First Reviewer:

The submitted manuscript aims to evaluate effects of TNFα and IFNγ on MCF7. In particular, the authors used conditioned medium collected from ILs-NK and hWJMSCs coculture and showed that this TNFα/IFNγ-containing conditioned medium increased apoptosis of MCF7. Although not explicitly stated in the manuscript, the significance of this work may lie in the potential use of MSC as delivery vector for cell therapy to enhance cytokine secretion from immune cells (NKs) in the tumor microenvironment.

Respond:

1. This research was really to continue the previous study conducted both CM-WJMSCs for cancer treatment (Widowati et al., 2015) and cell lysate from WJMSCs for cancer therapy (draft).
   I want to develop the cytotoxic effect of both CM and cell of WJMSCs. To increase cytotoxic activity of CM and cells of WJMSCs can be done by transfecting TNFα and IFNγ plasmid into WJMSCs. The final objective of this study is to transfect MSCs using TNFα and IFNγ plasmid, which is still on progress. The preliminary study proved that TNFα and IFNγ secreted by MSCs can induce apoptosis and other studies proved that MSCs transfected with TNFα and IFNγ can induce apoptosis of some cancer cell lines. Pure TNFα and IFNγ recombinant were used as positive control.
2. TNFα and IFNγ have been used as cancer treatment, the novelty of my research was to increase cytotoxic activity of WJMSCs using activated-NK cells. Activated-NK cells using Interleukins (IL2, IL15, Il18) showed the increase of activating receptor of NK cells (submitted manuscript), proliferation of NK cells, maturation of NK cells, cytotoxic activity via TNFα, IFNγ secretion. NK cells secrete Prf1, GzmB while WJMSCs did not secrete Prf1, GzmB. The secretion of TNFα, IFNγ, Prf, GzmB increased when WJMSCs and NK cells co-cultured. Cytokines-activated NK cells release death receptor (TNFα), effector molecules (IFNγ), Prf1, GzmB (cytoplasmic granule) directly kill cancer cells and indirectly activate B and T cells (Cheng et al, 2013).

Major points:

1. The results did not fully support the manuscript title. The title of the manuscript implies that authors intend to look for general effects of these inflammatory cytokines on breast cancer cell apoptosis. However, the cytokine concentration used in this study is only very high concentrations (175 ng/mL and 350 ng/mL). The dose-dependent effect of TNF-α and IFNγ, covering the low to high concentration, on breast cancer cell apoptosis should be provided.

Respond:

1.1. MSCs can be used for cancer therapy, this study aims to increase anticancer of CM-WJMSCs by improving IFNγ and TNFα secretion. My research: 1). Transfection IFNγ and TNFα plasmid into WJMSCs (on progress), 2), MSCs increase IFNγ, TNFα secretion via activated-NK. Previous research exhibited that coculture experiments BM-MSC and human NK cells. MSC enhanced the ability of IL-12/IL-18-stimulated NK cells to secrete IFN-γ in a dose-dependent manner (Thomas et al., 2014). Based on this research showed that IFN-γ secretion of WJMSCs can be increased by co-culture with ILs-activated NK cells. This research in line with previous research by Cheng et al (2013) that ILs (IL2, IL12, IL15, IL18) activated NK cells to release IFNγ, TNFα, PRF1, GzmB which inhibit cancer cells by inducing apoptosis.

1.2. My research demonstrated that ILs (IL2, IL15, IL18)-induced NK cells cocultured with WJMSCs increased IFNγ, TNFα, PRF1, GzmB. These secretoms directly inhibited cancer cells (my research has proved, that CM from co-culture NK and ILs-induced NK cells inhibited cancer proliferation, induced apoptosis by flowcytometry and RTPCR) and indirectly activate B and T cells (should be done by in vivo assay).

1.3. MSCs have been used as delivery agents for a variety of molecules that can inhibit tumor growth and interferon has been shown to have anti-proliferative and proapoptotic effects (Shah, 2012). MSCs have also been produced to deliver IFNγ which stimulated apoptosis and inhibited leukaemic cell proliferation in vitro (Li et al., 2006).

2. There have been numerous literatures linking TNFα with breast cancer progression and promotion, as demonstrated in both pre-clinical and patient data. For example, elevated TNF is associated with poor patient prognosis in TNBC. As well, the use of only one *in vitro* model (MCF7) may represent only a small fraction of breast cancer population. How about the effect of TNF-α and IFNγ on the TNBC?

Respond:

2.1. TNF-α can induce apoptotic (caspase-dependent) or necrotic (caspase- independent) cell death *in vitro*, depending on the cell type used (Ghavami *et al*., 2009). The IFNs mediate anticancer effect directly by modulating immunomodulatory response or directly by regulating tumour cell proliferation and differentiation (Chawla-Sarkar *et al*., 2001) and inhibition of tumour angiogenesis (Ikeda *et al*., 2002). TNF-α, IFN-γ secretion of NK cells directly inhibit cancer cells proliferation, induce apoptosis of cencer cells (Cheng et al., 2013).

2.2. Many journals mentioned that TNFα, IFNγ can induce cancer cells proliferation,

these results were affected by dose. My previous research exhibited that in low concentration of TNFα, IFNγ (60 ng/ml) was in-effective inhibit cancer cells and the IC_{50} of TNFα, IFNγ toward breast cancer cells (MCF7) were 364.78 ng/ml, 29.03 ng/ml (Widowati et al., 2016).

2.3. Effect TNFα, IFNγ on TNBC should be investigated to know its specific character in proliferation rate, metastasis potency

3. I am also curious about whether hWJMSCs are really necessary in increasing cytokine secretion from NKs and promoting apoptosis, as this may also be caused by just IL15 and IL18 stimulation of NKs.

Respond :

3.1. hWJMSCs secrete low level of TNFα, IFNγ, co-culture hWJMSCs and NK cells increase TNFα, IFNγ secretion. The higher NK cells ratio will increase TNFα, IFNγ secretion. ILs induction increase TNFα, IFNγ. This research result was in line with previous study where MSC enhanced the ability of IL-12/IL-18-stimulated NK cells to secrete IFN-γ in a dose-dependent manner (Thomas et al., 2014). CM from co-culture WJMSCs and ILs-activated NK cells was significantly different compare to CM-hWJMSCs and CM-(hWJMSCs+NK) to induce apoptosis (Table 4). CM-hWJMSCs and CM-(hWJMSCs+NK) induced apoptosis not significantly different.

4. The manuscript needs further editing, as many spelling, grammatical, and stylistic errors can be spotted throughout the manuscript. (to list out a few: multipoytency, hepatome, reserach; 295-03ng/ml). The consistency in writing format, in particular for the Reference Section, should be much improved. I would also suggest re-wording the manuscript title as the submitted title is not reflective of the content of study.

Respond :

4.1. Thank you very much for your comment to correct my paper

Second Reviewer:

This study can become an interesting study if the author is able to explain it properly. There are several questions for the author:

1. What is the novelty in this study? The effect of TNF alpha and IFN gamma on cancer cell had been known for several years. The author didn't explain clear novelty in this study

Respond:

The novelty: co-culture WJMSCs and cytokine activated-NK cells can increase the secretion of TNF alpha, IFN gamma and other secretomes. The result of this study was consistent with previous research that MSC enhanced the ability of IL-12/IL-18-stimulated NK cells to secrete IFN-γ in a dose-dependent manner (Thomas et al.,2014), where IFN-γ inhibit cancer proliferation. I measured not only IFN-γ but also TNFα, Prf1, GzmB secreted by NK cells, which inhibit cancer proliferation and induce apoptosis.

2. The data presentation for each experiment is not clear and make the reviewer questioning the evidence provided by the author.

Respond :

The data are presented as mean and standard deviation (each treatment was triplicates). The data was analyzed using ANOVA and followed by Tukey HSD post-hoc test. Among treatment can be compared based on Tukey HSD post hoc test. The data in table presented in superscript letter, different superscript letter implies significantly difference in the same column.

3. The author need to check the manuscript translation and grammar in an English language editor since there are many grammatical error and fragmented sentences in the paragraph that made the reviewer get difficulty to obtain the main message in this manuscript and the importance of this study.

Respond :

Thank you for your review, I will correct the grammar

4. The author should follow the Instruction to Author of target journal

Respond :

Thank you for your review, I will adjust based on guideline

5. The presentation of the data is inappropriate. If author want to show the significance increment or reduction in RNA expression level, graph or histogram is more suitable and will give more impressive presentation.

Respond :

Thank you for your review,

6. Not proper conclusion: need revision!

Respond :

TNFα and IFNγ induce Bax, p53, decrease Bcl-2 gene expression directly. CM of coculture hWJMSCs and ILs-activated NK increase secretion of TNFα, IFNγ, Prf1, GzmB furthermore inhibit breast cancer cells proliferation, induce apoptosis, increase Bax expression,

7. References have to be in Vancouver style !

Respond:

Thank you for your review, I will adjust based on guide line

Following sentences need revisions:

Indonesia is the highest mortality rate per 100,000 (36.2), the lowest is Singapore (13.6) (Kimman *et al*., 2012).

Replace:

Indonesia exhibits the highest mortality rate of 36.2 per 100,000 patients while the lowest mortality is Singapore around 13.6 per 100,000 patients (Kimman *et al*., 2012).

BC treatments are chemotherapy, surgery, endocrinotherapy, radiotherapy and molecular-targeted therapy (Yu *et al*., 2017), all therapies are expensive, remain low effectiveness (Kimman *et al*., 2012). The development effecient, effective therapy is highly required. Strategic therapy targets cancer cells directly both primary and metastatic side are strongly required (Yang *et al*., 2014). Metastasis is invasive features (Joyce & Pollard, 2009) and it escapes antitumor immunity (Kute *et al*., 2009; Smyth *et al*. 2006).

Replace:

This research was the continuing study to evaluate the direct and indirect effects of TNFα and IFNγ secreted in CM of co-culture hWJMSCs and NK cells toward apoptosis of BC cells (MCF7) and to increase the cytotoxic of NK cells induced by interleukins (IL-15, IL-18).

Dr. Wahyu Widowati:

We have reached a decision regarding your submission to Molecular and Cellular Biomedical Sciences, "Direct and Indirect Effect of TNFα, IFNγ Toward Apoptosis in Breast Cancer Cells".

Our decision is to: Accept Submission

Dr. Ferry Sandra
Department of Biochemistry and Molecular Biology, Faculty of Dentistry, Trisakti University
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Dear Ferry Sandra, PhD

I have completed proofreading the galley proof of my manuscript entitled "Direct and Indirect Effect of TNFa and IFNg Toward Apoptosis in Breast Cancer Cells"
this manuscript is ready to published

Thank you for cooperation