Human Umbilical Cord Mesenchymal Stem Cells (hUC-MSCs) Inhibits Acute Myeloid Leukemic Cell Line (K562) *in vitro*

Muneerah A Huwaikem[1], Farid Ahmed[2], Mohammed AlQahtani[2], Kalamegam Gauthaman[2 *]
[1]Department of Medical Laboratories Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
[2]Centre of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
*Corresponding author

11th Annual Conference on Stem Cell and Regenerative Medicine

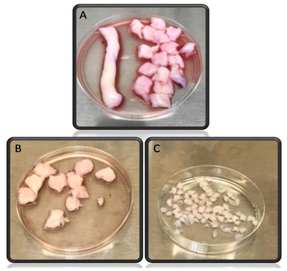
INTRODUCTION

- AML is a clonal disorder of the hematopoietic stem cells (HSCs) and is characterized by abnormal proliferation of the myeloid progenitors, subsequently leading to marrow failure [1].
- AML is the most common leukemia in adults. Despite therapeutic advances, its prognosis remains poor in adults compared to the younger patients.
- However, the relapse rate (recurring of disease) also remains higher despite chemotherapy. Therefore, treatment of AML remains a great challenge [2].
- Interestingly, the mesenchymal stem cells (MSCs) either engineered or naive are used to target various cancers to bring about its inhibition [3]. As such, in the present study we explored and evaluated the cancer inhibiting properties of the MSCs derived from the human umbilical cord (hUC-MSCs) on AML cell line (K-562) using *in vitro* studies in terms of morphological changes of the cancer cell, its proliferation/inhibition and related gene expression.

MATERIALS AND METHODS

- Human umbilical cord samples were collected following Institutional Ethical Committee approval [33-15/KAU]. hUC-MSCs were derived using explant culture method and the cell line K562 was obtained from ATCC.
- Derived hUC-MSCs were characterized for their stemness using cell morphology and MSCs related CD markers expression (FACS). CD marker antibodies cocktails (Miltenyi Biotech) composed of CD marker (5µl per individual CD marker) were used to assess the hUC-MSCs related markers. MSCs positive cocktail 1 (labelled as Mix 1, containing CD29 APC, CD90-FITC and CD73-PERCP), MSCs positive cocktail 2 (labelled as Mix 2, containing CD44-APC and CD105-FITC) and MSCs negative cocktail 3 (labelled as Mix3, containing CD34-APC and CD45-PE). CD marker expressions were analyzed using FACS (FACS Aria II, BD Biosciences).
- Anti-cancer effect of hUC-MSCs was evaluated by co-culture with AML cells (K562 cells) plated at equal seeding density (2 x 10^4 cells/well) in a 24-well plate followed by culture for 24h, 48h and 72h. Changes in cell morphology, cell proliferation (MTT assay) and gene expression (quantitative real-time polymerase chain reaction, qRT-PCR) were assessed.
- The morphological characteristics of the hUC-MSCs and K562 cells in co-culture were evaluated during the three different duration times (24h, 48h and 72h) using phase contrast microscope (Nikon ECLIPCE TS100, Japan) at low (x4) and high (x10) magnifications.
- The K562 cells proliferation was evaluated using MTT assay. A density of (2 x 10^4 cells/well) were seeded in 24-wells culture plates containing its own culture medium and treated with hUC-MSCs, and then incubated at 37°C in a 5 % CO_2 atmosphere for three periods of 24h,48h and 72h. MTT reagent (yellow tetrazolium MTT 3-(4, 5-dimethylthiazol-2-yl-2, 5-diphenyltrazolium bromide) was added to the cells followed by adding Dimethylsulfoxide to measure the optical density with absorbance microplate reader spectrophotometer (SpectraMax i3) at a wavelength of 750 nm and 630 nm.
- qRT-PCR was done using the Fast SYBR Green Master Mix. The data were normalized to housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the relative expression level and fold induction of each target gene were calculated using a comparative CT method [(1/ ($2^{\Delta CT}$) formula, where ΔCT is the difference between CT target and CT reference].

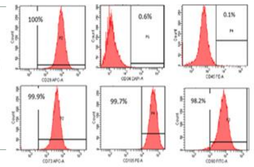
Figure 1: Deriviation of hUC-MSCs using explant method



Images of explant method. (A) Human UC of 15 cm was cut into 1 cm portions; (B) 1 cm UC portions are washed with PBS to remove blood; (C) UC fragments 2-3 mm^3.

RESULTS

Figure 2: CD markers expression of hUC-MSCs. FACS images showing positive expression of CD29, CD73, CD90 and CD105 and negative expression of CD34 and CD45 respectively.



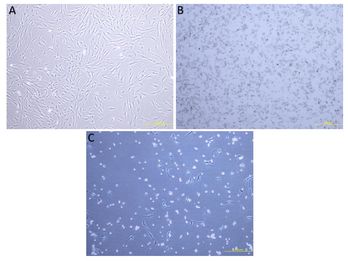


Figure 3: Phase contrast micrograph of cells after 72h of incubation.

(A) hUC-MSCs showed long fibroblastic-like shape indicated by black arrow (4x magnifications).

(B) K562 cells in suspension culture showing the blast cell appearance; spherical and undifferentiated cells (4x magnification).

(C) Co-culture of K562 cells and hUC-MSCs showed accumulation of K562 cells around hUC-MSCs, and decreasing of K562 cells.

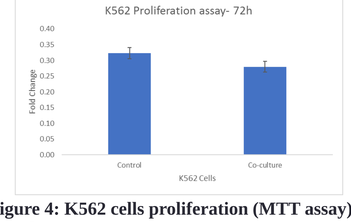


Figure 4: K562 cells proliferation (MTT assay) in co-culture.

K562 cells showed significant decreases in their proliferation (normalized to control) when exposed to hUC-MSCs in co-culture for 72.

RESULTS

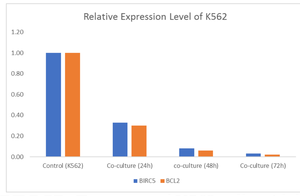


Figure 5: qRT-PCR. Treatment of K562 cells with hUC-MSCs in co-culture for 72 h showed decreased expression of anti-apoptotic gene (BCL2 gene) and SURVIVIN genes.

CONCLUSION

The present study demonstrated that hUC-MSCs were spindle shaped in early passages while through late passages the cells were mostly fibroblastic-like shaped. Furthermore, hUC-MSCs were plastic-adherent cells and showed the expression for CD markers related to human MSCs, in addition to different proliferation rates through different passages. K562 cells showed blast cell appearance; spherical and undifferentiated cells as well as considerable co-culture of hUC-MSCs with K562 cells showed that K562 cells were affected by hUC-MSCs through their accumulation around hUC-MSCs and decreasing in their number. The K562 cells proliferation rate for 72h were significantly decreased as well as gene expression of anti-apoptotic (BCL2) and SURVIVIN genes for K562, after treated with hUC-MSCs cells were significantly decreased compared to control. Identification of decreasing in K562 cells proliferation and their specific gene expression after treatment with hUC-MSCs will directly highlight the anti-cancer effects (properties) of hUC-MSCs on AML, which will provide us with new insights and add to the scientific knowledge, cell therapy and regenerative medicine.

ACKNOWLEDGMENT

We thank Almighty Allah, then we thank Biomedical Research Ethics Committee of the Faculty of Medicine of King Abdulaziz University, Department of Obstetrics and Gynecology at King Abdulaziz University Hospital and Embryonic Stem Cell Research Unit, King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia for giving us the chance to make this study.

References

[1] ISLAM, A. 2017. Induction treatment of acute myeloid leukemia in an elderly patient with intramarrow injection/administration of cytarabine. Second case report. Clin. Case Rep. Clinical Case Reports, 5, 1496-1502.

[2] SAULTZ, J. N. & GARZON, R. 2016. Acute Myeloid Leukemia: A Concise Review. Journal of clinical medicine, 5.

[3] BONOMI, A., BUCCARELLI, M., DALESSANDRIS, Q., FALCHETTI, M., GIANNETTI, S., PASCUCCI, L., PACIONI, S., MORGANTE, L., PALLINI, R., RICCI-VITIANI, L., PESSINA, A., ALESSANDRI, G. & COCCÈ, V. 2017. Human mesenchymal stromal cells inhibit tumor growth in orthotopic glioblastoma xenografts. Stem Cell Research & Therapy, 8, 1-15.