propolis Extracts against Methicillin-Resistant Staphylococcus aureus (MRSA) in Vitro	*Cukurova Medical Journal* 2014;39(2): 224-233	Arina Novilla, As'ari Nawawi, Ganthina Sugihartina, **Wahyu Widowati**
9	2014	Effect of β-carotene on cell proliferation and differentiation of adipose-derived stem cells into endothelial progenitor cells	*BioTechnology an Indian Journal* BTAIJ, 9(10), 2014 [407-412] (SCOPUS)	**Wahyu Widowati** Caroline Tan Sardjono, Laura Wijaya, Dian Ratih Laksmitawati, Jeanne Adiwinata Pawitan Ferry Sandra
10	2014	Effect of oxygen tension on proliferation and characteristics of Wharton's jelly-derived mesenchymal stem cells	*Biomarkers and Genomic Medicine* (2014) 6, 43-48 (SCOPUS)	**Wahyu Widowati ,** Laura Wijaya , Indra Bachtiar , Rimonta F. Gunanegara , Sri Utami Sugeng , Yudha Aryadi Irawan , Sutiman B. Sumitro ,

				M. Aris Widodo
11	2014	Effect of green tea extract and epigallocatechin-3-gallate potency on lipid profile and coronary artery morphology of dyslipidemic rats.	*Oxid Antioxid Med Sci* 2014; 3(2):147-152	**Wahyu Widowati**, Hana Ratnawati, Roro Wahyudianingsih, Yelliantty Yelliantty Maesaroh Maesaroh, Dwiyati Pujimulyani, Tjandrawati Mozef
12	2015	Conditioned medium from normoxia (WJMSCs-norCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation	*Biomark Genom Med 2015; 7:8-17* (SCOPUS)	**Wahyu Widowati,** Laura Wijaya, Harry Murti, Halida Widyastuti, Dwi Agustina, Dian Ratih Laksmitawati, Nurul Fauziah, Sutiman B. Sumitro, M. Aris Widodo, Indra Bachtiar
13	2014	Green tea extract protects endothelial progenitor cells from oxidative insult through reduction of intracellular reactive oxygen species activity.	*Iran J Basic Med Sci* 2014; 17:702-709 (SCOPUS)	**Wahyu Widowati**, Rahma Micho Widyanto, Winsa Husin, Hana Ratnawati, Dian Ratih Laksmitawati, Bambang Setiawan, Dian Nugrahenny, Indra Bachtiar
14	2014	High Performance Liquid Chromatography (HPLC) Analysis, Antioxidant, Antiaggregation of Mangosteen Peel Extract (*Garcinia mangostana* L.*)*	*Int J Biosci, Biochem Bioinform 2014; 4(6):458-466*	**Wahyu Widowati,** Lusiana Darsono, Jo Suherman, Yelliantty Yelliantty, Maesaroh Maesaroh
15	2014	Inhibitory potential of rambutan seeds extract and fractions on adipogenesis in 3T3-L1 cell line.	*J Exp Integr Med* 2015;5(1):55-60	Sylvia Soeng, Endang Evacuasiany, **Wahyu Widowati,** Nurul Fauziah, Visi Tinta Manik, Maesaroh Maesaroh
16	2015	Antioxidant and hypoglycemic activities of extract and fractions of Rambutan seeds (*Nephelium lappaceum* L.).	*Biomed Engineering* 2015;1(1):13-18	Slyvia Soeng, Endang Evacuasiany, **Wahyu Widowati**, Nurul Fauziah
17	2014	Cytotoxic and antioxidant activities of catechins in inhibiting the malignancy of breast cancer.	*J Exp Integr Med* 2014; 3(2):141-146	Endang Evacuasiany, Hana Ratnawati, Laella K. Liana, **Wahyu Widowati**, Maesaroh Maesaroh, Tjandrawati Mozef, Chandra Risdian
18	2015	Ex Vivo Study Of Garcinia Mangostana L. (Mangosteen) Peel Extract and Xanthones as Anti-	*Int J Med Res Health Sci*. 2015;4(3):566-	Lusiana Darsono, Meilinah Hidayat, Maesaroh Maesaroh,

		Adipogenesisin HepG2 Cell Model.	571	Nurul Fauziah, **Wahyu Widowati**
19	2015	Potency of turmeric (*Curcuma longa L.*) extract and curcumin as anti-obesity by inhibiting the cholesterol and triglycerides synthesis in HepG2 cells.	*Int J Res Med Sci. 2015;3(5):1165 -1171*	Iwan Budiman, Rita Tjokropranoto, **Wahyu Widowati**,, Nurul Fauziah, Pande Putu Erawijantari
20	2015	Phytochemical assay, potential of antimalarial and antioxidant activities of green tea extract and its fraction.	*Biomed Engineering* 2015;1(1):1-7	Fanny Rahardja, Rita Tjokropranoto, **Wahyu Widowati,** Siska Lusiana, Adrian Suhendra, Susy Tjahjani, Iwan Budiman, MaesarohMaesaroh, NurulFauziah
21	2015	Inhibition of Inflammatory Agent Production by Ethanol Extract and Eugenol of *Syzygium aromaticum* (L.) Flower Bud (Clove) in LPS-Stimulated Raw 264.7 Cells.	*Res J Med Plant 2015; 9 (6): 264-274* (SCOPUS)	Djaja Rusmana, Mariska Elisabeth, **Wahyu Widowati**, Nurul Fauziah, Maesaroh Maesa
22	2015	Human platelet lysate enhances the proliferation of Wharton's jelly-derived mesenchymal stem cells.	*Biomark Genom Med* 2015; 7:87-97 (SCOPUS)	Andreas Ardhika Antoninus, **Wahyu Widowati**, Laura Wijaya, Dwi Agustina, Sugiarto Puradisastra, Sutiman B. Sumitro, M.Aris Widodo, Indra Bachtiar
23	2015	Tumoricidal Property of Normoxia and Hypoxia Cell-Free Lysate of Wharton's Jelly-Mesenchymal Stem Cells Toward Various Cancer Cells.	*Int J Cancer Res* 2015 ; 11 (4): 186-196 (SCOPUS)	**Wahyu Widowati**, Laura Wijaya, Dwi Agustina, Harry Murti, Nurul Fauziah, Sutiman B. Sumitro, M. Aris Widodo, Indra Bachtiar
24	2015	Potential of Unengineered and Engineered Wharton's Jelly Mesenchymal Stem Cells as Cancer Inhibitor Agent.	*Immun Endoc Metab Agents in Med Chem* 2015;15:128-137 (SCOPUS)	**Wahyu Widowati**, Diana Krisanti Jasaputra, Sutiman B. Sumitrob, M. Aris Widodo, Kelvin Yaprianto, Indra Bachtiar,
25	2015	Antioxidant properties of spice extracts.	*Biomed Engineering* 2015;1(1):24-29	**Wahyu Widowati,** Hana Ratnawati, Winsa Husin, Maesaroh Maesaroh2
26	2015	Phytochemical, Free Radical Scavenging and Cytotoxic Assay of *Cucumis melo* L. Extract and β-Carotene	*J Advanced Agricul Technolog* 2015; 2(2): 114-119	**Wahyu Widowati** Rachma Micho Widyanto, Dian Ratih Laksmitawati, Pande Putu Erawijantari, Laura Wijaya, and

				Ferry Sandra
27	2015	Anticancer Activity of 3-Hydroxystigmastan- 5(6)-en (β-Sitosterol) Compound from Salacca Edulis Reinw Variety Bongkok in MCF-7 and T47D Cell Line *J*	*Advanced Agricul Technolog* 2015; 2(2): 129-133	Leni Herliyani Afrianti Willy Pranata Widjaja, Neneng Suliasih, **Wahyu Widowati**, Nurul Fauziah, Maesaroh Maesaroh, and Pande Putu Erawijantari
28	2015	*In silico* Analysis of Plantaricin EF that Expressed by Plasmid-Associated Bacteriocin Production Gene of *Lactobacillus plantarum* IBL-2 for Anti-Candida Agent Potential.	*Res J Microbiol* 2015; 10 (12): 582-591 (SCOPUS)	Betty Nurhayati, Marlia Singgih Wibowo, Yantyati Widyastuti, Pande Putu Erawijantari, **Wahyu Widowat**i, Mohammad Rizki Fadhil Pratama and Tutus Gusdinar Kartawinata
29	2015	Free Radical Scavenging and α-/β-glucosidase Inhibitory Activities of Rambutan (*Nephelium lappaceum* L.) Peel Extract	*Indones Biomed J. 2015; 7(3): 157-62*	**Wahyu Widowati,** Maesaroh, Nurul Fauziah, Pande Putu Erawijantari, Ferry Sandra
30	2015	*In vitro* study of *Myristica fragrans* seed (Nutmeg) ethanolic extract and quercetin compound as anti-inflammatory agent	*Int J Res Med Sci. 2015 ;3(9):2303-2310*	Kartika Dewi, Budi Widyarto, Pande Putu Erawijantari, **Wahyu Widowati**
31	2015	Antioxidant Potential of Black, Green and Oolong Tea Methanol Extracts	*Biol Med Nat Product Chem 2015; 4(2):38-43*	**Wahyu Widowati**, Tati Herlina, Hana Ratnawati, Gabriella Constantia, I Dewa Gde Sathya Deva, Maesaroh Maesaroh
32	2016	Selective Cytotoxic Potential of IFN-γ and TNF-α on Breast Cancer Cell Lines (T47D and MCF7)	*Asian Journal of Cell Biology 2016; 11(1):1-12 (SCOPUS)*	**Wahyu Widowati,** Harry Murti, Diana Krisanti Jasaputra, Sutiman B. Sumitro, M. Aris Widodo, Nurul Fauziah, Maesaroh Maesaroh Indra Bachtiar
33	2016	Tea Flavonoids Protect Endothelial Progenitor Cells (EPCs) from Oxidative Damage by Reducing Reactive Oxygen Species (ROS) Level	*Natural Product Sciences 2016; 22(2):87-92 (SCOPUS)*	**Wahyu Widowati,** Laura Wijaya, Dian Ratih Laksmitawati, Rahma Micho Widyanto, Pande Putu Erawijantari, Nurul Fauziah, Indra Bachtiar, Ferry Sandra
34	2016	Modulation of the Adipogenesis-related Gene Expression by Ethanol Extract of Detam 1 Soybean (Glycine	*Bangladesh J Pharmacolog 2016; 11:697-*	Meilinah Hidayat, Sijani Prahastuti, Maesaroh Maesaroh,

		max) and Jati Belanda (Guazuma ulmifolia) Leaves in 3T3-L1 Cells	*702* (SCOPUS)	Nurul Fauziah, Balqis Balqis, **Wahyu Widowati**
35	2016	Anti-inflammatory Flavonoid C-glycosides from Piper aduncum Leaves	*Planta Medica 2016; 2016 http://dx.doi.org/10.1055/s-0042-108737* (SCOPUS)	Bui Thi Thuy Luyen, Nguyen Phuong Thao, Bui Huu Tai, **WahyuWidowati**, Nurul Fauziah, Maesaro Maesaroh, Tati Herlina, Young Ho Kim
36	2016	Cytotoxic Activity of Mangosteen (*Garcinia mangostana* L.) Peel Extract and α-Mangostin toward Leukemia Cell Lines (HL-60 and K-562)	*Journal of Natural Remedies* 2016; 16(2):52-59 (SCOPUS)	Arina Novilla, Dedi S. Djamhuri, Nurul Fauziah, Maesaroh Maesaroh, Balqis Balqis,**Wahyu Widowati**
37	2016	Anti-Inflammatory Potential of Gandarusa (Gendarussa vulgaris Nees) and Soursoup (Annona muricata L) Extracts in LPS Stimulated-Macrophage Cell (RAW264.7)	*Journal of Natural Remedies* 2016;16(2):73-81 (SCOPUS)	Dian Ratih Laksmitawati, Ajeng Prima Prasanti, Nadia Larasinta, Gloria Agitha Syauta, Rivanny Hilda, Hesty Utami Ramadaniati, Anisa Widyastuti, Nadia Karami, Merry Afni, Dwi Davidson Rihibiha, Hanna Sari W. Kusuma, **Wahyu Widowati**
38	2016	Anti-inflammatory Effect of Mangosteen (*Garcinia mangostana* L.) Peel Extract and its Compounds in LPS-induced RAW264.7 Cells	*Natural Product Sciences 2016; (Accepted) (SCOPUS)*	**Wahyu Widowati**, Lusiana Darsono, Jo Suherman, Nurul Fauziah, Maesaroh Maesaroh, Pande Putu Erawijantari
39	2016	Interleukins and VEGF Secretome of Human Wharton's Jelly Mesenchymal Stem Cells-Conditioned Medium (hWJMSCs-CM) in Different Passages and Oxygen Tensions	*Int J Pharmtech Res (Under review) (SCOPUS)*	**Wahyu Widowati**, Halida Widyastutri, Laura Wijaya, Harry Murti, Dian Ratih Laksmitawati, Maesaroh Maesaroh, Sutiman B. Sumitro, M. Aris Widodo, Indra Bachtiar
40	2016	Anti-inflammatory Properties of Oolong Tea (Camellia sinensis) Methanol Extract and EGCG (Epigallo Catechin Gallate) in LPS-Induced RAW264.7 Cells	*Journal of Complementary and Integrative Medicine (Under review)* (SCOPUS)	Arina Novilla, Dedi Somantri Djamhuri, Betty Nurhayati, Nurul Fauziah, I Dewa Gde Sathya Deva, **Wahyu Widowati**
41	2016	Anti-inflammatory Activities of	*Iranian J Basic*	Ni Made Dwi

		Coumarin and Indonesian Cassia (Cinnamomum burmanni (C. Nees & T. Ness)) Extract in Macrophage Cell Line	*Med Sci (Accepted)* (SCOPUS)	Sandhiutami, Moordiani Moordiani, Dian Ratih Laksmitawati,Nurul Fauziah, Maesaroh Maesaroh, **Wahyu Widowati**
42	2016	Suppression of Pro-Inflammatory Cytokines and Mediators Production by Ginger (Zingiber officinale) Ethanolic Extract and Gingerol in Lipopolysaccharide-Induced RAW264.7 Murine Macrophage Cells	*Indian J Nat Products Resources (Under review)* (SCOPUS)	Sri Nadya Saanin, Roro Wahyudianingsih, **Wahyu Widowati**
43	2016	α-/β-Glucosidase and α-Amylase Inhibitory Activities of Roselle Ethanol Extract (Hibiscus sabdariffa L.)	*Mol Cellular Biomed Sci* (*Accepted*)	Marisca Evalina Gondokesumo, Hanna Sari W. Kusuma, **Wahyu Widowati**
44	2016	Phytochemical screening and antioxidant assay of Phyllantus niruri extract and flavonoid compounds rutin, quercetin	*Med J Indonesia* (*Under review*)	Djaja Rusmana,Roro Wahyudianingsih, Mariska Elisabeth, Balqis, Maesaroh,**Wahyu Widowati**
45	2016	Conditioned Medium from Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) Has Lower Activity in Inhibiting Proliferation and Inducing Apoptosis on Breast Cancer Cells Line (T47D, MCF7) Compared to Normoxia (WJMSCs-norCM)	*Iranian J Basic Med Sci* (*Under review)* (SCOPUS)	**Wahyu Widowati**, Harry Murti, Halida Widyastuti, Dwi Agustina, Dian Ratih Laksmitawati, Merry Afni, Ervi Afifah, Sutiman B. Sumitro, M. Aris Widodo, Indra Bachtiar
46	2016	Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward TNF-α, IFN-γ, Perforin, Granzyme Concentration in Breast Cancer Cells	Tissue and Cell (*Under review)* (SCOPUS)	**Wahyu Widowati**, Diana Krisanti Jasaputra, Sutiman B. Sumitro, M. Aris Widodo, Merry Afni, Seila Arumwardana, Dwi Davidson Rihibiha, Indra Bachtiar,

A. Pengalaman Penyampaian Makalah Secara Oral Pada Pertemuan/Seminar Ilmiah

No	Nama Pertemuan Ilmiah / Seminar	Judul Artikel Ilmiah	Waktu dan Tempat
1	Seminar Nasional dan Pameran Integratif Pangan Kesehatan dan Lingkungan,	Potensi Antioksidan Berbagai Jenis Ekstrak The (*Camelia sinensis* L.)	17-18 Maret 2011. Universitas Padjadjaran Bandung

2	Globalization of Jamu Brand Indonesia. The Second International Syposium on Temulawak, The 40 th Meeting National Working Group on Indonesian Medicinal Plant.	Free Radicals Scavenging Activities of Spices and Curcumin .	29-30 March, 2011. Research and Community Services Institution of Bogor Agrucultural University. Bogor
3	Pertemuan Ilmiah Tahunan Nasional I, PEFARDI . Implementasi Ilmu Farmasi Kedokteran Dalam Praktek Kedokteran dan Aplikasi Klinik Hasil Pengembagan Obat Herbal.	Free Radicals Scavenger Potency of Betel Leaves (*Piper betel* L.) Extract and Various Fractions.	Universitas Kristen Maranatha, Bandung 7-8 Mei , 2011
4	The 2nd International Conference on Pharmacy and Advanced Pharmaceutical Sciences.	. Effect of Proliferative and Differentiative of Beta Carotene on Adipose-derived Stem Cell .	Faculty of Pharmacy Universitas Gadjah Mada. Yogyakarta, 19-20 July 2011.
5	The 2nd International conference on Pharmacy and Advanced Pharmaceutical Sciences.	Protective Effect of EGCG on Oxidative Damage in Endothelial Progenitor Cells.	Faculty of Pharmacy Universitas Gadjah Mada. Yogyakarta, 19-20 July 2011.
6	15th International Congress Phytopharm	The Role of Turmeric (*Curcuma longa* L.) Extract and EGCG in Enhancing Proliferation and Differentiation of Endothelial Progenitor Cells from Adipose-derived Stem Cell.	2011, 25-27 July, Nurmberg, Germany.
7	15th International Congress Phytopharm	The Comparison of Free Radicals Scavenging Activity of Tea Extracts and Tea Flavonoids.	2011, 25-27 July, Nurmberg, Germany.
8	The International Conference on Bioscience and Biotechnology. October 11th-12th, 2011, Yogyakarta, Indonesia	Antioxidant Activities of Extrcats and Effervescence Product from Mangosteen Peel Extract (*Garcinia mangostana* L.).	October 11th-12th, 2011, Yogyakarta, Indonesia
9	International Conference on Bioscience and Biotechnology.	Green Tea Extract Protects Endothelial Progenitor Cells from Oxidative Damage Through Reduction of Intracellular Reactive Oxygen Species Activity. The	October 11th-12th, 2011, Yogyakarta, Indonesia
10	The International Conference on Bioscience and Biotechnology.	Potential Cytotoxic on Breast Cancer Cells Line and Antioxidant of Water Extract of *Catharanthus*	October 11th-12th, 2011, Yogyakarta, Indonesia

		roseus [L] G.Don., *Dendropthoe petandra* L., *Curcuma mangga* Val., Piper betle L.	
11	The International Seminar on Translational Research in Cancer Chemoprevention,	Antioxidant Potency and Phytochemical Constituent of Piperaceae Extracts.	October 11th-12th 2011,Jakarta, Indonesia.
12	The International Seminar on Translational Research in Cancer Chemoprevention,	Cytotoxic and Apoptotic Inducer Activities of Water Extract of *Catharanthus roseus* [L] G.Don., *Dendropthoe petandra* L., *Curcuma mangga* Val.	October 11th-12th 2011,Jakarta, Indonesia.
13	The 2 nd International Seminar on Chemistry	The Protective Role of Tea Flavonoids towards Oxidative Stress on Endothelial Progenitor Cells from Peripheral Blood-derived Stem Cell.	2011, 24-25 November, Jantinangor Campus, Universitas Padjadjaran.
14	The 2 nd International Seminar on Chemistry. 2011	Potential Antioxidant and Antimalarial Activities of Green Tea (*Camelia sinensis* L.) Ethanolic Extract and Fractions .	2011, 24-25 November, Jantinangor Campus, Universitas Padjadjaran
15	The 2 nd International Seminar on Chemistry 2011.	The Antioxidant Activity and Phenolic Compound Changes of Effervescence Product and Mangosteen Peel (*Garcinia mangostana* L.) Aqueous Extract .	2011, 24-25 November, Jantinangor Campus, Universitas Padjadjaran.
16	The 2 nd International Seminar on Chemistry 2011.	Green Tea Extract Protects Endothelial Progenitor Cells Against H202-Induced Oxidative Stress.	2011, 24-25 November, Jantinangor Campus, Universitas Padjadjaran
17	The 2 nd International Seminar on Chemistry 2011.	Superoxide Dismutase and EarlyApoptotic Inducer Activities of Ethanolic Extract of *Catharanthus roseus* [L] G.Don. and *Dendropthoe petandra* L.	2011, 24-25 November, Jantinangor Campus, Universitas Padjadjaran
18	Simposium Nasional Herbal Medik.	Antioxidant And Hypolipidemic Potency Of Epigallocatechin-3-Gallate (Egcg) In Dyslipidemic Rat.	7 Januari 2012. Bandung.
19	Simposium Nasional Herbal Medik.	The Antioxidative And Cytotoxic Properties Of Piperaceae Extracts On Cervical Cancer Cell Line (Hela).	7 Januari 2012. Bandung.
20	Seminar Nasional POKJANAS TOI XLII.	The Free Radical Scavenging Potency of	15-16 Mei 2012. Cimahi, Bandung

		Catechins. 2012.	
21	4th International Conference on Medicinal Plants & Herbal Products	Antioxidant, Anticancer and Apoptotic Inducer Activities of Pipercaceae Extracts on HeLa Cells Line	6th-8th September 2012 Rockville, MD, USA
22	2nd Bandung Biomolecular Medicine Conference	Effect Oxygen Level Towards Proliferation Rate and Surface Marker of Human Umbilical Cord's-derived Mesenchymal Stem Cells	5-7 October 2012, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia
23	Simposium Nasional Kimia Bahan Alam ke-XX (*SimNas*KBA-2012)	Potensi Antikanker Payudara dan Antioksidan Superoksida Dismutase Dari Senyawa Golongan Katekin	9-10 Oktober 2012, Fakultas Kedokteran dan Ilmu Kesehatan (FKIK) Kampus III UIN Syarif Hidayatullah Jakarta
24	2nd Bandung Biomolecular Medicine Conference October 5-7, 2012	Effect Oxygen level towards proliferation rate and surface marker of human umbilical cord's derived mesenchymal stem cells	Faculty of Medicine Padjadjaran University, Indonesia
25	International Seminar on Natural Products Medicines, November 22 - 23, 2012.	Antioxidant and Antimalarial Potency of Oolong Tea (Camelia sinensis L.) Extract and Fractions	School of Pharmacy Bandung Institute of Technology
26	The International Conference on Pharmacy and Advanced Pharmaceutical Sciences June 18-19, 2013	Green Tea Extract Potency Towards Lipid Profile And Histopatological Coronary Artery On Dyslipidemic Rats	Faculty of Pharmacy, Gadjah Mada University, Yogyakarta
27	ICABBBE 2013 : International Conference on Agricultural, Biotechnology, Biological and Biosystems Engineering 7-8 October, 2013	Mangosteen Peel (Garcinia mangostana L.) Extract for Effervescent Tablet	World Academy os Science Engineering and Technology. Paris, France
28	ICABBBE 2013 : International Conference on Agricultural, Biotechnology, Biological and Biosystems Engineering 7-8 October, 2013	Hypolipidemic And Antioxidant Effects of Black Tea Extract and Quercetin in Atherosclerotic Rats	World Academy os Science Engineering and Technology, Paris, France
29	Seminar Nasional Bahan Obat Alam, 26 November 2013	Uji Fitokimia, Antioksidan Ekstrak Rempah-rempah	Laboratorium Biosains, Universitas Brawijaya Malang
30	International Conference	High performance liquid	4th International

	on Environmental, Biomedical and Biotechnology - ICEBB 2014,	chromatography (HPLC) analysis, antioxidant, antiaggregation of mangosteen peel extract (Garcinia mangostana L.)	Conference on Environmental, Biomedical and Biotechnology – ICEBB 2014, Nottingham, UK.
31	ICBAE 2015	Phytochemical, Free Radical Scavenging and Cytotoxic Assay of Cucumis melo L. Extract and β-Caoreten	International Conference on Biotechnology and Agriculture Engineering – ICBAE 2015, Kyoto, Japan April 6-7, 2015
32	World Congress on Pharmacology	The Protective Effect of Tea Flavonoids on Oxidative Damage in Peripheral Blood-derived Endothelial Progenitor Cells	July 20-22, 2015, at Brisbane, Australia.
33	2nd International Conference on Advance Molecular Bioscience and Biomedical Engineering's scientific programme	Selective cytotoxic potential of IFN-γ and TNF-α on breast cancer cell lines (T47D and MCF7)	**ICAMBBE 2015,** Malang, East Java
34	PCS Cell Science and Stem Cell Conference (CSSC--2016)	The Cytotoxic Effect of Conditioned Medium from Interleukins-Induced human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs-ILs-CM), ILs Induced-Natutal Killer Cells (NK-ILs), Interleukins (ILs) toward Breast Cancer (BC) and Natural Killer (NK) Cells	11-12 June 2016 Barcelona, 11-12 June 2016
35	3rd International Conference of Advance Molecular Bioscience and Biomedical Engineering 2016 (ICAMBBE 2016)	Effects of Co-culture IL2-induced NK (IL2-NK) and human Wharton's Jelly Mesenchymal Stem Cells (hWJMSCs) toward TNF-α, IFN-γ, Perforin, Granzyme Concentration, Cytotoxic and Apoptotic Inducing Activity in Breast Cancer Cells	4-5 August 2016 Malang, East Java

E. PENGALAMAN PEROLEHAN HKI
Dalam 5 – 10 Tahun Terakhir

No	Judul/Tema HKI	Tahun	Jenis	Nomor P/ID
1.	IL-2 meningkatkan sitotoksik sel NK terhadap kanker payudar			Terdaftar

F. Pengalaman Penulisan Buku dalam 5 Tahun Terakhir

No	Judul Buku	Tahun	Jumlah Halaman	Penerbit
1.	Efek Toksik Logam, Pencegahan dan Penanggulangan Pencemaran Penerbit	2008	410	Andi Offset. ISBN:978-979-29-0449-2

G. PENGALAMAN MENDAPATKAN LUARAN LAINNYA DARI HASIL PENELITIAN

No	Judul luaran	Jenis luaran	Tahun perolehan	Deskripsi singkat
1	Pemanfaatan limbah tanaman buah-buahan	TTG	2015/2016	Persiapan produksi cream anti aging

H. PENGALAMAN REVIEWER

	REVIEWER JURNAL NASIONAL/INTERNASIONAL	NAMA JURNAL	EDITOR
1	The Effect of *Solenostemma argel* leaves extract on status of induced Lipid Constituents in Albino Rats (2014)	*Merit Research Journal Of Medicine And Medical Sciences (MRJMMS)* http://www.meritresearchjournals.org/mms/index.htm	Larry Dave Editor
2	Green tea polyphenols attenuate high fat diet induced oxidative damage through SIRT3-dependent deacetylation (2015)	*Biomedical and Environmental Sciences* https://mc03.manuscriptcentral.com/bes (SCOPUS)	Yuanyuan Xu Editor
3	Sequence analysis of AKT1,AKT2 and AKT3 kinases (2015)	*International Journal of Medical and Clinical Research* *Bioinfo Publication* *http://www.bioinfopublication.org*	
4	The *in vivo* protective effects of soybeans on DMN-induced hepatotoxicity in rats (2015)	*International Journal Of Environmental Science And Toxicology Research (IJESTR)* *http://www.internationalinventjournals.org/*	Chris Awe, Editorial Assistant
5	Immunoglobulin G subtype responses to UB05, a dominant Plasmodium falciparum antigen by individuals living in a high transmission endemic area of the Cameroonian rainforest (2015)	*Issues in Biological Sciences and Pharmaceutical Research* http://www.journalissues.org/IBSPR/	
6	Improving the efficiency of neural-differentiation of umbilical cord blood–derived mesenchymal stem cell cultivated under appropriate condition (2015)	*Iranian Journal of Basic Medical Sciences* http://ijbms.mums.ac.ir/reviewer (SCOPUS)	Bibi Sedigheh Fazly Bazzaz, Ph.D. Editor-in-Chief Iranian Journal of Basic Medical Sciences

7	Growth suppression effect of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord Wharton's jelly (2015)	Iranian Journal of Basic Medical Sciences. http://ijbms.mums.ac.ir/reviewer (SCOPUS)	Bibi Sedigheh Fazly Bazzaz, Editor-in-Chief Iranian Journal of Basic Medical Sciences
8	Efektivitas Ekstrak Etanol Kulit Manggis terhadap Parasitemia pada Mencit yang Diinokulasi Plasmodium Berghei (2015)	Journal of Medicine and Health jmh.maranatha.edu	Stella Tinia Hasianna,dr.,M.Kes, IBCLC Sekretaris Journal of Medicine and Health
9	Therapeutic efficacy of spleen-derived mesenchymal stem cells in mice with acute pancreatitis (2015)	*Journal of Stem Cell Research & Therapy* http://omicsonline.org/reviewer-credits.php	Prasanna Krishnamurthy Editor
10	Expression Of Physiologic Biomolecules Following Anti-Malarial Cytokine-Cpg Motif Oligodeoxynucleotide Gene Therapy (2015)	*Merit Research Journal Of Medicine And Medical Sciences* http://www.meritresearchjournals.org/mms/index.htm	Larry Dave Editorial officer,
11	Phenolic compounds Isolated from Opuntia ficus-indica fruits (2015)	*Natural Product Sciences* http://www.e-nps.or.kr (SCOPUS)	Dr. Editor-in-Chief Editor-in-Chief
12	Xantin Oxidase Inhibition Activity of *Peperomia pellucida* L., *Acalypha indica* L., and *Momordica charantia* L. Ethanol Extract (2016)	*The Indonesian Biomedical Journal* http://inabj.org/index.php/ibj/reviewer/submission/48?key=tE8y9zX8	Nurrani Mustika Dewi Secretariat of InaBJ
13	Increased Platelet Count And Mean Platelet Volume Independent Of Gender In Iranian Patients With Colon Cancer (2016)	*International Research Journal of Medicine and Biomedical Sciences* http://www.journalissues.org/IRJMBS/	Prof. Cyril Azimi Editor
14	Wharton's jelly-derived mesenchymal stem cells as a therapeutic option for patients with ALS (2016)	*General Medicine* http://acrgroup.edmgr.com/	Mina Kelleni, Ph.D., M.D. Editor General Medicine
15	Cytotoxicity of Alpinia galanga Rhizome Crude Extract on NIH3T3 Cells (2016)	*The Indonesian Biomedical Journal* http://inabj.org/index.php/ibj/reviewer/submission/48?key=tE8y9zX8	Nurrani Mustika Dewi Secretariat of InaBJ
16	Cytotoxic and apoptotic effects of different extracts of Artemisia biennis Willd. on K562 and HL-60 cell lines (2016)	*Iranian Journal of Basic Medical Sciences.* http://ijbms.mums.ac.ir/reviewer (SCOPUS)	Bibi Sedigheh Fazly Bazzaz, Ph.D. Editor-in-Chief
17	The Probiotic Effect towards Aspirin-induced Gastric Ulcer Healing Process as Measured by Mucous Thickness, Reepithelialization, Gastric Glands Formation, and Angiogenesis in Animal Model (2016)	Journal of Medicine and Health jmh.maranatha.edu	Stella Tinia Hasianna,dr.,M.Kes, IBCLC
18	Pancreatic and beta islet cell transplantation - evidence base, outcomes, advantages and	*Journal of Diabetes & Metabolism*	Mark T. Marino Editor

	disadvantages (2016)	http://omicsonline.org/reviewer-credits.php	
19	Increased Platelet Count and Mean Platelet Volume Independent of Gender in Iranian Patients with Colon Cancer (2016)	*International Research Journal of Medicine and Biomedical Sciences* http://www.journalissues.org/IRJMBS/	Prof. Cyril Azimi Editor
20	Anti-inflammatory Action of Green Tea and Epigallocatechin-3-gallate (2016)	*Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry* *www.benthamscience.com*	Zubia Aleem Khan Assistant Manager Publications Bentham Science Publishers
21	Expression of pro-inflammatory genes in lesions, spleens and blood neutrophils after burning injuries in BALB/c mice	*Iranian Journal of Basic Medical Sciences.* http://ijbms.mums.ac.ir/reviewer (SCOPUS)	Bibi Sedigheh Fazly Bazzaz, Ph.D. Editor-in-Chief

H. PENGALAMAN INVITED SPEAKER

No	INVITED SPEAKER/SHORT LECTURE	NAMA SEMINAR	TEMPAT, TANGGAL PELAKSANAAN
1	Conditioned Medium from Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) Has Lower Activity in Inhibiting Proliferation and Inducing Apoptosis on Breast Cancer Cells Line (T47D, MCF7) Compared to Normoxia (WJMSCs-norCM)	Seminar dan Kongres Nasional ke-3 Asosiasi Sel Punca Indonesia (ASPI) 2016 "Indonesia Stem Cell Summit"	6-7 Februari 2016, IPB International Convention Center, Bogor
2	Potency of Cytotoxic and Free Radical Scavenger Activities of Aqueous Extract of *Catharanthus roseus* [L] G.Don., *Dendropthoe petandra* L., *Curcuma mangga* Val., *Piper betle* L.	College of Pharmacy	January 12th, 2014 Chungnam National University, Daejeon 305-764, South Korea
3	The Role of Turmeric (*Curcuma longa* L.) Extract and EGCG in Enhancing Proliferation and Differentiation of Endothelial Progenitor Cells from Adipose-derived Stem Cell	Department of Food and Nutrition, College of Life Science & Nano Technology	January 13th, 2014 Hannam University 70 Hannam-ro, Daedeok-gu, Daejeon, South Korea
4	Kiat Menembus Publikasi Internasional Terakreditasi	Pertemuan Dosen Poltekes Bandung	Politeknik Kesehatan Bandung, Jl Babakan Loa Gunung Batu Cimahi, 18 Januari 2016

I. Pengalaman Ceramah Sebagai Pengabdian pada Masyarakat dalam 5 tahun terakhir

No	Tahun	Kegiatan	Tempat Penerapan	Respon masyarakat
1	2011	Ceramah "Peranan Wanita dalam menjaga kelestarian lingkungan"	HKBP	Meningkatkan pengetahuan masyarakat
2	2012	Ceramah "Tetap Produktif Usia Lanjut"	HKBP	Meningkatkan pengetahuan masyarakat
3	2012	Ceramah "Waspada Kanker Payudara"	PIKKA-PTKAI	Meningkatkan pengetahuan masyarakat
4	2012	Ceramah "Peran Wanita dalam Mencegah Banjir"	PIKKA-PTKAI	Meningkatkan pengetahuan masyarakat
5	2013	Keamanan Pangan	PIKKA-PTKAI	Meningkatkan pengetahuan masyarakat
6	2014	Ceramah "Mengenal Penyakit Dimensia dan Alzheimer	24 Januari 2014 Kelompok Ibu-Ibu Direktorat Safety and Security PT KAI Bandung	Meningkatkan pengetahuan masyarakat
7	2014	Ceramah "Resiko Makanan Tambahan Bagi Kesehatan"	12 November 2014 Kelompok Ibu-Ibu Direktorat Sarana dan Prasarana PT KAI Bandung	Meningkatkan pengetahuan masyarakat
8	2014	Ceramah "Mengenal Penyakit Epilepsi"	9 September 2014 Kelompok Ibu-Ibu Direktorat Sarana dan Prasarana PT KAI Bandung	Meningkatkan pengetahuan masyarakat
9	2015	Ceramah "Mengenal Penyakit Alzheimer"	16 September 2016 Kelompok Ibu-Ibu Sarana PT KAI Bandung	Meningkatkan pengetahuan masyarakat

I. Penghargaan yang Pernah Diraih dalam 10 tahun Terakhir (dari pemerintah, asosiasi atau institusi lainnya)

No	Jenis Penghargaan	Institusi Pemberi Penghargaan	Tahun
1	The second winner for oral presentation at The Second International Conference on Pharmacy and Advanced Pharmaceutical Sciences,	Universitas Gajah Mada,	2011
2	Presenter Terbaik Seminar Nasional dan Pameran Integratif Pangan Kesehatan dan Lingkungan, 17-18 Maret 2011.	Universitas Padjadjaran Bandung	2011

J. Panitia Kegiatan Sosial

No	Jenis Kegiatan	Kedudukan	Tahun
1	Pertemuan Kelompok "Persatuan Istri Karyawan Karyawati Kereta Api (PIKKA)" Topik Tentang Penanganan Korban Tindak Kekerasan di Yayasan Jaringan Relawan Independen	Ketua Panitia Penyelenggara	2012

IV. Bimbingan mahasiswa Program Kreatifitas Mahasiswa Penelitian

1.	Potensi Senyawa Flavonoid Teh Sebagai Antioksidan Dan Antiagregasi Platelet Dengan Inducer Adenosin Difosfat (Adp)	2012

Semua data yang saya isikan dan tercantum dalam biodata ini adalah benar dan dapat dipertanggungjawabkan secara hukum. Apabila di kemudian hari ternyata dijumpai ketidaksesuaian dengan kenyataan, saya sanggup menerima risikonya. Demikian biodata ini saya buat dengan sebenarnya untuk memenuhi salah satu persyaratan sebagai dosen pembimbing mahasiswa Fakultas Kedokteran Universitas Maranatha Bandung.

Bandung, Oktober 2016

(Dr. Wahyu Widowati, M.Si)

Lampiran 10.2 Anggota Peneliti

A. Identitas Diri

1.	Nama Lengkap (dengan gelar)	:	Dr. Diana Krisanti Jasaputra, dr, M Kes L/**P**√
2.	Jabatan Fungsional	:	Lektor
3.	Jabatan Struktural	:	Pembantu Dekan FK UK Maranatha
4.	NIK/NIP	:	110292
5.	NIDN	:	0414076701
6.	Tempat dan Tanggal Lahir	:	Bandung, 14 Juli 1967
7.	Alamat Rumah	:	Perim. Bumi Indah 69 Cimahi-Bandung
8.	Nomor Telp/Fax/HP		0226032549 / 0818625980
9.	Alamat Kantor		Jl. Prof. drg. SuriaSumantri MPH No. 65 Bandung 40164
10.	No. Telepon/Fax		(022-2012186 / 022-2017621
11.	E-mail	:	dianakjasaputra@yahoo.com
12.	Lulusan yang telah dihasilkan	:	S1 = 100 S2 = tidak ada S3 = tidak ada
13.	Mata kuliah yang diampu	:	1. Farmakologi 2. Biomedical Research and Advanced Medicine (Karya Tulis Ilmiah)

B. Riwayat Pendidikan

	S1	**S2**	**S3**
Nama Perguruan Tinggi	Universitas Kristen Maranatha	Universitas Padjadjaran	Universitas Padjadjaran
Bidang Ilmu	Pendidikan Dokter	Farmakologi	Farmakologi
Tahun masuk-lulus	1985-1994	1998-2001	2005-2010
Judul Skripsi/Tesis/ Disertasi		Pengaruh pemberian *Taraxacum officinale* Weber et Wiggers terhadap SGOT, SGPT, Malondialdehid, dan Gambaran Histopatologi Hepar Mencit yang diinduksi CCl4	Mekanisme *Phyllanthus niruri* L. Dan *Taraxacum officinale* Weber et Wigger dalam menurunkan kadar IL-4 dan meningkatkan IFN gamma pada tikus model alergi
Nama Pembimbing/ Promotor		Prof. Dr. H.R. Muchtan Sujatno, dr, SpFK (K) Prof Sidik Wahyuni, dr, PhD.	Prof. Dr. H.R. Muchtan Sujatno, dr, SpFK (K) Prof. Dr. Endang Sutedja, SpKK (K) Dr. As'ari Nawawi, M Sc.

C. Pengalaman Penelitian dalam 5 Tahun Terakhir

			Pendanaan	
No	Tahun	Judul Penelitian	**Sumber**	**Jml (juta Rp)**
1	2006	Mekanisme *Phyllanthus niruri* L. dan *Taraxacum officinale* Weber et Wiggers dalam menurunkan dominasi jalur Th2 dan meningkatkan jalur Th1 penderita Dermatitis Atopik	Risbin Iptekdok	90
2	2007	Pengembangan Fitofarmaka Buah Merah (Pandanus Conoideus Lam) Sebagai Antioksidan Dan Antikanker (sebagai anggota peneliti)	DIKTI (HB)	35
3	2008	Uji Efektivitas Ekstrak Air dan Ekstrak Etanol Herba Meniran *(Phyllantus niruri L.)*, Herba Jombang *(Taraxacum officinale Weber et Wiggwrs)* Kombinasinya pada Dermatitis Alergika dengan Hewan Coba Mencit (sebagai ketua peneliti)	LPPM	8,164.050
3	2010	Uji Toksisitas Subkronik Simunox Sidomuncul	PT Sidomuncul	57,5
4	2011	Uji Toksisitas Subkronik Sari Kunyit Sidomuncul	PT Sidomuncul	66
5	2012	Mekanisme Kerja Beberapa Tanaman Obat untuk *Diabetes Melitus* dan *Obesitas*	DIKTI (HB)	45
6	2013	Uji Toksisitas Subkronik Sari Daun Sirsak Sidomuncul	PT Sidomuncul	75
7	2013	PENGARUH TERAPI AJUVAN DAUN JATI BELANDA TERHADAP DISLIPIDEMIA	LPPM	10,471

D. Pengalaman Penulisan Artikel Ilmiah dalam Jurnal dalam 5 tahun Terakhir

No	Judul Artikel Ilmiah	Volume/Nomor/Tahun	Nama Jurnal
1.	The Anti hepatotoxic and Anti inflammation Effect to Alergic Dermatitis Mice, and Acute Toxicity of *Taraxacum officinale* Weber et Wiggers	*Jurnal Kedokteran Maranatha*,; 2003; 3(3). Authors : **Jasaputra, Diana Krisanti**, Endang Evacuasiany, Yohanes S.A.,P. Aitara, Hermawan, Iwan,	*Jurnal Kedokteran Maranatha*, Volume 3, No 3, Juli 2003, halaman 24 – 42, ISSN 1411 - 9641
2.	Immunomodulator for Allergic Diseases	*Jurnal Kedokteran Maranatha*. 2005; 4(2) ISSN 1411-9641, Authors : **Jasaputra, Diana Krisanti**	*Jurnal Kedokteran Maranatha*, Volume 4, No 2 , Februari 2005, halaman 74-141, ISSN 1411-9641
3.	Antiinflammation effect and safety of *Phyllanthus niruri* L. and *Taraxacum officinale* Weber et Wiggers to	*Jurnal Kedokteran Maranatha*, 2007 Authors :	*Jurnal Kedokteran Maranatha*, Volume , No ,

	Allergic Dermatitis Mice	**Jasaputra, Diana Krisanti**, Rosnaeni	halaman , ISSN 1411-9641
4.	THE Th1/Th2 IMBALANCE, ATOPIC ECZEMA, AND HERBAL MEDICINE	Jurnal Medika Planta. 2010 1(1):93-97 Authors : **Jasaputra, Diana Krisanti**., Dewi.Kurniawati., Tri Budhi Baskara	Jurnal Medika Planta, 2010 Volume 1, No 1, page 93-97, ISSN 2086-7514.
5.	ANTIINFLAMMATORY EFFECT OF DANDELION INTO ATOPIC DERMATITIS MODELS USING MICE	Jurnal Medika Planta 2010; 1(1): 9-23 Authors : **Jasaputra, Diana Krisanti**, Laella K. Liana, Muchtan Sujatno, Soen, Mary	Jurnal Medika Planta, 2010. Volume 1, No 1, page 9-23, ISSN 2086-7514.
6.	COMPARISON EFFECT OF ANGSANA LEAF, PARE FRUIT, BUNCIS, SAMBILOTO LEAF ETHANOL EXTRACT AND THEIR COMBINATION (JAMU D) TO BLOOD GLUCOSE IN DIABETES MICE MODEL	Jurnal Medika Planta,. 2010; 1(1): 49-56 Authors : **Jasaputra,,Diana Krisanti** Slamet Santosa, Muchtan Sujatno, Rhenata Dylan	Jurnal Medika Planta, 2010. Volume 1, No 1, page 49-56, ISSN 2086-7514.
7.	HERBAL MEDICINE FOR AGING	Jurnal Medika Planta 2010; 1(2):85-92 Authors : **Jasaputra, Diana Krisanti**., Dewi Kurniawati, Tri Budhi Baskara	Jurnal Medika Planta, 2010. Volume 1, No 2, page 85-92, ISSN 2086-7514.
8.	THE COMPARISON OF EFFECT OF WATER AND ETHANOL EXTRACT OF MENIRAN HERB TO THE AMOUNT OF EOSINOPHIL PERCENTAGE ON MICE AS ATOPIC DERMATITIS MODEL	Jurnal Medika Planta, 2010; 1(2) 2010 :13-20. Authors : **Jasaputra, Diana Krisanti**., Slamet Santosa, Sugiarto Puradisastra, Hana Ratnawati, Rosnaeni, Emmanuel, R.A Alexia Kusuma Editha, Marselina A.A., Sarah Kastilani	Jurnal Medika Planta, 2010. Volume 1, No 2, page 13-20, ISSN 2086-7514.
9	HERB FOR DIABETES	Jurnal Medika Planta; 2011. 1(3) Authors : **Jasaputra, Diana Krisanti,** Adrian Suhendra, Rita Tjokropranoto, Agistia Lembayung P, Laura Darmawan, I Gede Mahatma Pratama, Sanggam T.H.H.	Jurnal Medika Planta, 2011. Volume 1, No 3,, ISSN 2086-7514.
10.	HERBAL MEDICINE FOR OBESITY	Jurnal Medika Planta; 2011. 1(3)	Jurnal Medika Planta, 2011.

		Authors : **Jasaputra, Diana Krisanti**	Volume 1, No 3,, ISSN 2086-7514.
11.	COMPLIMENTARY THERAPY OF DIABETES MELLITUS	Jurnal Medika Planta; 2011. 1(3) Authors : **Jasaputra, Diana Krisanti**	Jurnal Medika Planta, 2011. Volume 1, No 3,, ISSN 2086-7514.

E. Pengalaman Penyampaian Makalah Secara Oral Pada Pertemuan/Seminar Ilmiah dalam 5 tahun Terakhir

No	Nama Pertemuan Ilmiah / Seminar	Judul Artikel Ilmiah	Waktu dan Tempat
1	National Symposium and Workshop On Anti-Aging Medicine (NASWAAM) by Udayana Unversity	Herb for diabetes, one aspect of anti aging therapy	Maret 2011 Denpasar Bali
2	Simposium Herbal Medik: Saintifikasi Obat Herbal dan Aplikasi Kliniknya oleh Perhimpunan Dokter Herbal Medik Indonesia, FK UNPAD, FK UKM	MEKANISME Phyllanthus niruri L. DAN Taraxacum officinale Weber et Wiggers DALAM MENURUNKAN KADAR IL-4 DAN MENINGKATKAN KADAR IFN-gamma PADA TIKUS MODEL ALERGI	April 2011 Bandung Jawa Barat
3	Pertemuan Ilmiah Tahunan Nasional Pefardi	Complimentary Therapy of Diabetes Mellitus	Mei 2011 Bandung Jawa Barat
4	Perhimpunan Dokter Herbal Medik Indinesia, Cabang Jawa Barat	Some plants that affect immunomodulatory and Phytopharmaca clinical trials as an immunomodulator	Januari 2012 Bandung Jawa Barat
5	The National Symposium and Workshop on Anti-Aging Medicine (NASWAAM) diselenggarakan oleh Universitas Udayana, bekerja sama dengan UKM, INCAAM, SCI.	Protective effect of ethanol extract Purwoceng to phone electromagnetic wave exposure as a risk factor for andropause	Maret 2013 Bali Indonesia
6	The 3rd Continuing Professional Development "Applications of Evidence Based Medicine in Clinical Practice."	Herbal medicine in primary services Practice physician	September 2013 Bandung Indonesia
7	National Symposium and Workshop On Anti-Aging Medicine (NASWAAM) by Udayana Unversity	Potensi daun jati belanda sebagai terapi adjuvan agen hipokolesterolemik pada penderita dislipisemia dalam kaitannya dengan proses penuaan	Maret 2014 Denpasar Bali

Semua data yang saya isikan dan tercantum dalam biodata ini adalah benar dan dapat dipertanggungjawabkan secara hukum. Apabila di kemudian hari ternyata dijumpai ketidaksesuaian dengan kenyataan, saya sanggup menerima risikonya.Demikian biodata ini saya buat dengan sebenarnya untuk memenuhi salah satu persyaratan dalam pengajuan Penelitian Hibah Kompetensi 2015.

Bandung, Oktober 2016

(Dr. Diana Krisanti Jasaputra, dr, M Kes)