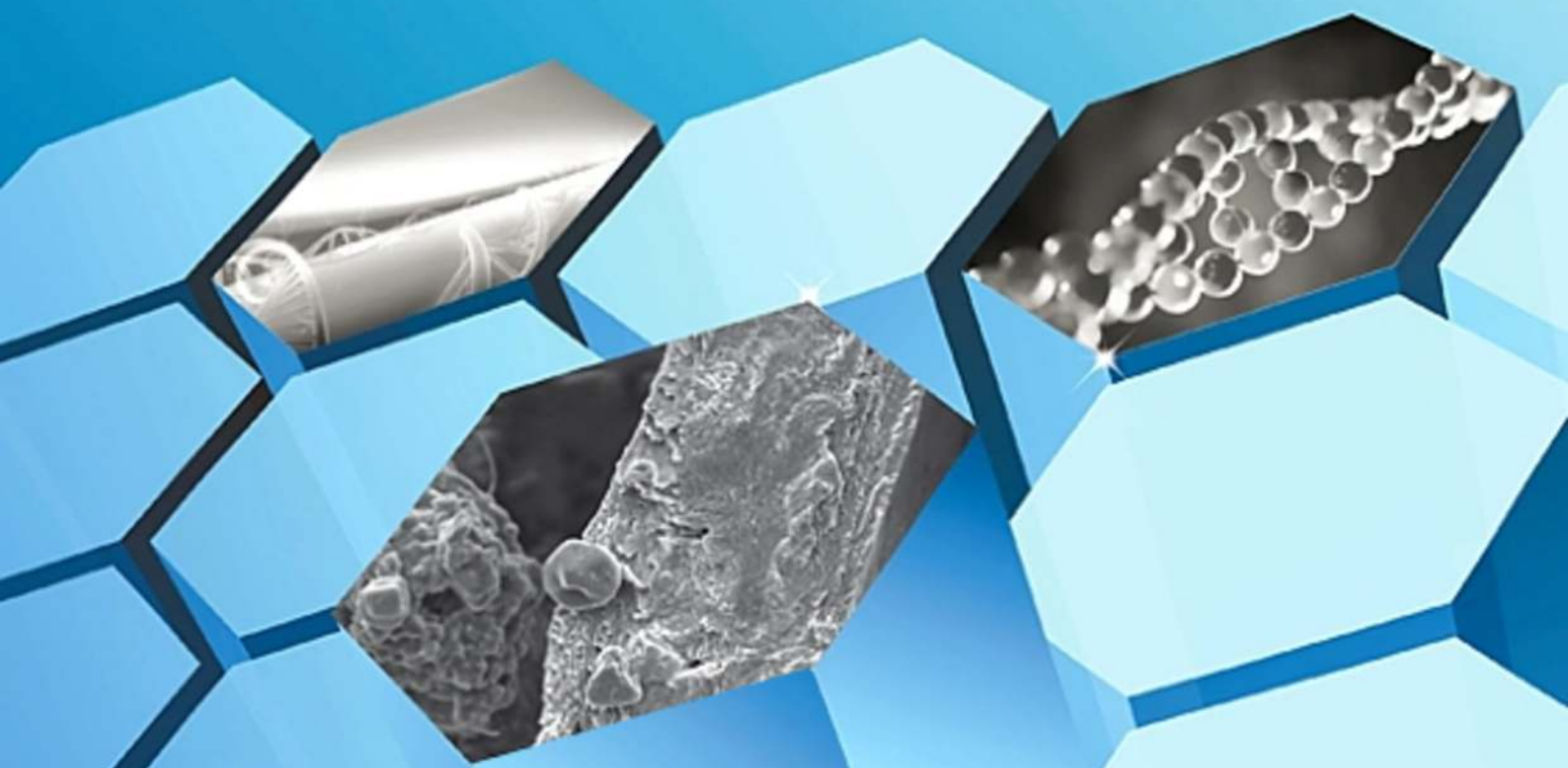
Biomarkers and Genomic Medicine



Indexed in Chemical Abstracts Service, EMBASE, EMCARE, and SCOPUS

Biomarkers and Genomic Medicine

OPEN ACCESS

Volume 7, Issue 1

Pages 1-42 (March 2015)

Previous vol/issue Next vol/issue

Actions for selected articles
Select all / Deselect all

Download PDFs

Export citations

Show all article previews

Original Articles

Research article • *Open archive*

Associations of positive epidermal growth factor receptor expression and *K-RAS* gene mutations with various clinicopathological parameters and survival of colorectal carcinoma patients

Warsinggih Warsinggih, Irawan Yusuf, Ida Bagus Tjakra Wibawa Manuaba, Aryono Pusponegoro

Pages 1-7

Download PDF Article preview

Research article • *Open archive*

Conditioned medium from normoxia (WJMSCs-norCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation

Wahyu Widowati, Laura Wijaya, Harry Murti, Halida Widyastuti, ... Indra Bachtiar

Pages 8-17

Download PDF Article preview

Research article • *Open archive*

In-vitro antioxidant properties of the hydro-methanol extract of the seeds of *Swietenia mahagoni* (L.) Jacq.

Tushar Kanti Bera, Kausik Chatterjee, Debidas Ghosh

Pages 18-24

Download PDF Article preview

Research article • *Open archive*

Transforming growth factor β1 is better than α smooth muscle actin for the prediction of renal fibrosis in patients with nephritic lupus

Hani Susianti, Kusworini Handono, Atma Gunawan, Karyono Mintaroem, ... Handono Kalim

Pages 25-30

Download PDF Article preview

Research article • *Open archive*

Reduction of histopathological images through a decrease in H_2O_2 levels in diabetic rats with polysaccharide peptides

Teuku Heriansyah, Titin Andri Wihastuti, Djanggan Sargowo, Mohammad Aris Widodo, ... Fitriani Intan Puspitasari

Pages 31-37

Download PDF Article preview

Short Communication

Short communication • *Open archive*

Association of prolactin and beta-lactoglobulin genes with milk production traits and somatic cell count among Indian Frieswal (HF × Sahiwal) cows

Umesh Singh, Rajib Deb, Sushil Kumar, Rani Singh, ... Arjava Sharma

Pages 38-42

Download PDF Article preview

ISSN: 2214-0247

Biomarkers and Genomic Medicine (2015) 7, 8–17

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.j-bgm.com

ORIGINAL ARTICLE

Conditioned medium from normoxia (WJMSCs-norCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation

Wahyu Widowati [a,*], Laura Wijaya [b], Harry Murti [b], Halida Widyastuti [b], Dwi Agustina [b], Dian Ratih Laksmitawati [c], Nurul Fauziah [d], Sutiman B. Sumitro [e], M. Aris Widodo [f], Indra Bachtiar [b,**]

[a] *Medical Research Center, Faculty of Medicine, Maranatha Christian University, Bandung, West Java, Indonesia*
[b] *Stem Cell and Cancer Institute, Jakarta, Indonesia*
[c] *Faculty of Pharmacy, Pancasila University, Jakarta, Indonesia*
[d] *Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, West Java, Indonesia*
[e] *Department of Biology, Faculty of Science, Brawijaya University, Malang, East Java, Indonesia*
[f] *Pharmacology Laboratory, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

Received 5 June 2014; received in revised form 10 August 2014; accepted 12 August 2014
Available online 2 October 2014

KEYWORDS
anticancer;
conditioned medium;
hypoxic;
normoxic;
Wharton's jelly mesenchymal stem cells

Abstract Mesenchymal stem cells (MSCs) have unique properties, including high proliferation rates, self-renewal, multilineage differentiation ability, wide multipotency, hypoimmunogenicity, noninduction of teratomas, and anticancer properties. MSCs can be isolated from embryonic and extraembryonic tissues as well as adult organs. Human Wharton's jelly stem cell-conditioned medium possesses anticancer properties and inhibits the growth of solid tumors. Lower oxygen concentration or hypoxic condition can increase the proliferation of MSCs, but there are no differences in surface markers. We determined the osteocyte, chondrocyte, and adipocyte differentiation of normoxic WJMSCs (nor-WJMSCs) and hypoxic 2.5%, hypoxic 5% (hypo-WJMSCs); from a different passage (P4 and P8), we determined the inhibitory effect of WJMSCs-norCM and WJMSCs-hypoCM on the proliferation of human cancer cells including cervical (HeLa), liver (HepG2), prostate (pc3), ovarian (skov3), and oral squamous (hsc3) cancer cell lines compared to normal cells including mouse fibroblast (NIH3T3), human

* Corresponding author. Medical Research Center, Faculty of Medicine, Maranatha Christian University, Jl. Prof. drg. Surya Sumantri no 65 Bandun 40164, West Java, Indonesia.
** Corresponding author. Stem Cell and Cancer Institute, JL.A. Yani no 2 Pulo Mas, Jakarta 13210, Indonesia.
E-mail addresses: wahyu_w60@yahoo.com (W. Widowati), nantenine@yahoo.com (I. Bachtiar).

http://dx.doi.org/10.1016/j.bgm.2014.08.008

fibroblast, and human mesenchymal stem cells (hMSCs). Surfacer marker expression of nor-WJMSCs-and hypo-WJMSCs from P4 and P8 were >95% for CD90, CD73 and CD105 and <2% for CD14, CD19, CD34, CD45, and HLDA-II. Nor-WJMSCs and hypo-WJMSCs from P4 and P8 underwent differentiation to osteocyte, chondrocyte, and adipocyte. WJMSCs-norCM and WJMSCs-hypoCM could inhibit proliferation of various cancer cell lines with minimum inhibitory concentration (IC_{50}) 51.690–81.440% and cause low inhibition of the normal cells with IC50 136.290–185.339%. WJMSCs-norCM and WJMSCs-hypoCM were not cytotoxic toward normal cells. Nor-WJMSCs and hypo-WJMSCs from P4 and P8 showed no significant differences in MSC surface marker expression or differentiation. WJMSCs-norCM and WJMSCs-hypoCM could inhibit proliferation in various cancer cell lines, and were safe for normal cells.

Introduction

Umbilical cord matrix contains an inexhaustible, noncontroversial source of stem cells.[1–4]. Postnatal stem cells are offered for use less often because of possible moral/ethical conflict.[4] The umbilical cord mesenchymal stem cells (UCMSCs) derived from human umbilical cord Wharton's jelly (WJMSCs) exhibit low immunogenicity and low immunity after cytotherapy.[5] The UCMSCs are more useful and simpler in donor accessibility isolation, expansion, proliferative capacity, and banking capability; can be used in clinical and experimental therapy[6,7]; have a higher proliferation rate and self-renewal capacity than adult tissue-derived MSCs[2,8]; and a short doubling time.[9,10] MSCs possess strong immunosuppressive properties and can be used for autologous and allogeneic therapy.[11,12]

Research has shown that MSCs, including UCMSCs, bone marrow MSCs, and adipose tissue MSCs, have anticancer activity and have been shown to inhibit the proliferation of cancer cells in both *in vitro* and *in vivo* assays.[5]

Hypoxic conditions (1–3% O_2) are more beneficial for MSCs because low oxygen tension is more suitable for MSC physiology in the bone marrow (2–7% O_2). Previous research has shown that MSCs cultured in hypoxic conditions (2–5% O_2) could maintain their viability.[13] The hypoxic microenvironment can lead to a substantial increase in the proliferation rate and population doubling time, but no differences in surface markers of MSCs has been shown. The hypoxic 2.5% O_2 yield has the highest proliferation and the lowest population doubling and population doubling time.[10]

The hypoxic condition induces molecular responses including angiogenesis, metabolic change, and metastasis; it also induces the secretion of growth factor and cytokine in MSCs, and elevated the secretion of transforming growth factor-β1 (TGF-β1). In addition, the hypoxic condition can enhance cancer cell growth through the MSCs effect by secretion and expression of TGF-β1.[14]

We conducted the continuing research to elucidate the osteocyte, chondrocyte, and adipocyte differentiation of normoxia-treated WJMSCs (nor-WJMSCs) and hypoxia-treated WJMSCs (hypo-WJMSCs) from early and late passage (P4 and P8), to evaluate the WJMSCs-nor conditioned medium (norCM) and WJMSCs-hypoCM toward cancer cell lines including HeLa, HepG2, pc3, skov3, and hsc3 compared to human fibroblast, NIH3T3, and human mesenchymal stem cells (hMSCs).

Materials and methods

Isolation and cultivation of WJMSCs

Isolation and cultivation of MSCs from umbilical cord was as described by Widowati et al,[10] fresh human umbilical cords (UC; $n = 5$) were obtained from women aged 25–40 years after full-term births (normal vaginal delivery), all donors signed a written informed consent, and guidelines were approved by the Institutional Ethics Committee at the Stem Cell and Cancer Institute, Jakarta, Indonesia and from the Institutional Ethics Committee collaboration between Maranatha Christian University, Bandung, Indonesia and Immanuel Hospital Bandung, Bandung, Indonesia.

Isolated WJMSCs from UC were rinsed in normal saline (0.9% w/v sodium chloride) and cut into very small explants (1–2 mm), then plated on tissue culture plastic plates. The explants were cultured in minimum essential medium-α with 2 mM GlutaMAX (Invitrogen, Carlsbad, CA, USA), supplemented with 20% fetal bovine serum (FBS; Invitrogen) and penicilline streptomycine 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°C, replacing medium every 5 days for 21 days. The cells were harvested and replated at a density 8×10^3 cells/cm^2 when cells reached 80–90% confluence. WJMSCs were cultured in 95% air (21% O_2), and 5% CO_2 for normoxic and hypoxic conditions (5% O_2 and 2.5% O_2).[10,15]

Cell surface phenotype and multipotent differentiation

To confirm the effect of oxygen concentration (hypoxia and normoxia) on MSCs characterization. The WJMSCs of passage 4 and 8 (P4 and P8) were characterized the surface marker CD105, CD73, CD90, CD34, CD45, CD14, CD19, and HLA-II using a flow cytometer. WJMSCs at 80% confluence were harvested and dissociated with trypsin-EDTA and centrifuged at 300*g* for 10 minutes. The pellet was resuspended with phosphate buffered saline (PBS) + 2% FBS, and cells were counted with a hemocytometer. Between 100 cells and 200 cells in 25 mL PBS were stained with the appropriate surface monoclonal antibodies. Isotype controls were used to determine background staining. These antibodies were as follows: PE (Phycoerythrin) conjugated: CD105 (abcam

53321-100), CD73 (BD550257), CD 90 (abcam 226), CD 34 (BD 348053), CD45 (BD 555482), CD14 (abcam 28061-100), CD 19 (abcam 1168-500), and HLA-DR (abcam 23901); FITC-conjugated: mIgG1 (BD349041 and BD 349043), and mIgG2 (BD349053) then added to each FACS tube: isotype mIgG2a-PE, CD105-PE, HLA class II-PE; isotype mIgG1-PE, CD73-PE, CD19-PE; isotype mIgG1-FITC, CD 34-FITC, CD45-FITC, CD14-FITC, after incubation at 4°C for 15 minutes. The cells were analyzed by flow cytometry with a FACS CaliburTM 3 argon laser 488 nm (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA) using CellQuest Pro Acquisition on the BD FACStationTM Software. The experiments and measurement of surface marker were performed in triplicate.[10]

For osteogenic differentiation, WJMSCs (P4 and P8) were seeded at density 1×10^4 cells/cm^2 in culture dishes using StemPro Osteogenesis Differentiation Kit (Gibco A10072-01, Invitrogen) for 3 weeks. Calcium deposits were visualized using Alizarin red S (Biochemicals and Life Science Research Products, Amresco 9436). For chondrogenic differentiation of WJMSCs were seeded at density 1×10^4 cells/cm^2 in culture dishes using StemPro Chondrogenesis Differentiation Kit (Gibco A10071-01, Invitrogen) for 2 weeks. Chondrocytes were visualized using Alcian blue (Amresco, 0298). Adipogenic differentiation of WJMSCs was done using StemPro Adipogenesis Differentiation Kit (Gibco A10070-01, Invitrogen, Carlsbad, CA, USA) for 2 weeks. We used Oil Red O (Sigma 00625, St Louis, USA) to confirm lipid droplets.[16–18]

Preparation of conditioned media from normoxia- or hypoxia-treated WJMSCs

WJMSCs of P4 were used for the experiments. The WJMSCs were seeded at a density of 8×10^3 cells/cm^2 under normoxia (20% O_2 and 5% CO_2) and hypoxia (5% O_2 and 20% CO_2) for 72 hours; when cultures reached 80–90% confluence, cells were harvested. The medium was collected and centrifuged at 3000*g* for 4 minutes at room temperature, and the supernatant was filtered by a 0.22-μm Millex–GV Filter Unit with Durapore (SLGV 033 RS, Millipore Corporation, Billerica, MA, USA) and used as WJMSCs-CM.[18,19]

Anticancer assay

The cancer cell lines of cervical (HeLa- ATCC CCL-2), liver (HepG2- ATCC HB-8065), prostate (PC3-ATCC CRL-1435), ovarian (SKOV3-ATCC HTB-77), oral squamous (HSC3; ATCC, Manassas, VA, USA), mouse fibroblast (NIH3T3-ATCC CRL-1658), human fibroblast (primary cells), and hMSCs (primary cells from Wharton's jelly) were obtained from Stem Cell and Cancer Institute, Jakarta, Indonesia. The cells were grown and maintained in Dulbecco modified Eagle's medium supplemented with 10% FBS (Invitrogen), 100 U/mL penicillin (Invitrogen), and 100 μg/mL streptomycin (Invitrogen), and incubated at 37°C in a humidified atmosphere and 5% CO_2.[20,21]

The cell viability assay uses an optimized reagent containing resazurin converted to fluorescent resorufin by viable cells that absorbs the light at 490 nm. Briefly, cells were seeded at density 5×10^3 in 96-well plates for 24 hours of incubation[20,21]; cells were supplemented by WJMSCs-norCM and WJMSCs-hypoCM in various concentrations (100%, 75%, 50%, and 0%) then incubated for 72 hours. The anticancer activity of WJMSCs-CM was noted for cancer cell lines including HeLa, HepG2, PC3, SKOV3, HSC3. We used NIH3T3, human fibroblast, and hMSCs as controls to determine the cytotoxic effect of WJMSCs-CM in normal cells. MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay (Promega, Madison, WI, USA) was used to determine cell viability. MTS was added at 10 μL to each well. The plate was incubated at 5% CO_2, 37°C for 4 hours. The absorbance of the cells was measured at 490 nm using a microplate enzyme-linked immunosorbent assay reader (Multiskan Go, Thermo Scientific, USA). The data were presented as number of viable cells and the percentage of viability.[20,21]

Statistical analysis

To verify the statistical significance of all parameters, the data were calculated and expressed in means and standard deviation (M ± SD). To compare treatments, analysis of variance (ANOVA) was used, and $p < 0.05$ were considered as statistically significant, along with Tukey honestly significant difference *post hoc* test and 95% confidence interval. The median inhibitory concentration (IC_{50}) of cytotoxic effect was analyzed using probit analysis. Statistical analysis used the SPSS version 20.0 program (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp, SPSS Inc., Chicago, IL, USA).

Results

MSC markers by cell surface phenotype

Flow cytometry analysis showed that for cultured cells under normoxia and hypoxia (5% O_2) for P4 and P8, WJ-MSCs were positive for MSCs marker CD105, CD73, and CD90 and negative for CD34, CD45, CD14, CD19, and HLA-II. The effect of oxygen level (normoxic and hypoxic 5% O_2) and passage (early passage P4 and late passage P8) on the surface marker of WJMSCs can be seen in Table 1. Surface marker expression of WJ-MSCs (P4 and P8) on hypoxia and normoxia were not significantly different ($p > 0.05$).

MSC differentiation

We examined the differentiation potentials (osteogenic, chondrogenic, and adipogenic differentiation) that were cultured in either normoxic (20% O_2 and 5% CO_2) or hypoxic (5% O_2 and 2.5%O_2) conditions. After 3 weeks, WJMSCs were cultured in osteogenesis differentiation medium, and the differentiated WJMSCs responses to either normoxia or hypoxia exhibited calcium deposits based on the staining of Alizarin red S. After 2 weeks, WJMSCs were cultured in chondrogenesis differentiation medium, and the differentiated WJMSCs in responses to either normoxia or hypoxia exhibited chondrocyte expression using Alcian blue. After 2 weeks, WJ-MSCs were cultured in adipogensis differentiation medium in response to either normoxia or hypoxia and exhibited lipid droplets using Oil Red O (Fig. 1).

Fig. 1 shows that WJ-MSCs incubated in normoxia (21% O_2) and hypoxia (5% O_2 and 2.5% O_2) differentiated to osteocyte, adipocyte, and chondrocyte.

Table 1 Effect of oxygen level and type of passage toward surface markers for umbilical cord mesenchymal stem cells derived from human umbilical cord Wharton's jelly.

Surface markers of WJMSCs	Passage 4		Passage 8	
	Normoxia	Hypoxia 5%	Normoxia	Hypoxia 5%
CD105	96.00 ± 1.39	96.88 ± 0.11	95.65 ± 1.77	95.07 ± 3.33
CD73	96.67 ± 2.25	97.30 ± 2.55	96.40 ± 3.11	97.63 ± 0.45
CD90	96.17 ± 1.00	96.70 ± 0.71	96.55 ± 1.77	96.57 ± 1.95
CD34	0.00 ± 0.00	0.04 ± 0.05	0.00 ± 0.00	0.00 ± 0.01
CD45	0.00 ± 0.00	0.05 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
CD14	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.01
CD19	−0.39 ± 1.12	0.12 ± 0.11	−0.80 ± 1.24	0.15 ± 0.21
HLA-II	0.23 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.05

Data are presented as mean ± standard deviation of surface markers of WJ-MSCs.
WJMSCs = mesenchymal stem cells derived from Wharton's jelly.

Cytotoxic activity

To determine the effect of both WJMSCs-norCM and WJMSCs-hypoCM toward various cancer cell lines (HeLa, HepG2, PC3, SKOV3, and HSC3) and normal cells (NIH3T3, human fibroblast, and hMSCs), the cell lines were cultured at density 5×10^3 in a 96-well plate. We determined the cell viability by MTS assay. WJMSCs-norCM and WJMSCs-norCM exhibited decreased viability in cancer cell lines in a concentration-dependent manner. The effect of WJMSCs-norCM and WJMSCs-hypoCM on the number of cancer cells can be seen in Table 2. The number of cancer cells decreased along with treatments, and the higher WJMSCs-CM concentration decreased the number of cancer cells. The effect of WJMSCs-norCM and WJMSCs-hypoCM toward inhibition of cancer cell viability can be seen in Table 3. As seen in Table 3, WJMSCs-norCM and WJMSCs-hypoCM could inhibit the proliferation of cancer cells including HeLa, HepG2, PC3, SKOV3, and HSC3 in a concentration-dependent manner.

The IC_{50} value of WJMSCs-norCM and WJMSCs-hypoCM (concentration of anticancer candidate, which could inhibit 50% cell proliferation) was found to be 51.690—81.440% (Table 4). Each sample (WJMSCs-norCM and WJMSCs-hypoCM) was done in triplicate and inhibition data were analyzed using probit analysis to obtain the IC_{50}. As shown in Table 4, WJMSCs-norCM and WJMSCs-hypoCM exhibited a cytotoxic effect toward HeLa, HepG2, PC3, SKOV3, and HSC3 cells, and the highest anticancer activity was WJMSCs-hypoCM in HepG2 with IC_{50} 51.690% and in HeLa with IC_{50} 61.425%. The highest anticancer activity of WJMSCs-norCM with IC_{50} was 64.424% in HepG2. WJMSCs-hypoCM had higher anticancer activity to inhibit HeLa, HepG2, pc3, and hsc3 cell lines compared to WJMSCs-norCM.

The selective cytotoxic effect of WJMSCs-norCM and WJMSCs-hypoCM was carried out in NIH3T3, human fibroblast, and hMSCs. The effect of WJMSCs-norCM and WJMSCs-hypoCM on the number of normal cells can be seen in Table 5. The effect of WJMSCs-hypoCM and WJMSCs-norCM on inhibition of normal cells can be seen in Table 6. The IC_{50} value of WJMSCs-norCM and WJMSCs-hypoCM was found to be 136.290—185.339% (Table 7). As shown in Tables 5—7, WJMSCs-norCM and WJMSCs-hypoCM demonstrated a low cytotoxic effect, inhibited low cell proliferation in normal cells, including NIH3T3, human fibroblast cells, and hMSCs. WJMSCs-norCM and WJMSCs-hypoCM were not toxic toward normal cells with IC_{50}>100% concentration of CM.

Discussion

WJ-MSCs was positive for CD105, CD73, and CD90 and negative for CD34, CD45, CD14, CD19, and HLA-II (Table 1). These data were consistent with previous research that hMSCs highly expressed CD105, CD73,[1,10] and CD90[22] and had low expression of CD34, CD45, CD14, CD19, and HLA-II.[1,10,22] The surface marker of WJMSCs of P4 and P8 both normoxic and hypoxic 5% O_2 were not significantly different ($p > 0.05$); these data are consistent with previous research that surface markers expression are positive for CD105, CD73, and CD90 (>95%) and negative for CD34, CD45, CD14, CD19, and HLA-II (<2%).[10,16]

Pluripotency was confirmed by the ability of WJ-MSCs cells to differentiate into osteocytes, chondrocytes, and adipocytes.[24] As demonstrated in Fig. 1, both nor-WJMSCs and hypo-WJMSCs differentiated to osteocytes, chondrocytes, and adipocytes; these findings were consistent with previous research that MSCs possess an extensive potential to proliferate and differentiate. into osteocytes, adipocytes, and chondrocytes.[22,24—26] MSCs can also be isolated from umbilical cord Wharton's jelly and markers expressed by flow cytometry; differentiate into osteoblast, adipocyte- and chondrocyte-like cells; and exhibit multipotent differentiation potential.[27]

After 2 weeks of exposure to adipogenesis induction medium, cells began to show a round shape and most of them contained cytoplasmatic vacuoles, intracellular accumulated lipids, and small oil droplets in the cytoplasm that were positive with Oil Red O staining (Fig. 1B, E, H, K, N, and Q). Control cells grew with proliferative medium were negative with Oil Red O staining (Fig. 1T and W).[18,28,29] These data were validated with previous research, which found that reverse transcription-polymerase chain reaction analysis of adipogenic gene expression also revealed similar degrees of upregulation of lipoprotein lipase, adipocyte fatty acid-binding protein 2 (aP2), and peroxisome proliferator-activated receptor γ2 (PPAR γ2).[18,30]

After 3 weeks of osteogenic induction, the cells produced mineralized matrix by Alizarin red S staining (Fig. 1A,

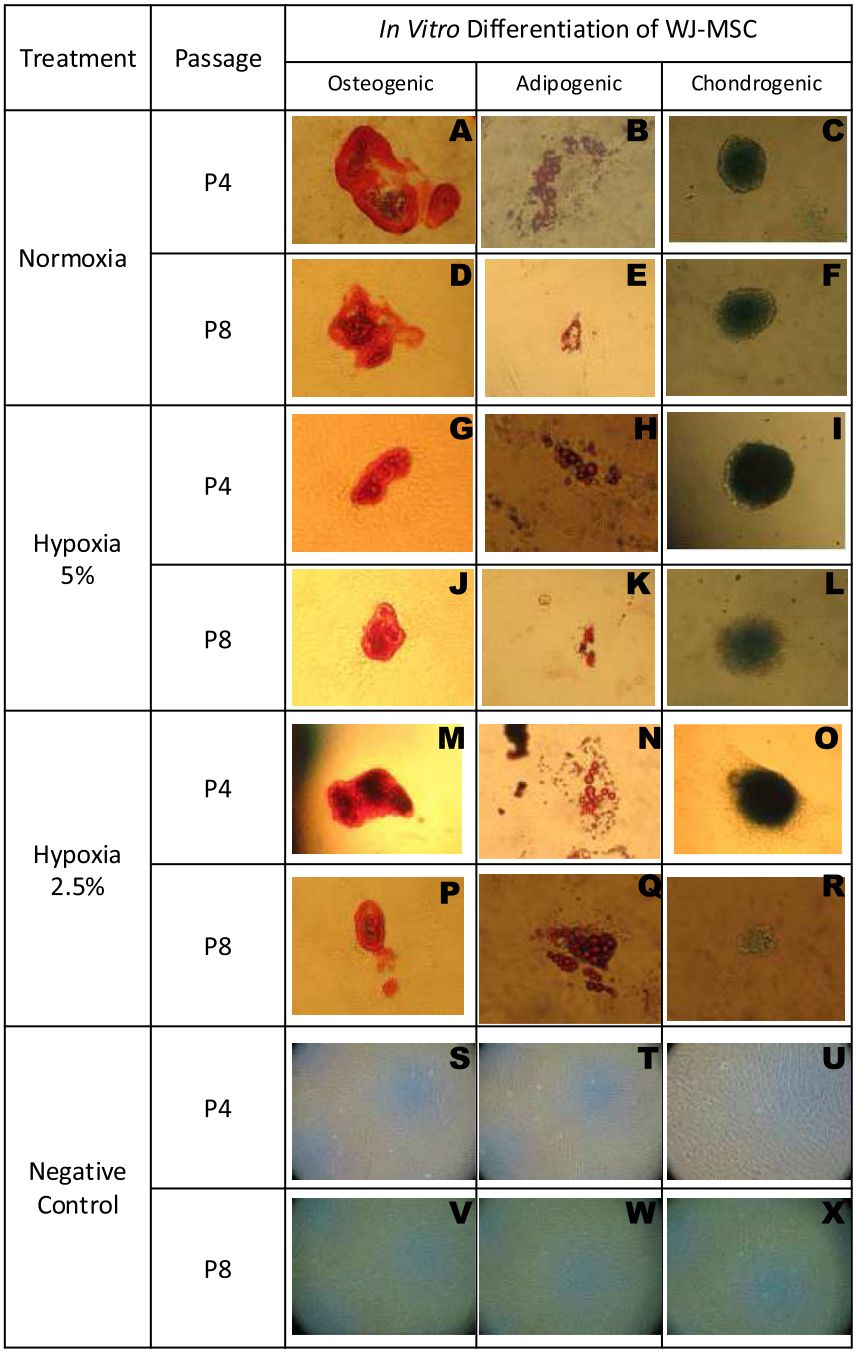


Figure 1 Morphological appearance of osteogenic, adipogenic and chondrogenic differentiation of WJ-norMSCs (normoxia-treated WJ-MSCs) and WJ-hypoMSCs (hypoxia-treated WJ-MSCs) at P4 and P8. For each differentiation protocol, undifferentiated cells were kept as controls (S,T,U,V,W,X).

D, G, J, M, and P), cells displayed bone-like nodular aggregates of matrix mineralization,[25] and were absent in control cultures[28] (Fig. 1S and V). These data are consistent with those of a previous study finding that reverse transcriptase-polymerase chain reaction analysis of osteogenic gene expression also revealed similar levels of upregulation of osteopontin and osteocalcin.[18]

The chondrogenic potential of the MSCs was confirmed with the presence of acidic proteoglycans; it was observed after 2 weeks of chondrogenic differentiation by Alcian blue staining,[23,31] for chondrogenic extracellular matrix containing hyaluronic acids[31] (Fig. 1C, F, I, L, O, and R), the negative controls of chondrogenic differentiation of WJMSCs were represented by MSCs of P4 and P8 not cultured into differentiation media[31] (Fig. 1U and X). According to Fig. 1, WJMSCs of early passage and late passage differentiated to osteocytes, chondrocytes, and adipocytes; these data were validated with the previous finding that MSCs could be expanded to 10 or 11 passages.[32]

Tables 2—4 show that WJMSCs-norCM and WJMSCs-hypoCM were able to inhibit the proliferation of various cancer cells (cervical, liver, prostate, ovarian, and oral squamosa) with

Table 2 Effect of WJMSCs-norCM and WJMSCs-hypoCM toward number of cancer cells.

Cancer cells	Number of cells							
	Normoxia				Hypoxia			
	FBS 10% (WJMSCs-norCM 0%)	WJMSCs-norCM 50%	WJMSCs-norCM 75%	WJMSCs-norCM 100%	WJMSCs-hypoCM 0%	WJMSCs-hypoCM 50%	WJMSCs-hypoCM 75%	WJMSCs-hypoCM 100%
HeLa	$21,900 \pm 591^{d}$	$16,273 \pm 3,340^{c}$	$11,863 \pm 419^{b}$	$1,343 \pm 211^{a}$	$24,913 \pm 2,012^{d}$	$16,216 \pm 240^{c}$	$10,280 \pm 326^{b}$	$1,670 \pm 55^{a}$
HepG2	$21,508 \pm 912^{c}$	$12,692 \pm 940^{b}$	$12,968 \pm 775^{b}$	$2,332 \pm 270^{a}$	$24,062 \pm 630^{c}$	$11,392 \pm 959^{b}$	$10,952 \pm 1,466^{b}$	$5,152 \pm 167^{a}$
PC3	$20,701 \pm 456^{d}$	$19,361 \pm 1,057^{c}$	$15,081 \pm 172^{b}$	$3,051 \pm 199^{a}$	$22,748 \pm 1,522^{d}$	$19,888 \pm 584^{c}$	$12,778 \pm 120^{b}$	984 ± 127^{a}
SKOV3	$18,105 \pm 1,385^{d}$	$11,229 \pm 864^{b}$	$12,902 \pm 196^{c}$	$1,892 \pm 96^{a}$	$17,959 \pm 2,082^{c}$	$17,115 \pm 725^{c}$	$12,632 \pm 150^{b}$	$2,982 \pm 795^{a}$
HSC3	$18,528 \pm 3,041^{c}$	$15,515 \pm 491^{b}$	$12,885 \pm 530^{b}$	918 ± 30^{c}	$17,625 \pm 3,168^{d}$	$13,985 \pm 266^{c}$	$7,865 \pm 230^{b}$	938 ± 334^{a}

Data are expressed as mean ± standard deviation, different letters in the same row (among concentrations of WJMSCs-norCM and among concentrations of WJMSCs-hypoCM) are significant differences at $p < 0.05$ (Tukey honestly significant difference *post hoc* test).
FBS = fetal bovine serum; WJMSCs = mesenchymal stem cells derived from Wharton's jelly; WJMSCs-hypoCM = hypoxia-treated WJMSCs conditioned medium; WJMSCs-norCM = normoxia-treated WJMSCs conditioned medium.
The data (number of cancer cells) are expressed as mean ± standard deviation, different superscript letters in the same row (a, b, c, d) are significant differences at $p < 0.05$ (Tukey honestly significant difference post hoc test). Among the means of groups (type of cancer cell lines in normoxia or hypoxia).

Table 3 Effect of WJMSCs-norCM and WJMSCs-hypoCM toward inhibition of cancer cells.

Cancer cells	Inhibition (%)							
	Normoxia				Hypoxia			
	FBS 10% (WJMSCs-norCM 0%)	WJMSCs-norCM 50%	WJMSCs-norCM 75%	WJMSCs-norCM 100%	WJMSCs-hypoCM 0%	WJMSCs-hypoCM 50%	WJMSCs-hypoCM 75%	WJMSCs-hypoCM 100%
HeLa	0.00 ± 2.70^{a}	25.69 ± 5.25^{b}	45.83 ± 1.91^{c}	93.87 ± 0.96^{d}	0.00 ± 8.08^{a}	34.91 ± 0.96^{b}	58.74 ± 1.31^{c}	93.30 ± 0.22^{d}
HepG2	0.00 ± 4.24^{a}	40.99 ± 4.37^{b}	39.70 ± 3.60^{b}	89.16 ± 1.26^{c}	0.00 ± 2.62^{a}	52.66 ± 3.98^{b}	54.49 ± 6.09^{b}	78.59 ± 0.69^{c}
PC3	0.00 ± 2.20^{a}	6.47 ± 0.51^{b}	27.15 ± 0.83^{c}	85.26 ± 0.96^{d}	0.00 ± 6.69^{a}	12.57 ± 2.57^{b}	43.83 ± 0.53^{c}	95.67 ± 0.56^{d}
SKOV3	0.00 ± 7.65^{a}	37.98 ± 4.77^{b}	28.74 ± 1.08^{b}	89.55 ± 0.53^{c}	0.00 ± 11.59^{a}	4.70 ± 4.03^{a}	29.66 ± 0.83^{b}	83.40 ± 4.43^{c}
HSC3	0.00 ± 16.41^{a}	16.26 ± 2.65^{b}	30.46 ± 2.86^{b}	95.05 ± 0.16^{c}	0.00 ± 17.98^{a}	20.65 ± 1.51^{b}	55.38 ± 1.31^{c}	94.68 ± 1.90^{d}

Data are expressed as mean ± standard deviation, different letters in the same row (among concentrations of WJMSCs-norCM, among concentrations of WJMSCs-hypoCM) are significant differences at $p < 0.05$ (Tukey honestly significant difference *post hoc* test).
FBS = fetal bovine serum; WJMSCs = mesenchymal stem cells derived from Wharton's jelly; WJMSCs-hypoCM = hypoxia-treated WJMSCs conditioned medium; WJMSCs-norCM = normoxia-treated WJMSCs conditioned medium.
The data (inhibition of cancer cells) are expressed as mean ± standard deviation, different superscript letters in the same row (a, b, c, d) are significant differences at $p < 0.05$ (Tukey honestly significant difference post hoc test). Among the means of groups (type of cancer cell lines in normoxia or hypoxia).

Table 4 The IC_{50} of WJMSCs-norCM and WJMSCs-hypoCM in various cancer cell lines for 72 hours of incubation.

Cancer cell lines	IC_{50} (%)	
	WJMSCs-norCM	WJMSCs-hypoCM
HeLa	67.77	61.43
HepG2	64.42	51.69
PC3	80.97	71.86
SKOV3	74.43	81.44
HSC3	74.19	66.93

IC_{50} = median inhibitory concentration; WJMSCs = mesenchymal stem cells derived from Wharton's jelly; WJMSCs-hypoCM = hypoxia-treated WJMSCs conditioned medium; WJMSCs-norCM = normoxia-treated WJMSCs conditioned medium.

various activities. These data were validated with previous studies that hMSCs can be used for neoplastic transformation and can be developed for novel anticancer therapeutics[33]; human Wharton's jelly stem cells inhibited certain solid tumors.[4,34–36] UCMSCs significantly inhibit proliferation of cancer cell lines by *in vivo* and *in vitro* assay.[4,37] Unengineered human and rat UC-MSCs significantly attenuate proliferation of breast cancer cell lines *in vitro* and *in vivo*,[4] rat mammary tumor cells,[37] human lung cancer cells,[38] mouse Lewis lung carcinoma cells,[39] and mouse pancreatic carcinoma cells.[5,40] Human umbilical cord mesenchymal stem cells (hUCMSCs) are able to inhibit breast cancer cell proliferation (MDA-MB-231) in a severe combined immunodeficiency (SCID) mouse model through secretion of dickkopf and suppression of the Wnt pathway.[35] hWJMSCs-conditioned medium (hWJSC-CM; 50%) or hWJSCs-cell lysate (hWJSC-CL) 15 μg/mL for 48–72 hours inhibit cancer cell proliferation in breast adenocarcinoma (MDA-MB-231), ovarian carcinoma (TOV-112D), and osteosarcoma (MG-63) cells. The cancer cell lines exhibited cell shrinkage, blebbing, and vacuolations compared to controls.[41] The inhibition was 20–26% and 31–46% for hWJSC-CM and hWJSC-CL, respectively, for all three cancer cell lines. Cell cycle assays show increases in sub-G1 and G2/M phases for all three cancer cell lines suggestive of apoptosis and metaphase arrest.[41] hWJSCs migrated to metastatic tumor sites in the lungs and reduced tumor burden after hWJSCs were administered intravenously 8 days after tumor transplantation in a rat model.[4,37,42] Engineered hWJSCs-expressed human interferon-β inhibited breast tumor growth in animal models.[43] hWJSCs inhibit human mammary carcinoma proliferation.[41]

Conditioned medium and cell-free lysate of hWJSCs (hWJSC-CM and hWJSC-CL) inhibit the growth of a range of cancer cells, including breast cancer (MDA-MB-231) and ovarian cancer cells (TOV-112D), as well as osteosarcoma cells (MG-63).[41] Exposure of the osteosarcoma cell lines SKES-1 and MG-63 to hWJSC-CL and hWJSC-CM results in cell death and significant growth inhibition *in vitro*. At the molecular level, there is a simultaneous upregulation of proapoptotic and autophagy-related genes, such as BAX, ATG-5, and BECLIN-1, and downregulation of prosurvival genes, such as BCL-2 and SURVIVIN. *In vivo*, there was a notable reduction in mammary tumor sizes and weights in immunodeficient mice at 6 weeks after the injections of

Table 5 Effect of WJMSCs-norCM and WJMSCs-hypoCM toward number of normal cells.

Cancer cells	Number of cells							
	Normoxia				Hypoxia			
	FBS 10% (WJMSCs-norCM 0%)	WJMSCs-norCM 50%	WJMSCs-norCM 75%	WJMSCs-norCM 100%	WJMSCs-hypoCM 0%	WJMSCs-hypoCM 50%	WJMSCs-hypoCM 75%	WJMSCs-hypoCM 100%
NIH3T3	$50{,}912 \pm 2.991^{b}$	50.379 ± 1.346^{b}	53.157 ± 1.405^{b}	36.979 ± 192^{a}	27.331 ± 459^{c}	26.447 ± 109^{c}	24.266 ± 164^{b}	21.070 ± 729^{a}
Human fibroblast	23.650 ± 2.219^{b}	18.725 ± 1.692^{a}	19.771 ± 793^{a}	16.517 ± 80^{a}	12.163 ± 151^{d}	11.142 ± 116^{c}	9.579 ± 45^{b}	8.564 ± 168^{a}
MSCs	18.976 ± 2.540^{b}	18.309 ± 658^{b}	19.832 ± 115^{b}	13.742 ± 133^{a}	26.844 ± 608^{d}	24.762 ± 249^{c}	21.926 ± 429^{b}	20.183 ± 476^{a}

Data are expressed as mean ± standard deviation, different letters in the same row (among concentrations of WJMSCs-norCM, among concentrations of WJMSCs-hypoCM) are significant differences at $p < 0.05$ (Tukey honestly significant difference *post hoc* test).
FBS = fetal bovine serum; WJMSCs = mesenchymal stem cells derived from Wharton's jelly; WJMSCs-hypoCM = hypoxia-treated WJMSCs conditioned medium; WJMSCs-norCM = normoxia-treated WJMSCs conditioned medium.
The data (number of normal cells) are expressed as mean ± standard deviation, different superscript letters in the same row (a, b, c, d) are significant differences at $p < 0.05$ (Tukey honestly significant difference post hoc test). Among the means of groups (type of normal cells in normoxia or hypoxia).

Table 6 Effect of WJMSCs-norCM and WJMSCs-hypoCM toward inhibition of normal cells.

Cancer cells	Inhibition (%)							
	Normoxia				Hypoxia			
	FBS 10% (WJMSCs-norCM 0%)	WJMSCs-norCM 50%	WJMSCs-norCM 75%	WJMSCs-norCM 100%	WJMSCs-hypoCM 0%	WJMSCs-hypoCM 50%	WJMSCs-hypoCM 75%	WJMSCs-hypoCM 100%
NIH3T3	0.00 ± 5.87^{a}	1.05 ± 2.64^{a}	-4.41 ± 2.76^{a}	27.37 ± 0.38^{b}	0.00 ± 1.68^{a}	3.23 ± 0.40^{a}	11.21 ± 0.60^{b}	22.91 ± 2.67^{c}
Human fibroblast	0.00 ± 9.38^{a}	20.82 ± 7.16^{b}	16.40 ± 3.35^{b}	30.16 ± 0.34^{b}	0.00 ± 1.24^{a}	8.40 ± 0.95^{b}	21.25 ± 0.37^{c}	29.59 ± 1.38^{d}
MSCs	0.00 ± 13.39^{a}	3.51 ± 3.47^{a}	-4.51 ± 0.61^{a}	27.58 ± 0.70^{b}	0.00 ± 2.26^{a}	7.76 ± 0.93^{b}	18.32 ± 1.60^{c}	24.81 ± 1.77^{d}

Data are expressed as mean ± standard deviation, different letters in the same column (among concentrations of WJMSCs-hypoCM, among concentrations of WJMSCs-hypoCM) are significant differences at $p < 0.05$ (Tukey honestly significant difference *post hoc* test).
FBS = fetal bovine serum; WJMSCs = mesenchymal stem cells derived from Wharton's jelly; WJMSCs-hypoCM = hypoxia-treated WJMSCs conditioned medium; WJMSCs-norCM = normoxia-treated WJMSCs conditioned medium.
The data (inhibition of normal cells) are expressed as mean ± standard deviation, different superscript letters in the same row (a, b, c, d) are significant differences at $p < 0.05$ (Tukey honestly significant difference post hoc test). Among the means of groups (type of normal cells in normoxia or hypoxia).

Table 7 The median inhibitory concentration IC_{50} of WJMSCs-norCM and WJMSCs-hypoCM in various normal cells for 72 hours of incubation.

Normal cells	IC_{50} (%)	
	WJMSCs-norCM	WJMSCs-hypoCM
NIH3T3	136.29	159.33
Human fibroblast	148.47	152.48
MSCs	140.44	185.34

IC_{50} = median inhibitory concentration; MSCs = mesenchymal stem cells; WJMSCs = mesenchymal stem cells derived from Wharton's jelly; WJMSCs-hypoCM = hypoxia-treated WJMSCs conditioned medium; WJMSCs-norCM = normoxia-treated WJMSCs conditioned medium.

hWJSC-CL and hWJSC-CM into these tumors. These findings suggest that hWJSC-CL and hWJSC-CM may interfere with the growth of mammary carcinoma and osteosarcoma cells via apoptosis and autophagy.[41] A similar cell death mechanism is observed during co-culture of WJMSCs with the prostate cancer cell line (PC3). In the presence of WJMSCs, PC3 cells exhibit caspase 9/3, PARP, and BAX induction, c-Jun NH2-terminal kinase (JNK) activation, as well as a decrease in phosphatidylinositol 3-kinase (PI3K)/AKT (also known protein kinase B (PKB)) and extracellular signal-regulated kinase (ERK) phosphorylation. Simultaneously, there is a downregulation of prosurvival gene expressions, such as BCL-2, BCL-XL, SURVIVIN, Mcl-1, and cIAP-1.[4,44–47]

The tumoricidal activity of hWJSCs-CM is probably mediated by certain soluble factors secreted by these cells into their extracellular environment, such as interleukins, cell adhesion molecules, hyaluronic acid, growth factors, and glycosoaminoglycans.[44,48,49] Indeed, proteomic analysis of hWJSC-CM shows significantly high levels of interleukins (IL-1α, IL-6, IL-7, and IL-8), stem cell factor, human growth factor, and intercellular adhesion molecule-1.[44] Moreover, the extracellular matrix of WJMSCs also contains dickkopf-1, a protein known to suppress the Wnt signaling pathway.[35,48] Likewise, bone marrow MSCs conditioned medium suppresses the proliferation of two hepatoma cell lines *in vitro* and induces tumor regression in a hepatoma SCID mouse xenograft model by means of Wnt signaling pathway regulations.[45,47,48] Engineered bone marrow MSCs are found to secrete IL-12, which inhibits the growth of melanoma and cervical cancer cells through the induction of a tumor-specific T cell response *in vivo*.[45] Moreover, bone marrow MSCs also express several suicide genes, which halt the proliferation of prostate cancer cells in an athymic murine model.[45] In addition to the upregulation of several proapoptotic and tumor suppressor genes in hWJSCs, transcriptomic studies have also found an increased expression of several cytokines in these cells, such as IL-12α, which are thought to induce apoptosis and thereby mediate the anticancer effects of hWJSCs, hWJSC-CM, and hWJSC-CL.[50,51]

The IL-12 gene promoted the activation of the cellular immune response via expression of a Th1-type cytokine profile and was associated with the inhibition of tumor growth.[3,52] IL-12 treatment represents a novel approach for gene therapy against cervical cancer.[51] IL-8 of hWJSCs killed the cancer cells.[41] Hyaluronan oligosaccharides

inhibited the growth of osteosarcoma cell lines (MG-63 and LM-8)[53] and glycosoaminoglyans inhibited the cell proliferation of osteoblasts and osteosarcoma cells.[54]

UC-MSCs expressed the multiple tumor suppressor gene.[5] hUCMSC are able to inhibit human breast cancer cells by attenuating primarily the AKT and mitogen-activated protein kinase pathways and stimulating the intrinsic apoptosis pathway.[5] hUCMSC attenuated the growth of cancer cells and mainly by attenuation of Erk-1/2 and PI3K/AKT signaling and intrinsic apoptosis.[5]

Nor-WJMSCs and hypo-WJMSCs from P4 and P8 showed no significant differences in MSCs surface marker expression and MSCs differentiation. WJMSCs-norCM and WJMSCs-hypoCM could inhibit cells proliferation in various cancer cell lines, and were not toxic for normal cells.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

The authors gratefully acknowledge the financial support from the Ministry of Research and Technology (Research Grant No KP-2013-0715 and KP-2014-0713). This research was also supported by the Stem Cell and Cancer Institute, Jakarta, Indonesia.

References

1. Weiss ML, Medicetty S, Bledsoe AR, et al. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells*. 2006;24:781—792.
2. Weiss ML, Troyer DL. Stem cells in the umbilical cord. *Stem Cell Rev*. 2006;2:155—162.
3. Weiss ML, Anderson C, Medicetty S, et al. Immune properties of human umbilical cord Wharton's jelly-derived cells. *Stem Cells Express*. 2008:2865—2874.
4. Ayuzawa R, Doi C, Rachakatla RS, et al. Naive human umbilical cord matrix derived stem cells significantly attenuate growth of human breast cancer cells in vitro and in vivo. *Cancer Lett*. 2009;280:31—37.
5. Tamura M, Kawabata A, Ohta N, et al. Wharton's jelly stem cells as agents for cancer therapy. *The Open Tissue Eng Regen Med J*. 2011;4:39—47.
6. Prasanna SJ, Gopalakrishnan D, Shankar SR, et al. Proinflammatory cytokines, IFNgamma and TNFalpha, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. *PLoS One*. 2010;5:e9016.
7. Puranik SB, Nagesh A, Guttedar RS. Isolation of mesenchymal-like cells from Wharton's jelly of umbilical cord. *IJPCBS*. 2012;2:218—224.
8. Can A, Karahuseyinoglu S. Concise review: human umbilical cord stroma with regard to the source of fetus-derived stem cells. *Stem Cells*. 2007;25:2886—2895.
9. Bongso A, Fong C-F, Gauthaman K. Taking stem cells to the clinic: major challenges. *J Cell Biochem*. 2008;105:1352—1360.
10. Widowati W, Wijaya L, Bachtiar I, et al. Effect of oxygen tension on proliferation and characteristics of Wharton's jelly-derived mesenchymal stem cells. *Biomarkers Genomic Med*. 2014;6:43—48.
11. Jones BJ, McTaggart SJ. Immunosuppression by mesenchymal stromal cells: from culture to clinic. *Exp Hematol*. 2008;36:733—741.
12. Menon LG, Shi VJ, Carroll RS. *Mesenchymal Stromal Cells as a Drug Delivery System*. Boston, MA 02115, USA: Stem Book; 2009:1—14. The Stem Cell Research Community.
13. Grayson WL, Zhao F, Bunnell B, et al. Hypoxia enhances proliferation and tissue formation of human mesenchymal stem cells. *Biochem Biophys Res Commun*. 2007;358:948—953.
14. Hung SP, Yang MH, Tseng KF, et al. Hypoxia-Induced secretion of TGF-β1 in mesenchymal stem cell promotes breast cancer cell progression. *Cell Transplantation*. 2013;22:1869—1882.
15. Nekanti U, Dastidar S, Venugopal P, et al. Increased proliferation and analysis of differential gene expression in human Wharton's jelly-derived mesenchymal stromal cells under hypoxia. *Int J Biol Sci*. 2010;6:499—512.
16. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. *Cytotherapy*. 2006;8:315—317.
17. Zheng L, Zhang D, Chen X, et al. Antitumor activities of human placenta-derived mesenchymal stem cells expressing endostatin on ovarian cancer. *Plos One*. 2012;7:e39119.
18. Jun EK, Zhang Q, Yoon BS, et al. Hypoxic conditioned medium from human amniotic fluid-derived mesenchymal stem cells accelerates skin wound healing through TGF-β/SMAD2 and PI3K/Akt pathways. *Int J Mol Sci*. 2014;15:605—628.
19. Nakahara M, Okumura N, Kay EP, et al. Corneal endothelial expansion promoted by human bone marrow mesenchymal stem cell-derived conditioned medium. *Plos One*. 2013;8:e69009.
20. Widowati W, Mozef T, Risdian C, et al. Anticancer and free radical scavenging potency of *Catharanthus roseus*, *Dendrophthoe petandra*, *Piper betle* and *Curcuma mangga* extracts in breast cancer cell lines. *Oxid Antioxid Med Sci*. 2013;2:137—142.
21. Widowati W, Wijaya L, Wargasetia TL, et al. Antioxidant, anticancer, and apoptosis-inducing effects of *Piper* extracts in HeLa cells. *J Exp Integr Med*. 2013;3:225—230.
22. Shen Z-Y, Zhang J, Song H-L, et al. Bone-marrow mesenchymal stem cells reduce rat intestinal ischemia-reperfusion injury, ZO-1 downregulation and tight junction disruption via a TNF-α-regulated mechanism. *World J Gastroenterol*. 2013;19:3583—3595.
23. Cardoso TC, Ferrari HF, Garcia AF, et al. Isolation and characterization of Wharton's jelly-derived multipotent mesenchymal stromal cells obtained from bovine umbilical cord and maintained in a defined serum-free three-dimensional system. *BMC Biotechnology*. 2012;12(8):1—12.
24. Jaiswal RK, Jaiswal N, Bruder SP, et al. Differentiation to the osteogenic or adult human mesenchymal stem cell adipogenic lineage is regulated by mitogen-activated protein kinase. *J. Biol. Chem*. 2000;275:9645—9652.
25. Oswald J, Boxberger S, Jørgensen B, et al. Mesenchymal stem cells can be differentiated into endothelial cells *in vitro*. *Stem Cells*. 2004;22:377—384.
26. Heino TJ, Hentunen TA. Differentiation of osteoblasts and osteocytes from mesenchymal stem cells. *Curr Stem Cell Res Ther*. 2008;3:131—145.
27. Zhang YN, Lie PC, Wei X. Differentiation of mesenchymal stromal cells derived from umbilical cord Wharton's jelly into hepatocyte-like cells. *Cytotherapy*. 2009;11:548—558.
28. Conconi MT, Burra P, Di Liddo R, et al. CD105(+) cells from Wharton's jelly show *in vitro* and *in vivo* myogenic differentiative potential. *Int J Mol Med*. 2006;18:1089—1096.
29. Amable PR, Teixeira MVT, Carias RBV, et al. Protein synthesis and secretion in human mesenchymal cells derived from bone marrow, adipose tissue and Wharton's jelly. *Stem Cell Res Ther*. 2014;5:1—13.

30. Wang D, Chen K, Du WT, et al. CD14+ monocytes promote the immunosuppressive effect of human umbilical cord matrix stem cells. *Exp Cell Res*. 2010;316:2414—2423.
31. Corotchi MC, Popa MA, Remes A, et al. Isolation method and xeno-free culture conditions influence multipotent differentiation capacity of human Wharton's jelly-derived mesenchymal stem cells. *Stem Cell Res Ther*. 2013;4:1—18.
32. Nakamizo A, Marini F, Amano T, et al. Human bone marrow—derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res*. 2005;65:3307—3318.
33. Serakinci N, Guldberg P, Burns JS, et al. Adult human mesenchymal stem cell as a target for neoplastic transformation. *Oncogene*. 2004;23:5095—5098.
34. Rachakatla RS, Marini F, Weiss ML, et al. Development of human umbilical cord matrix stem cell-based gene therapy for experimental lung tumors. *Cancer Gene Ther*. 2007;14:828—835.
35. Sun L, Wang D, Liang J, et al. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. *Arthritis Rheum*. 2010;62:2467—2475.
36. Chao K-C, Tang H-Y, Chen M-Y. Human umbilical cord mesenchymal stem cells suppress breast cancer tumourigenesis through direct cell—cell contact and internalization. *J Cell Mol Med*. 2012;16:1803—1815.
37. Ganta C, Chiyo D, Ayuzawa R, et al. Rat umbilical cord stem cells completely abolish rat mammary carcinomas with no evidence of metastasis or recurrence 100 days post-tumor cell inoculation. *Cancer Res*. 2009;69:1815—1820.
38. Matsuzuka T, Rachakatla RS, Doi C, et al. Human umbilical cord matrix-derived stem cells expressing interferon-beta gene significantly attenuate bronchioloalveolar carcinoma xenografts in SCID mice. *Lung Cancer*. 2010;70:28—36.
39. Doi C, Egashira N, Kawabata A, et al. Angiotensin II type 2 receptor signaling significantly attenuates growth of murine pancreatic carcinoma grafts in syngeneic mice. *BMC Cancer*. 2010;10(67):1—13.
40. Doi C, Maurya DK, Pyle MM, et al. Cytotherapy with naive rat umbilical cord matrix stem cells significantly attenuates growth of murine pancreatic cancer cells and increases survival in syngeneic mice. *Cytotherapy*. 2010;12:408—417.
41. Gauthaman K, Fong CY, Cheyyatraivendran S, et al. Human umbilical cord wharton's jelly stem cell (hwjsc) extracts inhibit cancer cell growth *in vitro*. *J Cell Biochem*. 2012;113:2027—2039.
42. Maurya DK, Doi C, Kawabata A, et al. Therapy with unengineered naive rat umbilical cord matrix stem cells markedly inhibits growth of murine lung adenocarcinoma. *BMC Cancer*. 2010;10:1—10.
43. Chamberlain G, Fox J, Ashton B, et al. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells*. 2007;25:2739—2749.
44. Fong CY, Gauthaman K, Suganya C, et al. Human umbilical cord Wharton's Jelly stem cells and its conditioned medium support hematopoietic stem cell expansion ex vivo. *J Cell Biochem*. 2012;113:658—668.
45. Seo SH, Kim KS, Park SH, et al. The effects of mesenchymal stem cells injected via different routes on modified IL-12-mediated antitumor activity. *Gene Ther*. 2011;18:488—495.
46. Cavarretta IT, Altanerova V, Matuskova M, et al. Adipose tissue-derived mesenchymal stem cells expressing prodrug converting enzyme inhibit human prostate tumor growth. *Mol Ther*. 2011;18:223—231.
47. Han I, Yun M, Kim E-O, et al. Umbilical cord tissue-derived mesenchymal stem cells induce apoptosis in PC-3 prostate cancer cells through activation of JNK and downregulation of PI3K/AKT signaling. *Stem Cell Res Ther*. 2014;5:1—9.
48. Qiao L, Xu Z, Zhao T, et al. Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model. *Cell Res*. 2008;8:500—507.
49. Angelucci S, Marchisio M, Di Giuseppe F, et al. Proteome analysis of human Wharton's jelly cells during in vitro expansion. *Proteome Sci*. 2010;8:18—25.
50. Kobayashi M, Fitz L, Ryan M, et al. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J Exp Med*. 1989;170:827—845.
51. Wolf SF, Temple PA, Kobayashi M, et al. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J Immunol*. 1991;146:3074—3081.
52. Paz FG, Marina VM, Ortega AM, et al. The relationship between the antitumor effect of the IL-12 gene therapy and the expression of Th1 cytokines in an HPV16-positive murine tumor model. *Mediators Inflamm*. 2014;2014:1—10.
53. Hosono K, Nishida Y, Knudson W, et al. Hyaluronan oligosaccharides inhibit tumorigenicity of osteosarcoma cell lines MG-63 and LM-8 in vitro and in vivo via perturbation of hyaluronan-rich pericellular matrix of the cells. *Am J Pathol*. 2007;171(1):274—286.
54. Nikitovic D, Zafiropoulos A, Tzanakakis GN, et al. Effects of glycosaminoglycans on cell proliferation of normal osteoblasts and human osteosarcoma cells depend on their type and fine chemical compositions. *Anticancer Res*. 2005;25:2851—2856.

Dear Dr. Wahyu Widowati,

We have received your article "Conditioned Medium from Normoxia (WJMSCs-norCM) and Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) in Inhibiting Cancers Cell Proliferation" for consideration for publication in Biomarkers and Genomic Medicine.

Your manuscript will be given a reference number once an editor has been assigned.

To track the status of your paper, please do the following:

1. Go to this URL: http://ees.elsevier.com/bgm/

2. Enter the login details

If you need to retrieve password details, please go to:
http://ees.elsevier.com/BGM/automail_query.asp

3. Click [Author Login]
This takes you to the Author Main Menu.

4. Click [Submissions Being Processed]

Thank you for submitting your work to this journal.

Kind regards,

Elsevier Editorial System
Biomarkers and Genomic Medicine

from: BGM <bgm.jeo@gmail.com>

sender: ees.bgm.0.2ac792.53bbebfe@eesmail.elsevier.com

date: 24 Jul 2014 12:26:51

To: <wahyu_w60@yahoo.com>

Cc: <bgm.jeo@gmail.com>

subject: Comments for Your Submission BGM-D-14-00024

Ms. Ref. No.: BGM-D-14-00024

Title: Conditioned Medium from Normoxia (WJMSCs-norCM) and Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) in Inhibiting Cancers Cell Proliferation

Biomarkers and Genomic Medicine

Dear Dr. Wahyu Widowati,

Your above paper has been reviewed. The reviewers' comments are included below, please carefully address the issues raised in the comments and resubmit the revised manuscript within 14 days.

While you submit a revised manuscript, please outline each change made as raised in the reviewer comments, and/or provide a suitable explanation to the comment that you not addressed.

To submit your revision, please do the following:

1. Go to: http://ees.elsevier.com/bgm/

2. Enter your login details

3. Click [Author Login]

This takes you to the Author Main Menu.

4. Click [Submissions Needing Revision]

I look forward to receiving your revised manuscript.

Yours sincerely,

Li-Chen Yen, Ph.D.

Managing Editor

Biomarkers and Genomic Medicine

Reviewers' comments:

Reviewer #1:

The manuscript entitled "Conditioned Medium from Normoxia (WJMSCs-norCM) and Hypoxia-Treated WJMSCs (WJMSCs-hypoCM) in Inhibiting Cancers Cell Proliferation" describes the conditioned medium (CM) isolated from hypoxia-treated WJMSCs is able to suppress the proliferation of various cancer cell lines. They also found that the expression of surface markers in MSCs is not affected by the CM treatment. Overall the experimental design and data are very

straightforward. The presentation might need lots of work especially the writing and editing. It is also recommended to rephrase the Abstract and check the grammar again.

1. Novelty:

There are several references describe how the MSCs are derived from various sources and their deduced conditional medium are able to support or suppress the tumoral growth. Also, the possible molecular mechanism that regulates the MSC reaction to hypoxic condition has been revealed. Hypoxic MSCs might secret TGF□ that interferes the tumor growth/metastasis. These information might help to define the reported studies and they ought to be mentioned in the Introduction or the Discussion sections.

2. Tables and Statistics:

The significances of the Statistic tests need to be labeled on the tables or mentioned clearly in the Result section. There are several minor errors need to be corrected. For example, in Table 1, there are 0.000 or 0.0033 and they should all be four or three digits. What do a, b, c and d stand for in Tables 2 and 3? It is recommended for the authors to read them closely and correct them.

3. Writing:

The Abstract needs to be rephrased. There are several inconsistent writings, for example, Page 6, lines 45-46: "CD 105, CD 73, CD 14, CD 19,, CD 34 and…", which is different from the description on the same page line 30: "CD14, CD19, CD34, CD45 and….". Similar inconsistency needs to be corrected through all the manuscript.

Reviewer #2:

In this study, the authors first characterized that WJMSCs differentiated to osteocyte, chondrocyte, adipocyte under normoxic and hypoxic conditions. They fruther determined the inhibitory effect of WJMSCs-norCM and WJMSCshypoCM on the growth proliferation of several human cancer cells compared to normal cells including mouse fibroblast (NIH3T3) and human fibroblast cells. They found that WJMSCs-norCM and WJMSCs-hypoCM could inhibit cell proliferation in various cancer cell lines, but no growth inhibitory effect on normal cells. Although this study is very preliminary, this is an interesting study that has some potential to be published. However, some points may help to improve the manuscript. For example, some minor typographical errors (like servical (HeLa)..) and need to recheck some paragraph-spacing.

Comments

1. The information of antibodies used in this study should be added in the Material and Methods.

2. NIH3T3, fibroblast and MSC are all belong to plastic fibroblast like cells; however, most cancers are cancers of the epithelial cells. The authors should selected normal epithelial cells to serve as controls.

3. The author should consider performing some experiments like Flow analysis to present the cytotoxic effects of WJMSCs-norCM and WJMSCs-hypoCM on cancer cells in vitro.

4. It will be of great interest to if identify the key factors which mediated the growth inhibition of WJMSCs-norCM and WJMSCs-hypoCM on human cancer cells in vitro.

Dear Dr. Wahyu Widowati,

I have successfully received your revision and will proceed further. I will notify you the final decision for your submission at the earliest.

Thank you for your kind cooperation.

Best regards,
LiChen Yen

Dear Dr. Wahyu Widowati,

Thank you for your support to BGM.

We have not received the signed copyright transfer agreement (CTA) for the following article yet. Please sign and send them back, and then we will schedule to include it in the regular issue. Thank you.

If you have any question, please feel free to contact me.

Best regards,
LiChen Yen

Dear Dr. Wahyu Widowati,

I like to notify you in advance that the holding editor has decided to accept your manuscript which has been well revised. However, before sending the final decision letter to you, we request you to address the following issue.

1. In Discussion, there are several identical paragraphs to other literatures (especially Ref-1&2 marked in the attached CrossCheck report), please rewrite these sentences in your own words.

2. Editorial revision: please see the notations in the attached file [BGM-D-14-00024R1(marked)].

While you complete the revision, please directly send me the manuscript via email and then I will proceed further. Looking forward to hearing from you. Thank you.

Best regards,

LiChen Yen

Managing Editor

Biomarkers and Genomic Medicine

Dear Dr. Wahyu Widowati,

Sorry for the late reply.

Thank you for providing the signed CTA. We have scheduled to include this article in BGM Vol. 7 No.1 (March, 2015).

About the correction you request, I am sorry that the author cannot revise the online-published article. In your case, this revision can be made only when I, Editorial Office, provide the correction for the print file. Thus I will assist correcting your article as you request when the issue is compiled.

If you still have any question, please feel free to contact me. Thank you.

Best regards,

LiChen Yen

Dear Dr. Wahyu Widowati,

I knew that you like to revise the Acknowledgment and Reference. But I am sorry that your article has been online-published and cannot be revised anymore at current stage. The only opportunity to revise it is when I correct the final version for print file. The issue vol.7 No.1 will be compiled around February, 2015, so I will correct your article as your request then.

If you have any question, please feel free to contact me. Thank you.

Best regards,
LiChen Yen

LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau *PEER REVIEW*

KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel)	:	Conditioned medium from normoxia (WJMSCs-norCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation
Jumlah Penulis	:	10 Orang
Nama-nama Penulis	:	Wahyu Widowati, Laura Wijaya, Harry Murti, Halida Widyastuti, Dwi Agustina, Dian Ratih Laksmitawati, Nurul Fauziah, Sutiman B. Sumitro, M. Aris Widodo, Indra Bachtiar
Status Penulis	:	Penulis Pertama / ~~Penulis ke ....~~ / Penulis Korespondensi **)
Identitas Jurnal Ilmiah	: a.	Nama jurnal : Biomarkers and Genomic Medicine
	b.	Nomor ISSN : 2214-0247
	c.	Vol., No., Bulan, Tahun : Vol 7; No 1; Oct 2015;
	d.	Penerbit : Elsevier Taiwan LLC.
	e.	DOI Artikel (jika ada) : 10.1016/j.bgm.2014.08.008
	f.	Alamat Web Jurnal : https://www.sciencedirect.com/journal/biomarkers- and-genomic-medicine
	g.	Terindeks di : Scopus, SJR 0.195

Kategori Publikasi Jurnal Ilmiah: *(beri tanda √ yang dipilih)*

- [√] ~~Jurnal Ilmiah Internasional~~ / Internasional Bereputasi **)
- [ ] Jurnal Ilmiah Nasional Terakreditasi
- [ ] Jurnal Ilmiah Nasional / Nasional terindeks ***)

HASIL PENILAIAN (*Peer Review*) :

No	Komponen Yang Dinilai		Nilai Maksimal JURNAL ILMIAH			Nilai Akhir Yang Diperoleh *)
			Internasional / Bereputasi [√]	Nasional Terakreditasi [ ]	Nasional ***) [ ]	
a.	Kelengkapan unsur isi karya	(10%)	4			3,4
b.	Ruang lingkup dan kedalaman pembahasan	(30%)	12			11,7
c.	Kecukupan dan kemutakhiran data/ informasi dan metodologi	(30%)	12			11,2
d.	Kelengkapan unsur dan kualitas penerbitan	(30%)	12			11,4
	Total	**100%**	**40**			37,7

Catatan Penilaian ARTIKEL oleh Reviewer :

a. Kelengkapan dan kesesuaian unsur: Paper ditulis dengan baik, hasil sesuai dengan judul penelitian, mengikuti kaidah penulisan

b. Ruang lingkup & kedalaman pembahasan: Penelitian tentang potensi conditioned medium dari WJMSCs yang dikultur hipoksia dan normoksia dalam menghambat berbagai jenis sel kanker

c. Kecukupan & kemutakhiran data serta metodologi..... Ide dasar penelitian baik membahas mekanisme WJMSCs-CM dalam menghambat proliferasi sel kanker

d. Kelengkapan unsur dan kualitas penerbit ..... Jurnal BBM terindeks scopus SJR 0,195 . Penerbit Elsevier

e. Indikasi plagiasi ..... Tidak terdapat indikasi plagiarism atau self plagiarism

f. Kesesuaian bidang ilmu ..... Paper bidang biomedis sesuai dengan bidang ilmu penulis

REVIEWER 1

(Prof. Dr. Chrismis Novalinda Ginting, M.Kes)
NIK : 0115127801
UNIVERSITAS PRIMA INDONESIA

LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau *PEER REVIEW*

KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel)	:	Conditioned medium from normoxia (WJMSCs-norCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation
Jumlah Penulis	:	10 Orang
Nama-nama Penulis	:	**Wahyu Widowati**, Laura Wijaya, Harry Murti, Halida Widyastuti, Dwi Agustina, Dian Ratih Laksmitawati, Nurul Fauziah, Sutiman B. Sumitro, M. Aris Widodo, Indra Bachtiar
Status Penulis	:	Penulis Pertama / ~~Penulis ke ....~~ / Penulis Korespondensi **)
Identitas Jurnal Ilmiah	: a.	Nama jurnal : Biomarkers and Genomic Medicine
	b.	Nomor ISSN : 2214-0247
	c.	Vol., No., Bulan, Tahun : Vol 7; No 1; Maret, 2015;
	d.	Penerbit : Elsevier BV
	e.	DOI Artikel (jika ada) : 10.1016/j.bgm.2014.08.008
	f.	Alamat Web Jurnal : https://www.sciencedirect.com/journal/biomarkers- and-genomic-medicine
	g.	Terindeks di : Scopus, SJR 0.195

Kategori Publikasi Jurnal Ilmiah: *(beri tanda √ yang dipilih)*

- [√] ~~Jurnal Ilmiah Internasional~~ / Internasional Bereputasi **)
- [ ] Jurnal Ilmiah Nasional Terakreditasi
- [ ] Jurnal Ilmiah Nasional / Nasional terindeks ***)

HASIL PENILAIAN (*Peer Review*) :

No	Komponen Yang Dinilai		Nilai Maksimal JURNAL ILMIAH			Nilai Akhir Yang Diperoleh *)
			Internasional / Bereputasi √	Nasional Terakreditasi	Nasional ***)	
a.	Kelengkapan unsur isi karya	(10%)	4			3,5
b.	Ruang lingkup dan kedalaman pembahasan	(30%)	12			11,3
c.	Kecukupan dan kemutakhiran data/ informasi dan metodologi	(30%)	12			11,5
d.	Kelengkapan unsur dan kualitas penerbitan	(30%)	12			11,9
	Total	**100%**	**40**			37,7

Catatan Penilaian ARTIKEL oleh Reviewer :

a. Kelengkapan dan kesesuaian unsur.....
Jurnal ini sudah cukup lengkap serta unsur dan isinya juga sudah sesuai

b. Ruang lingkup & kedalaman pembahasan .....
Ruang lingkup dalam pembahasannya sudah mendalam

c. Kecukupan & kemutakhiran data serta metodologi.....
Secara umum data yang digunakan sudah cukup dan juga sudah mutakhir

d. Kelengkapan unsur dan kualitas penerbit .....
unsur - unsur yang ada di dalam jurnal sudah lengkap dan kualitas penerbit pada jurnal sudah terdeteksi: Scopus Q4 SJR 0,195 dan sudah termasuk kategori baik.

e. Indikasi plagiasi .....
Telah ditemukan adanya unsur plagiasi.

f. Kesesuaian bidang ilmu .....
Jurnal ini sudah sesuai dengan bidang Ilmu yang diteliti

REVIEWER 2

(Prof. Dr. Ermi Girsang, M. Kes)
NIK : 0117057501
UNIVERSITAS PRIMA INDONESIA

LEMBAR HASIL PENILAIAN
SEJAWAT SEBIDANG atau *PEER REVIEW*

KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel)	:	Conditioned medium from normoxia (WJMSCs-norCM) and hypoxia-treated WJMSCs (WJMSCs-hypoCM) in inhibiting cancer cell proliferation
Jumlah Penulis	:	10 Orang
Nama-nama Penulis	:	**Wahyu Widowati**, Laura Wijaya, Harry Murti, Halida Widyastuti, Dwi Agustina, Dian Ratih Laksmitawati, Nurul Fauziah, Sutiman B. Sumitro, M. Aris Widodo, Indra Bachtiar
Status Penulis	:	Penulis Pertama / ~~Penulis ke ....~~ / Penulis Korespondensi **)
Identitas Jurnal Ilmiah	:	

a.	Nama jurnal	:	Biomarkers and Genomic Medicine
b.	Nomor ISSN	:	2214-0247
c.	Vol., No., Bulan, Tahun	:	Vol 7; No 1; Maret, 2015;
d.	Penerbit	:	Elsevier Taiwan LLC
e.	DOI Artikel (jika ada)	:	10.1016/j.bgm.2014.08.008
f.	Alamat Web Jurnal	:	https://www.sciencedirect.com/journal/biomarkers- and-genomic-medicine
g.	Terindeks di	:	Scopus, SJR 0.195

Kategori Publikasi Jurnal Ilmiah: *(beri tanda √ yang dipilih)*

- [√] ~~Jurnal Ilmiah Internasional~~ / Internasional Bereputasi **)
- [ ] Jurnal Ilmiah Nasional Terakreditasi
- [ ] Jurnal Ilmiah Nasional / Nasional terindeks ***)

HASIL PENILAIAN (*Peer Review*) :

No	Komponen Yang Dinilai		Nilai Maksimal JURNAL ILMIAH			Nilai Akhir Yang Diperoleh *)
			Internasional / Bereputasi [√]	Nasional Terakreditasi [ ]	Nasional *** [ ]	
a.	Kelengkapan unsur isi karya	(10%)	4			3,45
b.	Ruang lingkup dan kedalaman pembahasan	(30%)	12			11,5
c.	Kecukupan dan kemutakhiran data/ informasi dan metodologi	(30%)	12			11,35
d.	Kelengkapan unsur dan kualitas penerbitan	(30%)	12			11,9
	Total	**100%**	**40**			37,7

Catatan Penilaian ARTIKEL oleh Reviewer :

a. Kelengkapan dan kesesuaian unsur..... Paper ditulis dengan baik, hasil sesuai dengan judul penelitian, mengikuti kaidah penelitian

Jurnal ini sudah cukup lengkap serta unsur dan isinya sudah sesuai

b. Ruang lingkup & kedalaman pembahasan Penelitian tentang potensi conditioned medium dari WJMSCs yang dikultur hipoksia dan normoksia dalam menghambat berbagai jenis sel kanker.

Ruang lingkup dalam pembahasannya sudah mendalam

c. Kecukupan & kemutakhiran data serta metodologi Ide dasar penelitian baik membahas mekanisme WJMSCs - CM dalam menghambat proliferasi sel kanker

Secara umum data yang digunakan sudah cukup dan sudah mutakhir

d. Kelengkapan unsur dan kualitas penerbit

Jurnal BBM terindeks scopus SJR 0,195 . Penerbit Elsevier

Unsur- unsur yang ada di dalam jurnal sudah lengkap dan kualitas penerbit pada jurnal sudah terindeks scopus Q4 SJR 0,195 dan termasuk kategori baik

e. Indikasi plagiasi

Tidak terdapat indikasi plagiarism atau self plagiarism

tidak ditemukan adanya unsur plagiasi

f. Kesesuaian bidang ilmu

Paper bidang biomedis sesuai dengan bidang ilmu penulis

Jurnal ini sudah sesuai dengan bidang ilmu yang diteliti

Medan, Reviewer 2	Medan, Reviewer 1
(Prof. Dr. Ermi Girsang, M.Kes) NIK : 0117057501	(Prof. Dr. Chrismis Novalinda Ginting, M.Kes) NIK : 0115127801
UNIVERSITAS PRIMA INDONESIA	UNIVERSITAS PRIMA INDONESIA