Phenotype and Conditioning Medium on Umbilical Cord-Mesenchymal Stem Cell (UC-MSC)

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ABSTRACT

Background: *Stem cells are unspecialized cells that differentiate into more specialized cells for various tissues in the human body, to renew themselves by dividing by mitosis and differentiating into various types of cells, having great potential for tissue regeneration. The detection and isolation of specific cells is of great importance in understanding biological processes such as development, senescence, regeneration and cell pathogenesis. Thus, stem cells are present as an invaluable therapeutic option. Before stem cells can be applied, it is important to know the characteristics of the cell's specific function or phenotypic markers. Isolation and characterization of stem cells depend on morphological culture or immunostaining. Immunohistochemistry can provide an overview of marker expression in a cell under certain conditions. Surface markers are the basis for identifying the characteristics and isolation of stem cells. The basic characteristic of stem cells is one that influences the outcome of the therapy to be carried out. The expression of cell surface markers can also be a differentiator in identifying the origin of the stem cells used.*

Method: *MSC culture method was done with DMEM + 5% HPL. Umbilical cord-mesenchymal stem cell (UC-MSC) phenotypic differentiation.*

Results: *Phenotypes were found CD73+, CD90+, CD105+, Lin- while the parameters found were IL-6, SDF-1 , BDNF, FGF, NGF, IL-12p70, IL-23, IL-18, IL-10, VEGF, EGF, IL -17A, IFN-γ, IL-1β, PDGF, MCP-1.*

Conclusion: *The standard content of UC-MSC cells can be used for cell research.*

Keywords: *stem cell, UC-MSC, phenotype, parameters*

ABSTRAK

Latar belakang: *Stem sel adalah sel yang tidak terspesialisasi yang berdifferensiasi menjadi sel yang lebih khusus untuk berbagai jaringan pada tubuh manusia, untuk memperbarui diri dengan membelah mitosis dan berdiferensiasi menjadi berbagai ragam sel, memiliki potensi yang besar untuk regenerasi jaringan. Deteksi dan isolasi dari sel spesifik menjadi sangat penting dalam memahami proses biologis seperti perkembangan, penuaan, regenerasi, dan patogenesis sel. Maka, stem sel hadir sebagai pilihan terapi yang tidak ternilai. Sebelum stem sel dapat diaplikasikan, penting untuk mengenal karakteristik dari sel fungsi spesifik atau marker fenotip. Isolasi dan penilaian karakteristik dari stem sel bergantung pada morfologi kultur atau immunostaining. Immunohistokimia*

dapat memberikan gambaran umum tentang ekspresi penanda pada suatu sel dalam kondisi tertentu. Surface marker menjadi dasar dalam mengidentifikasi karakteristik dan isolasi dari stem sel. Karakteristik dasar dari stem sel merupakan salah satu yang berpengaruh dalam luaran terapi yang akan dilakukan. Adanya ekspresi marker cell surface juga dapat menjadi pembeda dalam melakukan identifikasi dari asal stem sel yang digunakan.

Metode: *Metode kultur MSC dilakukan dengan DMEM + 5% HPL. Umbilical cord-mesenchymal stem cell (UC-MSC) yang dilakukan differensiasi fenotipe.*

Hasil: *Fenotipe ditemukan CD73+, CD90+, CD105+, Lin- sedangkan Parameter yang ditemukan adalah IL-6, SDF-1 , BDNF, FGF, NGF, IL-12p70, IL-23, IL-18, IL-10, VEGF, EGF, IL-17A, IFN- γ, IL-1β, PDGF, MCP-1.*

Simpulan: *Kandungan standar sel UC-MSC dapat untuk dilanjutkan sebagai penelitian sel.*

Kata kunci: *stem sel, UC- MSC, fenotipe, parameter*

INTRODUCTION

Stem cells are unspecialized cells that differentiate into more specialized cells for various tissues in the human body. Stem cells have the characteristics and ability to self-renewal by mitotic division and differentiate into various types of cells.[1,2] Stem cells also have great potential for tissue regeneration. Stem cells can produce daughter cells that are identical to the parent cell (self-renewal), or produce cells with a certain potential (differentiated cells).[2] The origin of stem cells is generally understood to be derived from embryonic stem cells.[3] Embryonic stem cells appear before the germ layer, which makes it the basis for the mechanism for the occurrence of multipotent stem cells, one of which is mesenchymal stem cells (MSCs).[3,4] MSCs can be obtained from various sources, such as placental blood, peripheral blood, adipose tissue, pulp, and bone marrow.[5,7] Detection and isolation of specific cells are very important in understanding biological processes such as development, aging, regeneration, and cell pathogenesis. Therefore, stem cells are present as an invaluable therapeutic option.[8]

Before stem cells can be applied, it is important to recognize the characteristics of specific function cells or phenotypic markers.[9] The success of expansion can be judged by the characteristics and proliferation rate of MSCs produced. Supplements added, for example growth factors, as well as the selection of the right culture medium can affect the pluripotency of stem cells and cell proliferation.[10] In addition, the composition of the culture medium is also a factor that affects the success of MSC expansion in vitro. Each culture medium has a different composition so the effectiveness of the medium is also different for each type of stem cell.[11] MSCs are nonimmunogenic cells, therefore transplantation into allogeneic hosts may not require immunosuppression. Phenotyping needs to be done to assess the surface markers of MSCs. This phenotyping process is useful for the identification of MSCs. MSCs are identified by the expression of CD105 and CD73 molecules and are negative for hematopoietic markers. These characteristics of MSCs make them potentially ideal candidates for tissue engineering.[12] In cultures with MSC stem cells, which were sourced from horse fat tissue, optimal proliferation was reported in the combined Dulbecco's modified Eagle medium (DMEM) and minimum essential medium (MEM).[5,13] MSC characterization needs to be done to determine the plasticity or potency of the cultured MSC.[14] This article aims to determine this ability, it is possible to examine specific markers for MSCs and differentiate MSCs into various cell types. MSC characterization and phenotyping is the first step to identifying MSCs so that they can be used for further research. Identification of MSC secretome content is also important to assess differentiation and benefits in therapy. This identification and phenotyping process is an important step before using stem cells for liver disease therapy.

METHOD

This study is a descriptive study conducted by In Vitro in March 2020 at the Prodia Stem Cell (ProStem) laboratory in Jakarta. This study received ethical approval from the Health Research Ethics Commission of Dr. Moewardi Hospital with number 178/I/HREC/2020.

Table 1. Protocol of isolation and immunophenotyping of umbilical cord-mesenchymal stem cells (UC-MSCs)

Kit	Step	Protocol
Human mesenchymal stem cell analysis kit	1	Prepare mesenchymal stem cells (MSCs) $1x10^6$ cell/mL
	2	Take a 100 µmL pipette as a sample and place it in a new cup
	3	Each tube is marked with 20 µL positive cocktail and 20 µL negative cocktail
	4	Incubate for 30 minutes at room temperature. Protect from exposure to direct light
	5	Added 1 mL of phosphate buffered saline (PBS) and centrifuged 200 g for 10 minutes
	6	The supernatant is discarded
	7	Re-suspend the pellet by adding 0.5 mL of PBS
	8	Flow cytometry is used

Table 2. Protocol of hepar G2 cell line isolation

Step	Protocol
1	HepG2 cells were cultured in a 100 mm petri dish using growth medium Dulbecco's modified Eagle medium (DMEM) 5% + human platelet lysate (HPL) for 5 days until a confluency of > 80%
2	When confluent, cells were harvested by adding 3 mL of TrypLe solution and incubated at 37 °C; 5% CO_2 for 7 minutes
3	The petri dish is tapped gently so that the cells are completely released
4	TrypLe is neutralized with growth medium (1:1)
5	The entire solution is accommodated in a petri dish in a conical tube
6	The sample is centrifuged for 5 minutes at 500 g speed to obtain a cell suspension
7	The supernatant was discarded and the cells were topped up with 1 mL phosphate buffered saline (PBS) for the cell count

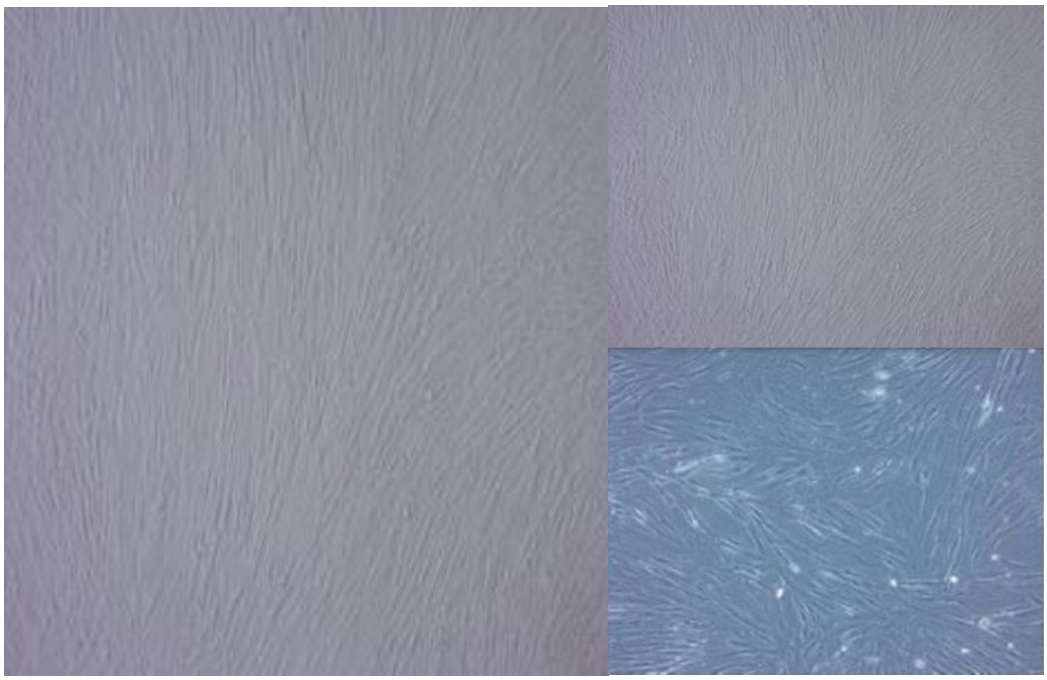

Figure 1. Isolation and Differentiation of the umbilical cord-mesenchymal stem cells

Umbilical cord samples were taken from the umbilical cord's healthy donor and were processed within 48 hours after cesarean section. Umbilical cords were delivered in a sterilized bottle. Under the biosafety cabinet, cords were washed with phosphate buffer saline (PBS) (Lonza17-516F) to remove any containing blood. Vessels were removed, cut the cord into small pieces, and placed in a culture dish in a growth medium. UC-MSC were cultured in a 100 mm petri dish (NUNC,150464-CS) using growth medium DMEM (Lonza,12-917F) + 5% HPL (Sigma-Aldrich, SCM152) for five days until a confluence of > 80% was reached. The addition of TryPLE (Gibco,12605028) removed tissues. The solution was centrifuged (Thermo Scientific, 41330587) for 5 minutes at 500 g speed to obtain a cell suspension, and 1 ml PBS (Lonza17-516F) solution was added for cell count. Stem cells can differentiate into fibroblasts

RESULT

This study showed markers CD 73+, CD 90+, C 105+ and Lin – after immunophenotyping. The expression of CD 73+ and CD 90+ was 99.9%, while CD 105+ was 98.5% (Figure 2). UC-MSC CM Characteristics were obtained with dominant parameters of SDF-1, BDNF and IL-6 (Figure 3). In the characteristics of the UC-MSC CM, the most significant expression was IL-6 of 1166.4 pg/ml, followed by SDF-1 expression of 861.95 pg/ml and BDNF of 126.60 pg/ml. Stem cell cultures can also develop into fibroblast cells (mesoderm) in this study (Figure 1).

Marker	Ekspresi
CD73+	99.9%
CD90+	99.9%
CD105+	98.5%
Lin-	0.6%

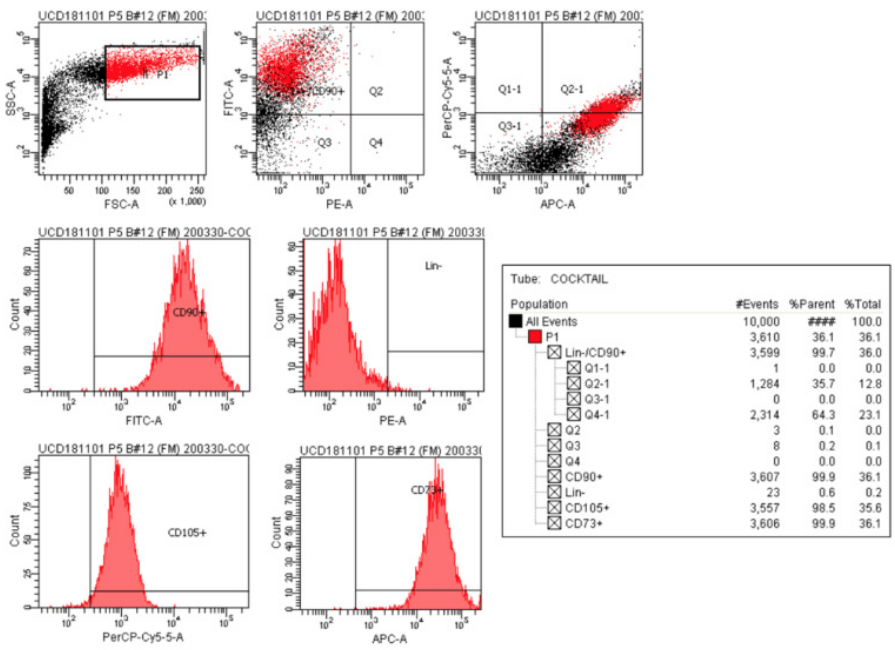

Figure 2. Mesenchymal Stem Cells Phenotyping

Mesenchymal Stem Cells phenotyping examination showed CD90, CD105, and CD73 expression. These results indicate the purity of the mesenchymal stem cells used.

Parameter	Unit	Result[1]
BDNF	pg/ml	126.60
SDF-1	pg/ml	861.95
FGF	pg/ml	48.13
VEGF	pg/ml	12.02
PDGF	pg/ml	1.33
FGF	pg/ml	48.13
EGF	pg/ml	6.25
NGF	pg/ml	31.52
IL-10	pg/ml	15.40
IL-1β	pg/ml	2.64
IFN-γ	pg/ml	4.06
MCP-1	pg/ml	0.08
IL-6	pg/ml	1166.4
IL-12p70	pg/ml	30.06
IL-17A	pg/ml	4.65
IL-18	pg/ml	13.44
IL-23	pg/ml	22.06

Figure 3. Identification of UC-MSC conditioned medium

SDF-1, BDNF and IL-6 have dominant levels in the characteristic examination of UC-MSC CM

DISCUSSION

Stem cells are cells that are the beginning of the growth of other cells that make up the whole body of organisms, including humans.[1] The human body is composed of > 100 trillion cells, which are divided into about 200 different types of cells with different functions.[2]

Mesenchymal stem cells (MSCs), also known as multipotent mesenchymal stromal cells, are mature progenitor cells derived from the neural crest and mesoderm.[8] MSCs can be found in bone marrow, adipose, umbilical, placental, fetal and liver tissues.[5-7] In bone marrow, the number of MSCs is about 0.001–0.01% of the total cells. MSCs play a role in non-hematopoiesis regeneration including the regeneration of osteoblasts, adipocytes and chondrocytes.[15]

The International Society of Cell Therapy (ISCT) stipulates that MSCs must meet the following criteria: Cells adhered to plastic under normal/standard culture conditions; Expressing positive CD105, CD73, CD90; Does not express (negatively) CD11b, CD14, CD34, CD45, CD79a, HLA-DR; and Ex-vivo can differentiate into osteocytes, adipocytes and chondrocytes.[2]

MSC generally have low immunogenicity due to low expression of class II MHC antigens and class I MHC molecules.[16] In addition, MSCs do not contain co-stimulatory molecules such as CD80, CD86 and CD40, which need recognition by immune cells.[17] according to this research, MSC also did not have the expression of CD89, CD86 and CD40.UC-MSC showed the presence of 3 dominant markers, CD 73+, CD 90+ and CD 105+. MSCs have positive markers for the presence of CD73, CD90 and CD105, indicating their ability to differentiate into adipocytes, osteoblasts and chondroblasts under certain conditions.[18]

The main molecules expressed in UC-MSC CM are SDF-1, BDNF and IL6. VEGF and IL-6 are growth factors.[19] IL-6 also functions as an anti-apoptotic molecule by increasing the expression of anti-apoptotic factors such as Bcl-2 and Bcl-xl during liver repair.[19,20] SDF-1 or CXCL12, is a cytokine expressed by normal liver cells. Its expression will increase in acute or chronic cell damage.[21] The various molecules produced by UC-MSC can potentially be used as regenerative

Table 3. Characteristic of stem cell

Characteristic	Explanation
Undifferentiated	Cells that do not yet have a specifix shape and function like other cells in the body's organs
Able to reproduce	Stem cells can replicate and produce cells with the same characteristics as the parent cell. This factor is considered important for maintaining the stem cell population in the body in to maintain homeostasis of body tissues
Can differentiate into > 1 cell type (multipotent/ pluripotent)	Pluripotent: able to differentiate into any cell body originating from all three embryonic layers (ectoderm, mesoderm, endoderm) Multipotent: only able to differentiate into several types of cells that are usually in a similar group, such as cells of the hematopoietic system or nervous system

therapy. However, further research is needed to determine the benefits of the clinical application of the UC-MSC and UC-MSC CM.

CONCLUSION

UC-MSCs have the main phenotype characteristics of MSCs and can be used as an alternative therapy for regenerative medicine. MSCs are proven to be able to differentiate into fibroblasts. The main components of UC MSC CM are SDF-1, BDNF and IL-6. The three main components have benefits in the regeneration process of cells. Further experimental studies are needed to assess the effects of UC-MSC and UC-MSC CM administration on cell regeneration.

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