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To cite this article: Novi Silvia Hardiany *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **217** 012035

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The impact of conditioned medium of umbilical cord-derived mesenchymal stem cells toward apoptosis and proliferation of glioblastoma multiforme cells

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Abstract. Glioblastoma multiforme (GBM) is the human malignant and highly invasive brain tumor. Tumor growth and invasive property are affected by mesenchymal stem cells (MSCs) as a part of tumor microenvironment. However, accumulating evidences describe that MSCs could act as pro and anti tumorigenic. The different action of MSCs in regulating tumor growth is mediated by secreted factor. This research purpose was to analyze the impact of MSCs secreted factor in conditioned medium (CM) on apoptosis and proliferation of GBM cells. Primary culture of umbilical cord-derived MSCs (UCSCs) was conducted in this research. CM-UCSCs was prepared by culturing the UCSCs in serum free α MEM for 24 hours. Those CM-UCSCs was 2-fold diluted by Dulbecco's Modified Eagle Medium (DMEM) high glucose (50% concentration) and used to treat human GBM T98G cells for 24 hours. Following this treatment, apoptosis was detected using annexin V-FITC and cells proliferation was analyzed using trypan blue exclusion test. The results showed that early apoptosis occurred in 14.4 % of control cells and 14.8 % of CM-treated T98G cells, as well as late apoptosis occurred in 7.8 % of control cells and 7.2 % of CM-treated T98G cells. Whereas, GBM cells proliferation was tend to higher in the CM treated cells (3.85×10^5 cells) compared to the control (2.97×10^5 cells). In conclusion, CM-UCSCs does not seems to affect apoptosis, but tend to increase cells proliferation. Further research is needed to elaborate the mechanism of UCSCs secretome in stimulating cells proliferation.

Keywords: mesenchymal stem cells, glioblastoma multiforme, apoptosis, cells proliferation

1. Introduction

Glioblastoma multiforme (GBM), which is highly invasive, is the most malignant brain tumor in the human adult. [1] GBM presents a complex microscopic feature which consists of tumor cells and their surrounding stroma. They are collectively referred to as the tumor microenvironment (TME).[2] The course of tumor growth is affected by mesenchymal cells (MSCs) and factors secreted by MSCs – the secretome – that are part of the tumor microenvironment. MSCs are a heterogeneous population of cells containing subpopulations with differing differentiation capacities.[3] These cells secrete an extensive variety of proteins such as growth factors, cytokines, and chemokines.[4.5] These secreted factors may inhibit the growth and metastasis of cancer cells in vitro and in vivo.[6]

On the other hand, contrasting evidence has been found in recent literature regarding the pro-tumorigenic properties of MSCs. The widely accepted notion is that MSCs may exert this effect mainly by secreting bioactive factors via paracrine mechanism. The intricate interaction of all these responses may lead to enhanced tumor progression, increased metastatic activity, and poor prognosis.[7] MSCs have been involved in the advance of tumor progression in many cancer types such as follicular lymphoma, head and neck carcinoma, hepatoma, glioma, gastric cancer, breast cancer, colorectal cancer, lung cancer and prostate cancer.[8] Hence, at present, the dual-role of MSCs in tumor progression is still mattered of debate. Many mechanisms have been reported to account for these contradicting observations, such as cytokine signaling, modulation of apoptosis, angiogenesis and immune balance.[7] Moreover, the role of MSCs in cancer progression depends on the specific MSCs subtype, in vitro or in



vivo conditions, factors secreted by MSCs, types of cancer cell lines and interactions between MSCs, cancer cells and host immune cells.[9] In addition, secretome of umbilical cord-derived MSCs (UCSCs) seem to play a role in enhancement of pluripotency capacity of GBM cells.[10] However it is still remain unclear whether those secretome could influence apoptosis and GBM cells proliferation. Therefore, the purpose of this study was to analyze the the impact of secretome in conditioned medium (CM) derived from UCSCs on apoptosis and proliferation of GBM cells.

2. Experimental Method

2.1. *The Culture of human glioblastoma T98G cells*

Human glioblastoma T98G cells line were grown on Flask T-25 cm² (Corning) with high glucose Dulbecco's Modified Eagles Medium (DMEM, Gibco) plus 3.7 g / L of sodium bicarbonate (Gibco), 10% Fetal Bovine Serum (FBS, Gibco), 1% streptomycin-penicillin (Biowest) and 1% amphotericin (Biowest) at 37 °C in a humid atmosphere with 95% air and 5% CO₂. Cell culture is grown until subconfluence. Medium was replaced every three days [10].

2.2. *Culture of umbilical cord stem cells*

Primary culture of umbilical cord-derived MSCs (UCSCs) was conducted in this research.[11] Umbilical cord mesenchymal stem cells were cultured on Flask T-25 cm² (Corning) in α -Minimum Essential Medium (α -MEM, Gibco) with 10% FBS (Gibco), 1% supplementation of Penicillin streptomycine (Biowest) and 1% amphotericin (Biowest). UCSCs were cultured at 37 °C, 20% O₂ and 5% CO₂. Cells subculture were reached on 90% confluence.[10]

2.3. *Conditioned medium of umbilical cord stem cells (CM-UCSCs) preparation & treatment.*

CM-UCSCs was prepared by culturing the UCSCs in serum free α MEM for 24 hours. Those CM-UCSCs was 2-fold diluted by high glucose DMEM (50% concentration) and used to treat human GBM T98G cells for 24 hours.[10]

2.4. *Apoptosis assay*

To detect apoptotic activity in cells, the cells were stained with Annexin V-FITC apoptosis assay (Abcam) and then observed using flow cytometry (Becton Dickinson, BD). The cells were prepared according to the kit protocol. The T98G cells control and CM-treated T98G cells were incubated with 10 μ l of propidium iodide (PI) and 5 μ l of Annexin V for 10 minutes. Cells in the early stage of the apoptosis were stained with only Annexin V. While, cells in the late stage were stained by both Annexin V and PI. Viable cells presented no staining by either Annexin V or PI. The experiment was replicated three times.

2.5. *Trypan blue exclusion test*

Ten micro liters of cells were mixed with 10 μ L of Trypan Blue staining (1: 1 ratio between cell suspension and 0.4% Trypan Blue solution). Nonviable cells will be colored blue while the viable cells will not be stained. The number of viable cells were counted using Cell Counter (LUNA).

3. Results and Discussion

Early apoptosis occurred in 14.4 % of control cells (Q4-1 region) and late apoptosis occurred in 7.8 % of control cells (Q2-1 region) as shown in figure 1.

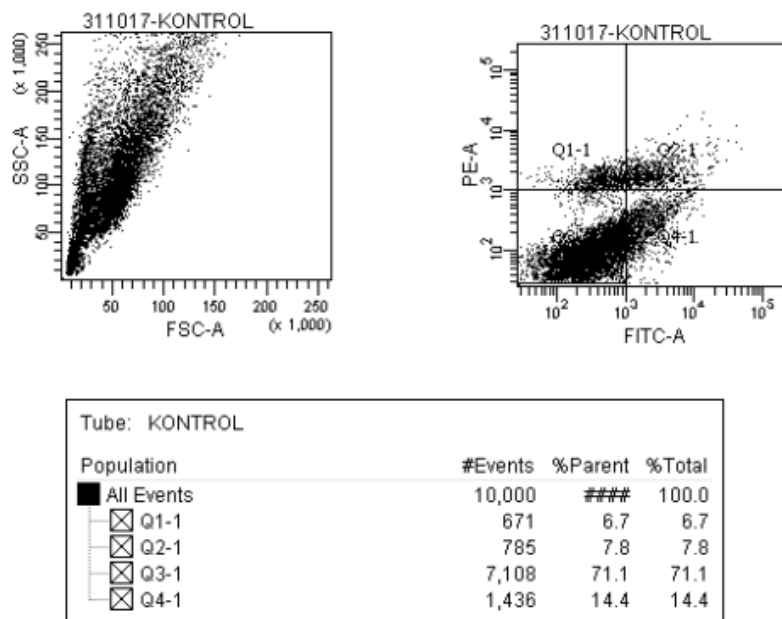


Figure 1. Apoptosis of control cells was detected using flow cytometry

In CM-treated cells, the number of early apoptotic cells and late apoptotic cells were not significant different with the control cells. About 14.8 % (Q4-1 region) of CM-treated T98G cells were in early apoptosis stage as well as late apoptosis occurred in 7.2 % (Q2-1 region) of CM-treated T98G cells (as shown in figure 2). Whereas, GBM cells proliferation was tend to higher in the CM treated cells (3.85×10^5 cells) compared to the control (2.97×10^5 cells) as shown in figure 3.

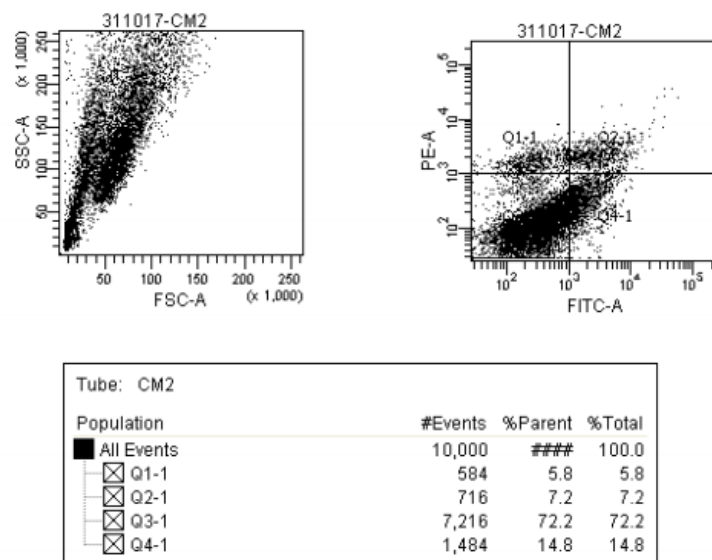


Figure 2. Apoptosis of CM-treated cells was detected using flow cytometry

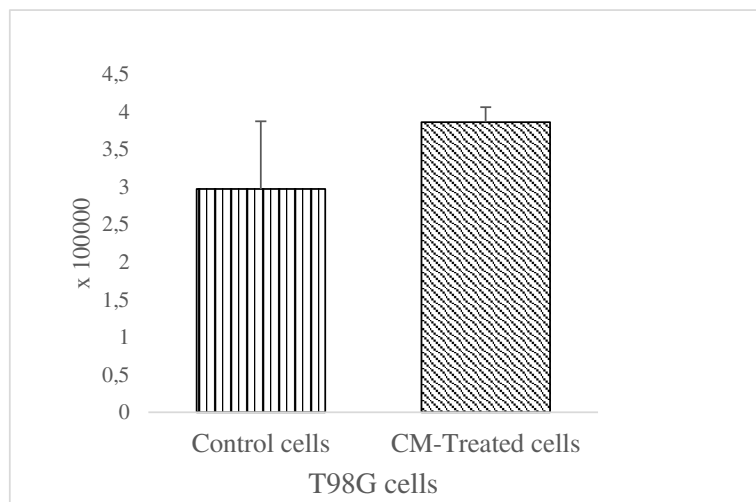


Figure 3. Cells proliferation of CM-treated T98G cells compared to control cells ($p > 0.05$) was detected based on trypan blue exclusion test using cell counter

Tumor microenvironment which contained secreted factors such as growth factor, cytokines, chemokine and mesenchymal stem cells could either affect apoptosis cancer cells or promote cancer cells proliferation.[12] Apoptosis occurs through inhibition of Akt signaling pathway in Kaposi's sarcoma model which was treated by bone marrow- derived MSCs (BM-MSCs) through systemic injection.[13]. Moreover conditioned medium of MSc could decrease expression of Bcl-2, cMyc and proliferating cell nuclear antigen (PCNA) which causes an increase in apoptosis of human liver cancer cell line HepG2 and H7402.[14]. Up regulation of p21 and caspase 3 were also stimulated by MSc in various cancer cells lead to G0/G1 phase arrest and apoptosis.[15]. However, co-injection of human bone marrow MSCs with only one of four breast cancer cell lines (MCF7) into mice led to stimulated tumor growth. Moreover, co-injection MSCs with all cell lines (MDA-MB-231, HMLR, MDA-MB-435, and MCF7) showed the increased of tumor metastasis. In addition, it was found that co-culture of BM-MSCs with the triple negative breast cancer cell line could inhibit tumor growth but increased invasion and metastasis in mice.[7] In another study, BM-MSCs were also found to trigger the proliferation, migration, and invasion of the prostate cell line PC3 in vitro.[6] Adipose-derived MSCs (AD-MSCs) developed the proliferation of human glioma U87 MG cells line. Co-injection of AD-MSCs with human glioma U87MG promotes tumor growth in vivo and it induces a reduction in apoptotic cell death.[2,13]. MSCs were involved in tumor progression by promoting angiogenesis, suppressing immune system, activating cancer-associated fibroblast (CAF) as well as inducing epithelial mesenchymal transition process which is responsible for metastasis.[16] Based on the description above, the role of MSCs in cancer cells are still debated and controversial.

Our findings proved that early and late apoptosis in T98G cells was not affected by secretome in conditioned medium of UCSCs. In early apoptosis, the membrane cells integrity is still intact whereas loss of membrane cells integrity is detected in late apoptosis. As indicated by numerous current model, late apoptotic cells seem like necrotic cells due to secretion of inflammatory substances as a consequence from the delay of cell clearance process.[17] The number of early and late apoptosis cells were not significant different between control cells and CM-treated cells in this study. Nevertheless, cells proliferation tend to increase in T98G cells which was treated by CM-UCSCs. Therefore secretome in CM-UCSCs of this study probably more stimulate tumor cells proliferation rather than apoptosis. This finding is parallel with de Castro et al[18] who conducted several glioblastoma lines cells with conditioned medium from human umbilical cord perivascular cells (HUCPVCs) which resulted in enhancement of viability, proliferation, and migration of tumor cells. Analysis of proteomic detected 699 proteins located in HUCPVCs conditioned medium. The examples of protein secreted from HUCPVCs were C-C motif chemokine 2 (CCL2), actin related protein complex subunit 5 (ARPC 5), translationally-controlled tumor protein (TPT 1), platelet-derived growth factor C (PDGFC), alpha-

actinin-4 (ACTN4), testican-1 (SPOCK1), neuropillin-2 (NRP2), disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), transforming growth factor-induced protein (TGF β 1), plasminogen activator inhibitor 1 (SERPINE1), semaphorin-7A (SEMA7A), periostin (POSTN), interleukin 6 (IL6).[18] Those proteins were responsible for proliferation, migration and invasion.[18] It is possible that those proteins are also contained in conditioned medium of UCSCs. Therefore proteomic analysis is needed to detect protein composition in our conditioned medium of UCSCs. Nevertheless, Akimoto et al[19] found that co culture of UCSCs with primary GBM cells and also U87MG cells line could induced apoptosis on GBM. It is assumed that inter-cell interactions play an important role for stimulating apoptosis beside the paracrine effects. Therefore, the observed effect on tumor apoptosis may be different if the GBM cells are treated with only MSC-secreted factors – not the cell itself such as in our study.

4. Conclusion

Secretome in conditioned medium of umbilical cord-derived mesenchymal stem cells tend to increase glioblastoma multiforme cells proliferation and not influence the apoptosis. Further research is needed to elaborate the mechanism of UCSCs secretome in stimulating cells proliferation.

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Acknowledgement

This research was funded by *Direktorat Riset & Pengabdian Masyarakat Universitas Indonesia (DRPM)* for *Publikasi Terindeks untuk Tugas Akhir Mahasiswa Universitas Indonesia (PITTA UI 2018)* grant.