The Role of Reprogrammed Somatic and Mesenchymal Stem Cells Through Wnt Signaling Pathway Modulators in Cardiac Regeneration

Bilal Saleh[1], Rabbia Muneer[1], Abiha Fatima[1], Irfan Khan[1,2], Joachim W. Herzig[3] Asmat Salim[1]

[1]Dr. Panjwani Center for Molecular Medicine & Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270 Pakistan.

[2]Center for Regenerative Medicine and Stem Cell Research, The Aga Khan University, Karachi, Pakistan.

[3]Department of Physiology and Pathophysiology Faculty of Medicine, Johannes Gutenberg University 7b Hardstrasse, Grenzach-Wyhlen, Germany.

ABSTRACT

Reprogramming of somatic and mesenchymal stem cells into cardiomyocytes represents a significant approach in regenerative medicine for treating cardiovascular disorders. However, several challenges during the reprogramming processes need to be addressed. Cardiomyocyte differentiation requires the activation of the Wnt signaling pathway at the initial stage and its inhibition at a later stage. In this study, fibroblasts and human umbilical cord-derived MSCs were treated with a Wnt pathway activator (SKL2001) and inhibitor (ETC-159) under identical experimental conditions. Both cell types were isolated, characterized based on their specific surface markers and multilineage potential. Selected small molecules were non-toxic up-to 100 µM concentrations. Preconditioning of MSCs and fibroblasts with SL2001 and ETC-159 was performed in different groups. The analyzed gene and protein expression revealed that the treatment successfully altered Wnt pathway-related genes, including Wnt, β-catenin, and other associated genes, with different fold changes in both, fibroblast and MSCs. However, treatment was not sufficient for preconditioning of fibroblasts toward cardiac-like cells, as no upregulation of early and late cardiac markers, including GATA-4, Tbx20, Mef2c, cMHC, and cTnI, was noted. After 14 days treatment of MSCs, upregulation of cardiac early and late markers including GATA-4, Mef2c, cMHC, cTnT and cTnC was observed. Immunocytochemistry of the treated MSCs further demonstrated the enhancement of cardiac-specific protein expression markers, such as GATA-4, Nkx2.5, cTnI, α-actinin, and connexin-43, suggesting the differentiation of MSCs into cardiac-like cells. The regenerative potential of these preconditioned cells was assessed in a rat myocardial infarction model. Echocardiograms taken at 2- and 4-weeks post-surgery confirmed the induction of myocardial infarction and showed improvement in cardiac functional parameters in the group, transplanted with preconditioned MSCs. Masson's trichrome and H&E staining of harvested heart tissue indicated the development of MI and their regeneration in the infarcted area, demonstrating the regenerative potential of the transplanted preconditioned MSCs. In conclusion, this study demonstrated that MSCs could be chemically induced to differentiate toward the cardiac lineage *in vitro*, and their pre-clinical potential was investigated *in-vivo*. However, further improvements are necessary to enhance the functionality of the differentiated cells to fully restore heart tissue.

Keywords: Reprogramming, Cardiovascular Disorders, Preconditioning, Cytotoxicity.

INTRODUCTION

Regenerative medicine particularly stem cell therapy is an advanced technique for regeneration of functional cardiomyocytes (Madeddu, 2021; Matsuzaki *et al*., 2021) with several benefits over the surgical procedure (Limongi *et al*., 2017; Limongi *et al*., 2021). Pre-clinical data has shown its potential for treatment, but certain

evidences raise questions on its efficacy. However, the data helps to understand the phenomenon of stem cell therapy and provide guidelines for future strategies to achieve full recovery of cardiac function (Madeddu, 2021; Xu *et al*., 2021).

Several studies have identified different compounds and transcription factors that possess high cardiac differentiation efficiency such as 5-azacytidine (Hasani *et al*., 2020), Iwr-1 (Karakikes *et al*., 2014), ascorbic acid and albumin (Burridge *et al*., 2014), CRFVPTZ (chemical cocktail) (Fu *et al*., 2015), 2'-deoxycytidine (Ali *et al*., 2020), zebularine, combination of different small molecules e.g. CHIR99021, SC1, Y27632, A83-01, BIX01294, AS8351, OAC2, and SU16F (Cao *et al*., 2016), overexpression of cardiac markers (Nkx2.5 and GATA-4) (Gao *et al*., 2011), induced pluripotent stem cell (iPSC) transcription factor, OCT-4 (Yannarelli *et al*., 2017), etc. These studies highlight these approaches in facilitating cellular differentiation, optimizing cell-type specificity, improving procedural efficiency, ensuring reproducibility, and targeting specific signaling pathways. However, certain limitations in the current methodologies resulted in the generation of heterogeneous population of cardiomyocytes (Karakikes *et al*., 2014) as well as cardiac progenitor cells (Minami *et al*., 2012), suggesting the need for further refinement in strategies and / or use of chemicals which are clinically safe, having no or limited side effects and higher cardiac differentiation capability.

OBJECTIVE OF STUDY

The aim of current study is to explore the effects of Wnt pathway modulators SKL2001 and ETC-159 on fibroblast and stem cells for cardiac differentiation. The findings from this research will contribute to a better understanding of cardiomyocyte differentiation patterns in hU-MSCs, somatic cells by modulating the Wnt signalling pathway.

METHODOLOGY

Fibroblasts and human umbilical cord-derived MSCs (hUC-MSCs) were treated with a Wnt pathway activator (SKL2001) and inhibitor (ETC-159) under identical experimental conditions. Both cell types were isolated, characterized based on their specific surface markers and multilineage potential followed by cytotoxicity analysis of both small molecules. Treatment of hUC-MSCs and fibroblasts with SL2001 and ETC-159 was performed in different groups. Gene expression analysis to investigate altering of Wnt pathway in treated hUC-MSCs and fibroblasts. Cardiac early and late markers were evaluated in treated hUC-MSCs and fibroblasts to identify the differentiation of fibroblast and hUC-MSCs. Immunocytochemistry of the treated MSCs further investigated to confirm cellular differentiation at protein level followed by the transplantation of treated hUC-MSCs in rate myocardial infracted (MI) model. Echocardiography was performed to examine cardiac functional parameters and histological analysis to identify regeneration potential following MI.

RESULTS

Selected small molecules were non-toxic up-to 100 µM concentrations. The analyzed gene and protein expression revealed that the treatment successfully altered Wnt pathway-related genes, including Wnt, β-catenin, and other associated genes, with different fold changes in both, fibroblast and MSCs. However, treatment was not sufficient for preconditioning of fibroblasts toward cardiac-like cells, as no upregulation of early and late cardiac markers, including GATA-4, Tbx20, Mef2c, cMHC, and cTnI, was noted. After 14 days treatment of hUC-MSCs, upregulation of cardiac early and late markers including GATA-4, Mef2c, cMHC, cTnT and cTnC was observed. Immunocytochemistry of the treated MSCs further demonstrated the enhancement of cardiac-specific protein expression markers, such as GATA-4, Nkx2.5, cTnI, α-actinin, and connexin-43, suggesting the differentiation of hUC-MSCs into cardiac-like cells. Echocardiograms taken at 2- and 4-weeks post-surgery confirmed the induction of myocardial infarction and showed improvement in cardiac functional parameters in the group, transplanted with treated hUC-MSCs. Masson’s trichrome and

H&E staining of harvested heart tissue indicated the development of MI and their regeneration in the infarcted area, demonstrating the regenerative potential of the transplanted treated hUC-MSCs.

CONCLUSION

This study demonstrated that MSCs could be chemically induced to differentiate toward the cardiac lineage *in vitro*, and their pre-clinical potential was investigated *in-vivo*. However, further improvements are necessary to enhance the functionality of the differentiated cells to fully restore heart tissue.

REFERENCES

1. Madeddu, P. (2021). Cell therapy for the treatment of heart disease: renovation work on the broken heart is still in progress. Free Radical Biology and Medicine, 164, 206-222.
2. Matsuzaki, Y., Wiet, M. G., Boe, B. A., and Shinoka, T. (2021). The real need for regenerative medicine in the future of congenital heart disease treatment. Biomedicines, 9(5), 478.
3. Limongi, T., Brigo, L., Tirinato, L., Pagliari, F., Gandin, A., Contessotto, P., Giugni, A. and Brusatin, G., (2021). Three-dimensionally two-photon lithography realized vascular grafts. Biomedical Materials, 16(3), 035013.
4. Limongi, T., Tirinato, L., Pagliari, F., Giugni, A., Allione, M., Perozziello, G., Candeloro, P. and Di Fabrizio, E. (2017). Fabrication and applications of micro/nanostructured devices for tissue engineering. Nano-Micro Letters, 9(1), 1-13.
5. Xu, S., Qiu, Y., and Tao, J. (2021). The challenges and optimization of cell-based therapy for cardiovascular disease. Journal of Translational Internal Medicine, 9(4), 234-238.
6. Hasani, S., Javeri, A., Asadi, A., and Taha, M. F. (2020). Cardiac differentiation of adipose tissue-derived stem cells is driven by BMP4 and bFGF but counteracted by 5-azacytidine and valproic acid. Cell Journal (Yakhteh), 22(3), 273.
7. Karakikes, I., Senyei, G.D., Hansen, J., Kong, C.W., Azeloglu, E.U., Stillitano, F., Lieu, D.K., Wang, J., Ren, L., Hulot, J.S., Iyengar, R., Li, R.A., and Hajjar, R. J. (2014). Small molecule-mediated directed differentiation of human embryonic stem cells toward ventricular cardiomyocytes. Stem Cells Translational Medicine, 3(1), 18-31.
8. Burridge, P. W., Matsa, E., Shukla, P., Lin, Z. C., Churko, J. M., Ebert, A. D., Lan, F., Diecke, S., Huber, B., Mordwinkin, N. M., Plews, J. R., Abilez, O. J., Cui, B., Gold, J. D., and Wu, J. C. (2014). Chemically defined generation of human cardiomyocytes. Nature Methods, 11(8), 855-860.
9. Fu, Y., Huang, C., Xu, X., Gu, H., Ye, Y., Jiang, C., Qiu, Z., and Xie, X. (2015). Direct reprogramming of mouse fibroblasts into cardiomyocytes with chemical cocktails. Cell Research, 25(9), 1013-1024.
10. Ali, S. R., Ahmad, W., Naeem, N., Salim, A., and Khan, I. (2020). Small molecule 2′-deoxycytidine differentiates human umbilical cord-derived MSCs into cardiac progenitors in vitro and their in-vivo xeno-transplantation improves cardiac function. Molecular and Cellular Biochemistry, 470, 99-113.
11. Cao, N., Huang, Y., Zheng, J., Spencer, C. I., Zhang, Y., Fu, J. D., Nie, B., Xie, M., Zhang, M., Wang, H., Ma, T., Xu, T, Shi, G., Srivastava, D., and Ding, S. (2016). Conversion of human fibroblasts into functional cardiomyocytes by small molecules. Science, 352(6290), 1216-1220.
12. Yannarelli, G., Pacienza, N., Montanari, S., Santa-Cruz, D., Viswanathan, S., and Keating, A. (2017). OCT4 expression mediates partial cardiomyocyte reprogramming of mesenchymal stromal cells. PloS one, 12(12), e0189131.
13. Minami, I., Yamada, K., Otsuji, T. G., Yamamoto, T., Shen, Y., Otsuka, S., Kadota, S., Morone, N., Barve, M., Asai, Y., Tenkova-Heuser, T., Heuser, J. E., Uesugi, M., Aiba, K., and Nakatsuji, N. (2012). A small molecule that promotes cardiac differentiation of human pluripotent stem cells under defined, cytokine-and xeno-free conditions. Cell Reports, 2(5), 1448-1460.