IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

Innovating Glioma Therapy Using Secretions from Umbilical Cord Mesenchymal Stem Cells to Target Homeobox and Growth Factor Genes

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Received: 25, Mar, 2024
Accepted: 14, Oct, 2024

ABSTRACT

Background: Glioblastoma is a prevalent and challenging malignant brain tumor. Secretome therapy using human umbilical cord mesenchymal stem cells (hUCMSCs) appears to be a promising treatment for glioblastoma. This study analyzed the potential of the hUCMSC secretomes (hUCMSCs-sec) for glioma therapy.

Materials and Methods: Characterization of hUCMSCs was performed by examining certain markers, including CD44, CD90, CD105, CD73, CD13, CD19, CD14, CD45, CD34, and HLA-D. The cells' ability to differentiate into adipocytes, chondrocytes, and osteocytes was evaluated. Cytotoxic effect on Glioblastoma (GBM) cells was analyzed using 2-[2-methoxy-4-nitrophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium (WST-8). mRNA relative expression, including homeobox (HOXA5, HOXB1, HOXC9 and HOXC10), insulin-like growth factor binding protein 2 (IGFBP2), Extracellular signal-regulated kinases (ERK), Epidermal growth factor receptor (EGFR), and Caspase 3 (Casp3), were quantified by quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR).

Results: The hUCMSCs-sec was successfully isolated and identified, showing positive markers and its capacity to differentiate into chondrocytes, adipocytes, and osteocytes. hUCMSCs-sec exerted a cytotoxic effect on GBM cells and upregulated the expression of Casp3, whereas it decreased the expression of HOX, IGFBP2, EGFR, and ERK in GBM cells.

Conclusion: The secretomes from hUCMSCs show potential for GBM cell therapy by improving the deregulation of HOX, inducing apoptosis, and inhibiting cell proliferation genes.

Keywords: Cytotoxic; mRNA expression; Glioblastoma therapy; human umbilical cord mesenchymal stem cells (hUCMSCs); Secretome

INTRODUCTION

Glioblastoma (GBM), anaggressive malignant tumor that arises from glial cells, is known for its rapid growth, infiltration, and frequent recurrence, making it one of the prevailing and lethal forms of brain tumors[1]. Gliomas are heterogeneous cell tumors, and their various subtypes have different levels of aggressiveness[2]. GBM forms its own blood-brain barrier, hindering drug exit due to irregular blood flow. This significantly affects the effectiveness of systemic drug delivery for treating the tumor[3]. Conventional therapies for GBM include

surgery, radiotherapy, and chemotherapy, and they are often limited, and they can have significant side effects. Damage to brain tissue due to radiation is one of the side effects that must be found for solutions[4].

Additionally, cell migration, immune evasion, and resistance to cell death further complicate the treatment of GBM and reduce the effectiveness of conventional therapy[3]. The rapid growth and migratory ability of cancer are influenced by the abundance of several growth factor genes, such as Extracellular signal-regulated kinase (ERK), epidermal Growth Factor Receptor (EGFR), and insulin-like growth factor binding protein 2 (IGFBP2)[6]. However, therapy can be challenging due to the overexpression of the Homeobox (HOX) gene that were associated with the development processes[7] and drug resistance[8]. A possible mechanism by which GBM growth is inhibited is by stimulating pro-apoptotic genes, including Caspase 3 (Casp3)[9].

Targeting the support system between the tumor and its microenvironment to combat GBM may involve leveraging secretomes from human umbilical cord mesenchymal stem cells (hUCMSCs). The cells secretomes contains various cytokines, growth factors, and extracellular vesicles with therapeutic potential[10]. These cytokines, as protein messengers, play a role in facilitating communication between Cancer Stem Cells (CSCs) and MSCs within the tumor microenvironment, thereby influencing cancer progression[11]. Thus, hUCMSCs-sec can move to the tumor area, providing local antitumor effects without disturbing healthy tissue in the surrounding environment[12].

Several studies have demonstrated the therapeutic effects of hUCMSCs-sec across various types of cancer. Mirabdollahi et al.[13] in their study reported that hUCMSCs-sec has toxicity against breast cancer cells (MCF-7), which is attributed to cellular apoptosis. hUCMSCs-sec has also been demonstrated to be non-tumorigenic and does not induce resistance to doxorubicin in lung cancer cells[14]. Another study by Widowati et al.,[9] affirm that the hUCMSCs has anti-cancer properties against various cancers. Nevertheless, research regarding the effects of hUCMSCs-sec as a cancer therapy still requires further exploration.

In this study, we harvest hUCMSCs and detect specific markers of hUCMSCs, including CD90, CD105, CD44, and CD73, along with evaluate their ability to differentiate into osteocytes, adipocytes, and chondrocytesand. The level of cytotoxicity or the ability of hUCMSCs-sec to inhibit growth and stimulate apoptosis of glioma cells (GBM). Analysis of specific gene expression in tumor tissues can help identify the most suitable targets for therapy[15]. Genes that are overexpressed or play a key role in tumor growth should be prioritized. The expression of the target genes are HOX gene family (HOXA5, HOXB1, HOXC9, HOXC10), IGFBP2, EGFR, ERK, and Casp3 in this study was analyzed by RT-PCR. It is hypothesized that the secretomes from hUCMSCs can modulate these target genes, leading to reduced tumor growth and enhanced apoptosis. Additionally, it is proposed that the secretomes enhance the sensitivities of cancer therapy by modulating the tumor microenvironment and reducing drug resistance.

MATERIALS AND METHODS

hUCMSCs Isolation

The hUCMSCs isolated from the umbilical cord underwent a washing process using normal saline with 0.9% w/v sodium chloridethen sectioned into small explants approx 1–2 mm. The hUCMSC cultivation method was described by Widowati et al[16]. Subsequently, these explants were seeded on plates. Minimum essential medium-α supplemented with 2 mM GlutaMAX from Invitrogen, along with fetal bovine serum 20% (Biowest S1810-500), and penicillin-streptomycin-amphotericin B (100 mg/mL, 100 U/mL, and 0.25 mg/mL; Elabscience PB180121) were used to maintain the cultures. A humidified atmosphere at 37°C with 5% CO_2 (Thermoscientific 8000DH) was used for incubation, with regular medium replacement every 2 days over a 21-day period. Upon reaching 80%–90% confluence, the cells were collected and reseeded at a concentration of 8×10^3 cells/cm^2 [16].

hUCMSCs Marker Characterization

Surface marker detection was conducted to confirm their characterization. Upon reaching 80% confluence, the cells were harvested and centrifuged for 10 min at 300 g. Then the pellet was resuspended in PBS 1x and FBS, then counted using a hemocytometer. Subsequently, 10 x 10^4 cells in 500 µL PBS were stained with CD90, CD73, CD105, CD13, CD14, CD44, CD19, CD34, CD45, and HLA-D. Analysis was conducted using FACS (Macsquant Analyzer 10, Miltenyi), and respective isotype controls were obtained from the hUCMSCs analysis kit following the manufacturer's protocol (BD stem flow™ kit, 562245). All surface marker measurements were performed in triplicate[16-18].

hUCMSCs Differentiation

hUCMSCs were cultured at a 5 x 10^3 cells density per 24-well plate. StemPro Chondrogenesis Differentiation Kits (Gibco, A10071-01), StemPro Osteogenesis Differentiation Kits (Gibco, A10072-01), and StemPro Adipogenesis Differentiation Kits (Gibco, A10070-01) were used for osteogenic, chondrogenic, and adipogenic differentiation. After 3 weeks, visualization was performed using Alizarin red S (Sigma, A5533) for osteogenic lesions, Alcian blue (Sigma, A5268) for chondrogenic lesions, and Oil Red O (Sigma, 00625) for adipogenic[16].

Cytotoxic hUCMSCs

The WST-8 from Elabscience (E-CK-A362) was used to determine the cytotoxicity of hUCMSCs-sec against the GBM cell line (ATCC HTB-14™), which were retrieved from Aretha Medika Utama, BBRC, Indonesia. The hUCMSC-sec is harvested from the secretion of hUCMSCs and then centrifuged to remove any remaining dead cells. A total of 5 x 10^3 GBM cells and Roswell Park Memorial Institute medium (RPMI 1640, Biowest, L0495) were plated for 24 h into 96-well plates. Then treated with hUCMSCs-sec at various concentrations (100%; 75%; 50%; 25%; 0) for 72 h. The absorbance was measured by a microplate reader (Multiskan Go, Thermo Scientific Inc.) at 490 nm after the addition of WST-8 to each well. The cytotoxicity test was conducted in triplicate, and data included the viable cell count, percentage of viability, and cell inhibition[16].

RT-PCR assay

The qRT-PCR method, as developed by Widowati et al.[19], was utilized. First, total RNA from Glioblastoma was extracted using a TRIzol reagent (Zymo research). Next, RNA was converted into cDNA via reverse transcription using a synthesis kit (Meridian Bioscience BIO-65054). The RNA and cDNA purity was assessed using a microplate reader, and the corresponding data are presented in Table 2. cDNA synthesized was amplified using PCR for 40 cycles (ESCO SCR-2A1). The PCR mixture contained nuclease-free water (NFW), SensiFAST SYBR NO-ROX (Meridian Bioscience BIO-98005), Primer forward, reverse, and cDNA template. The antisense fragment designs of HOXA5, HOXC9, HOXC10, IGFBP2, EGFR, ERK, and Casp3 were obtained from NCBI Entrez Nucleotide database (www.ncbi.nlm.nih.gov/nucleotide) (Table 1). The initial step of amplification was denaturation for 5 min at 95˚C, followed by 40 thermal cycles of 94˚C for 50 s, 40 cycles for 50 s at 58-61˚C, thenfor 50 s at 72˚C, with a final extension for 5 min of 72˚C. Finally, RT-PCR results were evaluated using detection techniques such as qPCR (quantitative PCR) using a kit (Clontech Biosciences, Advantage™ RT-for-PCR).

Statistical analysis

The parameters statistical significance was assessed using means and standard deviations (M± SD). Treatment comparisons were conducted via ANOVA, with significance set as $p<0.05$. The Tukey post hoc test with a p-value ($p<0.05$), was applied. Statistical analysis was performed using SPSS software (IBM SPSS Statistical, version 20.0), then the results were graphed using GraphPad Prism software (version 9.0)

Table 1: Primer sequence design of target genes in GBM cells

Gen	Primer Sequence (5' - 3')	Product length (bp)	Annealing (°C)	Cycle	Reference
Human GADPH	F: GCCAAAAGGGTCATCATCTC R: TGAGTCCTTCCACGATACCA	178	58	40	NM_001357943.2
Human Casp3	F: AGAACTGGACTGTGGCATTGAG R: GCTTGTCGGCATACTGTTTCAG	191	58	40	NM_001354783.2
Human ERK	F: TATTCGAGCACCAACCATCG R: TGCTGAGGTGTTGTGTCTTC	101	59	40	NM_002745.5
Human HOXA5	F: AAGTCATGACAACATAGGCG R: ATTTCAATCCTCCTTCTGCG	129	59	40	NM_019102.4
Human HOXC10	F: CCCAATGAAATCAAGACGG R: CCTTTATCTCCTCTTTCGCT	133	59	40	NM_017409.4
Human EGFR	F:C CTTACTTTCCTTCGACCTCTG R: GTCAGTTCCTGGAAGACCTTAC	173	60	40	NM_005228.5
Human IGFBP2	F: TGTTTGGGTCTAGCTTGGTC R: TTCAGTCGGCTCATACCAAC	133	60	40	NM_001313992.2
Human HOXC9	F: TCATCCTTCGATTCTGAAACCA	65	61	40	NM_006897.3

*Data were obtained from The NCBI Entrez Nucleotide database (www.ncbi.nlm.nih.gov/nucleotide)

Table 2: RNA and cDNA purity and concentrations

	Sample	Concentration (ng/µL)	Purity (λ260/ λ280)
RNA	1	11.60	2.3652
	2	8.48	2.5981
	3	8.32	2.9868
	4	8.16	2.8171
	5	9.20	2.9012
	6	9.68	2.8382
cDNA	1	529.92	2.0779
	2	405.92	2.0128
	3	178.48	2.0264
	4	942.64	2.0287
	5	655.52	2.0223
	6	936.32	2.0266

*The various samples (1, 2, 3, 4, 5, 6) indicate (1) Negative control, (2) Non-FBS control, (3) 0.33% hUCMSCs-sec, (4) 1.25% hUCMSCs-sec, (5) 5% hUCMSCs-sec, (6) 20% hUCMSCs-sec.

RESULT

hUCMSCs Characterization and Differentiation

The hUCMSCs cell surface markers were successfully characterized. High expressions of CD44, CD90, CD105, and CD73were detected in human hUCMSCs, whereas negative expression of HLA-DR, CD11b, CD34, CD45, and CD19 was observed (Figure 1 and Table 3).

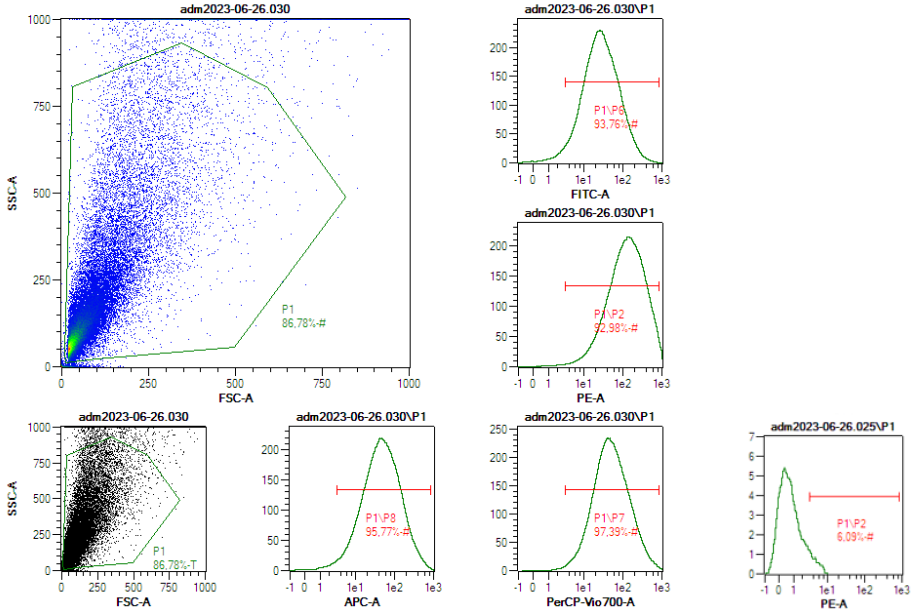


Figure 1. Dot-blot representative of hUCMSC surface markers

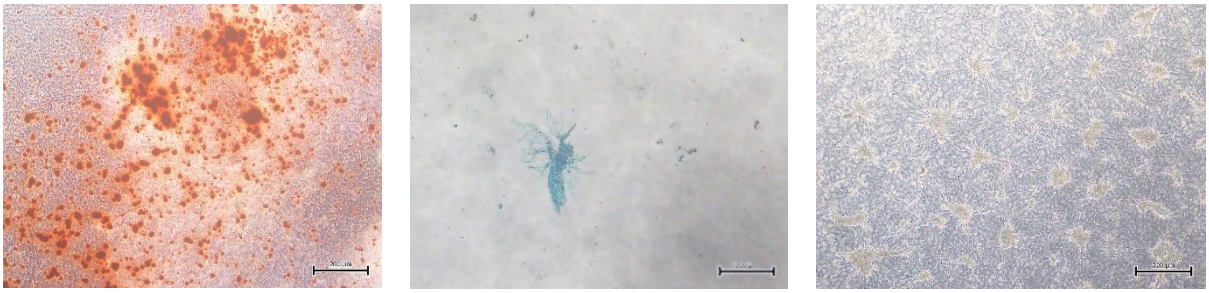
Table 3: Characterization analysis of the hUCMSCs passage 3 surface markers

	PE-CD44	FITC-CD90	PERCP-CD105	APC-CD73	NegativeLineage
1	92,98	93,76	97,39	95,77	6.07
2	92,35	93,97	95,74	93,85	6.07
3	92,56	94,99	97,23	95,44	6.07

* hUCMSCs surface markers positive for CD44, CD90, CD105, and CD73 and negative for lineage (CD19, CD45, CD34, CD11b, and HLA-DR)

Mesenchymal stem cells, which resemble fibroblasts in appearance (Figure 2), are multipotent stem cells. hUCMSCs can differentiate into different cell types, including fibroblast-like cells, due to their pluripotent potential. Figure 3 shows the ability of hUCMSCs to diversify into another cells; chondrogenic, osteogenic, and adipogenic.

Figure 2. Morphology of hUCMSCs that grew into fibroblast-like cells at 200 µm



(a) (b) (c)

Figure 3. Differentiation of hUCMSCs into (a) adipocyte, (b) chondrocyte, and (c) osteocyte types

Cytotoxic Effect of hUCMSCs-sec on GBM cells

The presence of hUCMSCs-sec on GBM cells demonstrated a notable effect on their viability. The viability of GBM increased proportionally with increasing concentration of added hUCMSCs-s. According to Figure 4, treatment number 3 exhibited significantly lower viability than the negative control, with a value of 60.14 ± 6.20%. This result implies that hUCMSCs-sec was most effective at inhibiting GBM at low concentrations. The lowest concentration of hUCMSCs-s inhibited 39.86 ± 6.20% GBM compared with untreated GBM. The outcome of the cytotoxic assay revealed that the most effective and safe concentration of hUCMSC-sec for treating glioma was 20%.

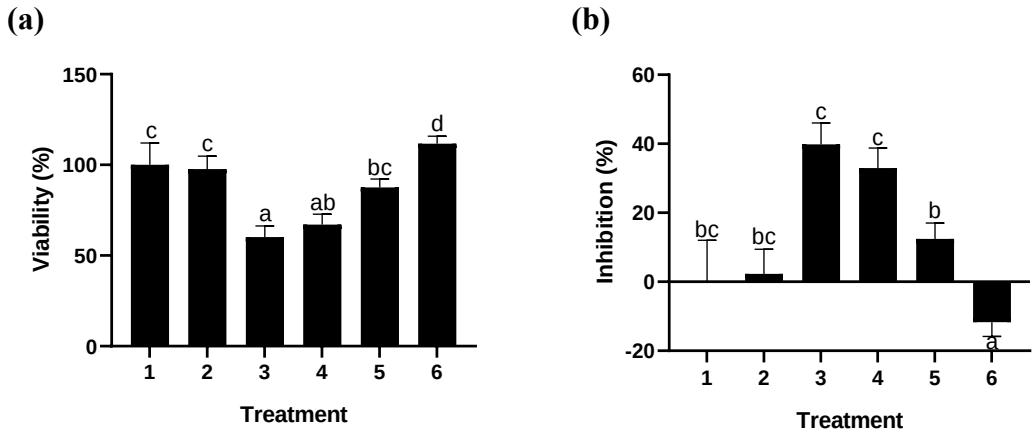


Figure 4. HUCMSCs-sec cytotoxic effect on GBM cells

The various treatments (1, 2, 3, 4, 5, 6) indicate (1) negative control, (2) control starved, (3) cell + hUCMSCs-sec = 25%, (4) cell + hUCMSCs-sec = 50%, (5) cell + hUCMSCs-sec = 75%, and (6) cell + hUCMSCs-sec = 100% *Data were represented as mean ± standard deviation (SD); tests were conducted in three replicates. Different codes (a, ab, bc, c, d) for viability and (a, b, bc, c) for inhibition specify significant differences between treatments.

HUCMSCs-sec effect on mRNA relative expression in GBM cells

Treatment with the hUCMSCs secretome significantly modulates the expression of key genes, including HOXA5, HOXC9, HOXC10, IGFBP2, EGFR, ERK, and Casp-3, in GBM cells compared with the negative control and non-FBS conditions, as illustrated in Figure 5. Notably, the levels of HOX genes were downregulated upon exposure to the hUCMSC secretome. Moreover, crucial growth factors implicated in glioma progression, such as ERK, EGFR, and IGFBP2, were downregulated following treatment with hUCMSCs secretome, whereas Casp-3 expression was increased. These findings highlight the optimal concentration of 1.25% for effectively treating GBM with hUCMSC secretome.

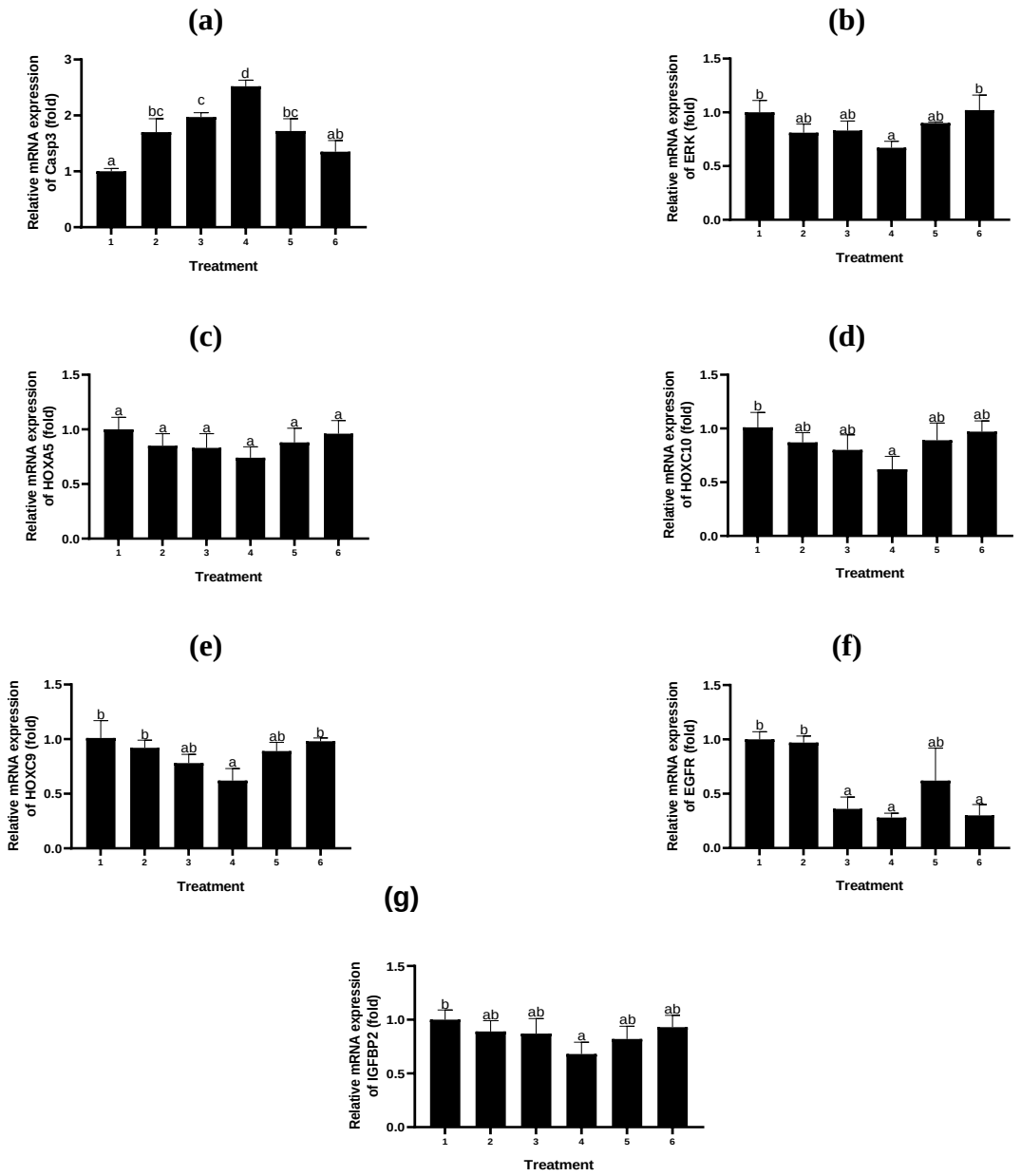


Figure 5. HUCMSCs-sec effect on mRNA relative expression in GBM cells

(a) Casp3, (b) ERK, (c) HOXA5, (d) HOXC10, (e) HOXC9, (f) EGFR, and (g) IGFBP2 expression levels in secretome-treated GBM cells. The various treatments (1, 2, 3, 4, 5, 6) indicates: (1) Negative control, (2) Non-FBS control, (3) 0.33% hUCMSCs-s, (4) 1.25% hUCMSCs-s, (5) 5% hUCMSCs-sec, (6) 20% hUCMSCs-s. Data were represented as mean ± standard deviation (SD); tests were conducted in three replicates. Different letters specify significant differences among treatments.

DISCUSSION

The cell surface markers characterization on hUCMSCs was successful (Figure 1). Similar findings were reported in a previous study by Widowati et al.[17], highlighting the high expression of CD90, CD105, CD44, and CD73 and the negative expression of CD19, CD45, CD11b, CD34, and HLA-DR in human UCMSCs.

The expressions of CD90 and CD105 indicate that hUCMSCs meet the criteria for valid cells derived from the mesenchymal cell lineage. CD90, also referred to as Thy1, plays a vital role in cell-cell interactions within the matrix and contributes to wound healing[20]. CD44 acts as a receiver of hyaluronic acid and interacts with other ligands, including osteopontin, matrix metalloproteinase, collagen, fibronectin, and laminin[21]. These markers indicate that hUCMSCs possess differentiation properties comparable to those of MSCs and can serve as a means of identification.

As noted by Amidi et al.[22], hUCMSCs exhibit negative expression of CD34 and CD45 markers, which are generally referred to as hematopoietic markers[23]. The absence of these markers indicates that hUCMSCs have minimal blood cell contamination. In addition, hUMSCs did not express CD11b and HLA-DR markers. CD11b is an antigen surface marker found specifically on macrophages[24], whereas HLA-DR is commonly present on antigen-presenting cells, which have the ability to activate the immune system[25]. hUCMSCs lack expression suggest anti-inflammatory traits and enhanced compatibility, eliminating the need for further genetic alterations.

hUCMSCs can differentiate into different cell types, including fibroblast-like cells, due to their pluripotent potential. Mesenchymal stem cells, which resemble fibroblasts in appearance (Figure 2), are multipotent stem cells. Denu et al.[26] have shown that fibroblasts and MSCs exhibit similar morphology and cell surface antigens.

hUMSCs exhibit the capacity to differentiate. Previous studies have shown consistent outcomes using identical staining methods for the differentiation of human adipose-derived stromal stem cells (hASCs)[27]. Similar findings were found in Widowati, et al.[17], which stated that Wharton's Jelly Mesenchymal Stem Cells (WJMSCs) from UCMSCs can differentiate into chondrogenesis, osteogenesis, and adipogenesis.

Oil red O staining revealed a dark red hue in hUCMSCs, indicating lipid presence, a key marker of adipogenesis. This staining method is commonly employed to identify adipocytes, which are crucial for energy homeostasis and serve as the body's primary lipid reservoirs[28]. Alcian blue staining revealed chondrogenic differentiation of hUCMSCs, highlighting blue chondrocytes, indicating glycosaminoglycan (GAG) presence in the cartilage matrix[29]. This stain, which has a high affinity for GAG, detects changes in histology and GAG synthesis, demonstrating the ability of hUCMSCs to differentiate into chondrocyte, which is crucial for cartilage tissue development[30]. Alizarin Red staining of hUCMSCs resulted in a distinct red coloration, confirming osteogenic differentiation and the presence of calcified calcium minerals in the bone matrix[31].

The characterized hUCMSCs cells had their secretomes extracted and were tested on GBM. The highest concentration of GBM treatment with hUCMSCs-sec did not exert any inhibitory effect. Conversely, the addition of hUCMSCs-sec at its highest concentration increased the viability of GBM. As shown in Figure 4, treatment number 6 increased cell viability by 111.72% compared with the negative control. The observed increase in viability may be attributed to the well-known self-renewal capacities of hUCMSCs, along with their multipotent ability to differentiate[32]. The impact of hUCMSCs-sec on tumor progression remains largely unclear, and their roles in this context are uncertain and subject to controversy.

According to Hendijani et al.[14], the secretomes of WJ-MSCs neither stimulated the rapid growth of lung cancer cells nor influenced the programed cell death potential of tumor cells, but it was associated with an increased likelihood of advanced necrosis. However, their in vitro findings indicated that the MSC secretomes was non-tumorigenic and did not confer resistance to doxorubicin in lung cancer cells, making it suitable for use in medical treatment. This investigation aligns with the findings of Karaoz et al.[33], demonstrating that co-culture of cancer cell lines with secretomes did not yield notable

enhancement in either proliferation assays or PCNA staining. Conversely, a study by Widowati et al.[34] stated that both normoxic and hypoxic medium of hWJMSCs could inhibit various malignant cell lines (ovarian, liver, tongue squasoma, and cervical cancer) and were not toxic to normal cells (human MSCs, human fibroblast, and mouse fibroblast).

Owing to the exceptional homing capability of MSC tumor sites, there has been an expansion in cancer treatment approaches, shifting from exclusively tumor cell-focused to modifying the tumor microenvironment[35]. Hence, this investigation aimed to assess gene expression in glioblastoma and examine the impact of hUCMSCs-sec treatment, considering the concentration determined for treatment via cytotoxic assay.

A gene that plays a vital role in the various cancers development is the homeobox (HOX) gene. Protein products derived from HOX genes promote carcinogenesis by exhibiting upregulation in cancer cells. They disrupt multiple signaling pathways by influencing the downstream targets of these pathways[36]. HUCMSCs-sec downregulated the HOXA5, HOXC10, and HOXC9 genes expression in GBM cells, as depicted in Figure 5c-e, with an optimum concentration of 1.25% for treatment. The role of MSC secretomes in relation to HOX genes in GBM remains relatively unexplored. However, Cimino, et al.[37] stated that targeting the HOX genes could enhance the response to radiation therapy in GBM. This study is consistent with the characteristics of MSCs described by Lin et al.[32], who asserted that vesicle compounds obtained from MSCs can serve as a medicinal approach for cancers resistant to treatment.

The ERK is triggered by diverse extracellular stimuli, including growth factors, cytokines, hormones, oxidative stress, and heat-acting receptors such as tyrosine kinases (RTKs) or EGFRs[38]. ERK and EGFR have been demonstrated to play significant roles in cell motility and survival. Consequently, ERK and EGFR overexpression is a common phenomenon in numerous cancer cells[39]. The hUCMSCs-sec has been shown to decrease ERK and EGFR levels in GBM. As illustrated in Figures 5b and 5f, gene expression was optimally decreased at a treatment concentration of 1.25%. This study is consistent with the research conducted by Ebadi et a.l[40], indicating that the secretomes of hAMSCs can reduce the levels of EGFR and ERK in HT-29 colon cancer cells.

Furthermore, Accumulating evidence indicates that IGFBP2 exerts a nuclear regulatory impact closely linked to Signal Transducer and Activator of Transcription 3 (STAT3) and EGFR . Phillips et al.[41] support this assertion, whose study illustrates that IGFBP2 is involved in modulating the EGFR-STAT3 pathway during the course of glioma development. When treated with an IGFBP2-neutralizing antibody, there is also a decrease in the EGFR activation and STAT3, including B-cell lymphoma-extra large (Bcl-xL) and Lysine-Specific Demethylase 1 (LSD1). It can therefore be inferred that IGFBP2, a multifunctional oncogenic protein, is actively involved in processes associated with cancer growth. It contributes to oncogenic activities by participating in various signaling pathways crucial for both tumor initiation and progression of tumors[6]. Our research demonstrated that the application of hUCMSCs-sec to GBM cells decreases IGFBP2 levels with an optimum treatment concentration of 1.25% (Figure 5g).

To assess apoptosis in hUCMSCs-sec, an examination of the Casp3 gene expression was examined. Casp3 is commonly recognized for its activated proteolytic functions in the implementation of apoptosis within cells[42]. The expression of Casp3 was successfully enhanced in GBM cells treated with hUCMSCs-sec (Figure 5a). The most effective treatment was observed with the addition of hUCMSCs-sec at a concentration of 1,25%, resulting an increase level of 2.52 ± 0.11 compared with the control. This study aligns with the findings of Rezaei-Tazangi et al.[43], who reported increased Casp3 and Casp9 activities in HT-29 cells upon the addition of the WJMSC secretomes. Additionally, another investigation indicated a notable upregulation of both BCL2-associated X-protein (BAX) and Casp3, along with a significant downregulation of anti-apoptotic (BCL2) genes in WJMSC secretome-treated MCF-7 cells[44].

The qRT-PCR findings indicate that the hUCMSCs-sec treatment induced Casp3 expression while suppressing HOXA5, HOXC9, HOXC10, IGFBP2, EGFR, and ERK genes associated with cancer growth. Notably, the secretomes treatments from hUCMSCs

exhibit a substantial impact on gene expression levels in treated cells. These outcomes support the therapeutic potential of treatments derived from hUCMSCs for glioblastoma cells.

CONCLUSION

This study successfully isolated and characterized hUCMSCs. The hUCMSCs-sec demonstrated therapeutic effects in influencing the growth of GBM cells through cytotoxic effects and downregulation of the expression of HOXA5, HOXC9, HOXC10, IGFBP2, EGFR, and ERK and upregulation of Casp3 in GBM cells. Additional investigations are warranted to explore the potential synergies between the drugs and hUCMSCs-sec, with the aim of enhancing the GBM therapy effectiveness.

ACKNOWLEDGMENT

We acknowledge the support from the Aretha Medika Utama (BBRC), Indonesia, for providing laboratory facilities and valuable insights. Special thanks to Adilah Hafizha Nur Sabrina, Nindia Salsabila Mia Dewi, Vini Ayuni, and Fadhilah Haifa Zahiroh from Aretha Medika Utama (BBRC) for their invaluable assistance.

Funding

The Minister of Education, Culture, Research, and Technology of the Republic of Indonesia supported this research under grant number 148/E5/PG.02.00.PL/2023.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest that may influence this study or the interpretation of results.

Ethical Approval

This ethical approval was received from the Research Ethics Committee of the Faculty of Medicine, Maranatha Christian University (No: 097/KEP/VII/2020, dated on July 25th, 2020).

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