L-Carnitine Promotes Cardiomyogenic Differentiation of C-Kit+Bone Marrow Progenitor Cells via MAPK-ERK Signaling Pathway

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Abstract- Many studies have shown that bone marrow (BM) stem/progenitor cells have the highest probability of cardiomyocyte differentiation. Regarding the major role of C-kit$^+$ BM stem cells in cell therapy of patients with heart disease and getting cells with higher differentiation potential, this study aimed to investigate the capacity and effect of L-carnitine (LC) on cardiomyogenic differentiation of C-kit$^+$ BM cells through MAPK/ERK signaling pathway. For this purpose, C-kit$^+$ was enriched from the BM mononuclear cell population using a magnetic activating cell sorting technique. The purity of the separated C-kit$^+$ cells was then evaluated by flow cytometry. In the next step, C-Kit$^+$ cells were treated in a cardiomyogenic differentiation culture medium for 21 days once in the presence and once in the absence of 0.2 µM LC (the experimental and control groups). To evaluate the cardiomyogenic differentiation potential of C-kit$^+$ cells, the Desmin cell marker was determined by immunocytochemistry. The expressions of both GATA4 and ERK proteins were measured using western blotting and flow cytometry, respectively. The results show that 95.7 percent of the cells separated by the MACS technique expressed a C-kit$^+$ cell marker. Additionally, it was found that 0.2 mM LC significantly increased the expression of GATA4 protein in the cardiomyogenic differentiated cells. The expression of ERK protein also suggested a significant increase of about 1.60 times in the experimental group in comparison with the control group ($*P<0.05$). In brief, it was found that treating C-kit$^+$ BM cells with LC increases cardiomyogenic differentiation by increasing the expression of GATA4. Notably, this effect can take place through MARK/ERK signaling pathway. The results of this research can be valuable in suggesting a treatment solution for cardiovascular diseases.

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Keywords: C-kit+ bone marrow progenitor cells; Cardiomyogenic differentiation; L-carnitin; MAPK-ERK signaling pathway

Introduction

Heart failure (HF) is a complex syndrome and is known as the end point of all cardiovascular disorders (1). Myocardial infarction (MI) and cardiac hypertrophy are the main causes of HF. MI is caused by cardiac myocyte loss due to persistent ischemia. It has been reported that the apoptotic system is turned on during progressive HF. Necrosis of the myocardial muscle is the main cause of cardiomyocyte loss. P38-mitogen-activated protein kinase (MAPK), reactive oxygen species (ROS), and NF-κB activation has been reported to be effective in cardiomyocyte apoptosis during MI (2). As claimed by the World Health Organization (WHO), it will be the major cause of mortality in the near future. MI cellular injury leaves a scar formation and leads to mechanical dysfunction. Cell Regeneration in cardiomyocytes is limited; the available treatments for MI focus on reducing myocardial oxygen needs and ischemic consequences, increasing its supply, and improving myocardial function.

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There is a strong need to improve treatments and find new methods to modify outcome. Stem cell therapy is a promising outlook for cardiovascular disease (CVD), especially IM (3,4).

C-kit (also known as CD117) is a tyrosine kinase family receptor in hematopoietic stem cells. C-kit is temporarily expressed in cardiomyocyte progenitors and infrequent cell populations of the adult heart. Recently C-kit has been marked, Bi-potent cardiovascular progenitors. Reports indicate that cells expressing C-kit can differentiate into cardiomyocytes, endothelial, and smooth muscle cells (5). In line with previous findings, C-kit+ cardiac progenitors have the capability of multilineage differentiation and the potential to become cardiovascular-committed C-kit+ cells (6).

L-Carnitine (LC) (β-hydroxy-ϒ-trimethylaminobutyrate, 3-hydroxy-4-N, N, N-trimethylaminobutyrate or L-3-hydroxy-4-N-trimethylaminobutyric acid) and its metabolites are familiar dietary supplements for athletes. LC is a vitamin-like substance that was discovered in muscles in 1905 for the first time. LC can be supplied through exogenous and endogenous sources. Investigations have shown the capability of LC to inhibit apoptosis (7,8). LC has the ability to balance the metabolism of cardiac cells by increasing mitochondrial β-oxidation. LC simplifies the transportation of long-chain fatty acid into the mitochondrial matrix and has protective effects via the reduction of cardiac myocyte's oxidative stress, necrosis, and inflammation. Exogenous LC has protective effects against ischemia, arrhythmia, ventricular dysfunction, and toxic myocardial injury. Besides, it can reduce the cardiovascular pathogenicity arising from hypertension, diabetic ketoacidosis, hyperlipidemia, hyperglycemia, etc. (9).

Signaling pathways are intracellular signal cascades that mediate processes which include cell proliferation, cell metabolism, protein translation, and cell survival. Comprehension of signaling protein functions is an important issue in cardiovascular pathophysiology (10). MAPK/extracellular signal-regulated kinase (MAPK/ERK) pathway is associated with differentiation, cell proliferation, migration, aging, and apoptosis. There are four different cascades in MAPK signaling pathway, which names are; p38-MAPK, extracellular signal-related kinases (ERK1/2), ERK5, and Jun amino-terminal kinases (JNK1/2/3) (11). According to reports, there is an expression of MAPK signaling related to stem cell cardiomyogenesis and there are findings that indicate, phosphorylated ERK (p-ERK) expression is existing in myocardial tissues of pathologic cardiac hypertrophy (12,13). Based on research conducted on zebrafish, MAPK/ERK signaling pathway is indispensable for cardiac regeneration (14).

Present study aimed to modify cardiac function through cell therapy and cardiomyocyte differentiation with LC-treated bone marrow (BM)-resident C-kit+ cells and find the role of MAPK-ERK signaling pathway.

Materials and Methods

All cell culture plates and materials were purchased from SPL Life Sciences Co., Ltd. (Gyeonggi-do, Korea) and Gibco (UK), respectively. Other materials were specified in the text.

In this study, the cells were divided into two groups: group I as the control group (cardiac differentiated C-kit$^+$ cells without any LC treatment) and group II as the experimental group (cardiac differentiated C-kit$^+$ cells with LC treatment).

Isolation of C-kit$^+$ cells

After giving ethical consent from the Committee on the Ethics of Tabriz University of Medical Sciences, Tabriz, Iran (Ethic Code No: IR. TBZMED.VCR. REC.1398.197), 6 rats were euthanized using Ketamine/Xylazine (87/13 mg/kg). After surgery and removal of the femur and tibia, BM contents were flushed using washing buffer (phosphate-buffered saline (PBS) supplemented with 5% fetal bovine serum (FBS)). Extracted fluid from BM was diluted 2:1 with PBS (pH 7.4). BM mononuclear cells (BM-MNCs) were obtained by gradient separation by a Ficoll Hipaque gradient (1.087 g/ml). BM-MNCs, which were located between the plasma and the Ficoll due to their low density, were separated and washed several times with a washing buffer. Cells were suspended in DMEM medium with 10% FBS. Then living cells were counted under a microscope using a Hemocytometer slide.

C-kit$^+$ cells were separated from the BM-MNCs population by magnetic cell sorting (MACS). BM-MNCs were incubated with FCR blocking (Cat No. 130059901, Milteny Biotech Company) and CD117 Micro Beads antibody (Cat No. 130-091-224, Milteny Biotech Company). C-kit$^+$ cells were separated using a MINI-MACS (Milteny Biotech) device. After isolation, C-kit$^+$ cells were seeded in a 12-well culture plate in the presence of a cardiomyogenic differentiation medium (Catalog No. 011AR, R and D systems) (15).

Flow cytometry (FCM)

The purity of c-kit$^+$ cells was assessed by FCM. The

FCM method was previously explained by Farahzadi *et al.*, (16). For this purpose, C-kit$^+$ cells were incubated with CD117 antibody conjugated with FITC for 30 minutes at 4^o C. Next, Fluorescence-activated cell sorting (FACS) instrument (BD Biosciences, San Diego, CA) was used to quantify the fluorescence intensity of cells.

Immunocytochemistry (ICC)

To assess the cardiomyogenic differentiation of C-kit$^+$ cells, the ICC method was used to detect Desmin-specific surface markers. Cells were fixed with 4% paraformaldehyde for 30 minutes. To prevent nonspecific reactions, 10% fetal calf serum (FCS) was added for 20 minutes. Cells were exposed to the mAbs against Desmin (cardiomyocyte characterization kit, USA; Cat no: SCR059) for 24 hours at 4° C and subsequently, anti-mouse IgG-PE conjugate secondary antibody (1/500) for 2 hours at 25° C. Then, cell nuclei were stained with DAPI at a working dilution of 1:1000 for 30 secs and the fluorescent cells were visualized under a fluorescence microscope (17).

Treatment of C-kit$^+$ cells with LC

The suitable concentration of LC was previously examined by Fathi *et al.*, (17). In this

Western blot analysis

Cells from both control and experimental groups were incubated with RIPA Lysis Buffer for 15 min on ice. After centrifugation for 15 min, the protein content of the samples was determined according to the *BCA protein* assay kit. Proteins (50 µg per lane) were loaded onto SDS-polyacrylamide gels (SDS-PAGE) and blotted on PVDF membranes. Western blots were performed using primary mouse monoclonal antibody anti-β-actin (1: 1000) and anti-GATA4 (1: 500). The chemiluminescence detection kit was used according to the instructions of ROCHE chemiluminescence detection kit (18,19).

Investigation of the ERK1/2 protein expression by FCM

To evaluate the expression of ERK1/2 protein, the FCM method was used. For this reason, 20×10^4 cells/well were washed with washing buffer and incubated with 0.2% Triton X-100 for 15 min. Next, the cells were stained with 5 µL of ERK1/2 antibody solution for 30 min and analyzed by flow cytometry (20,21).

Statistical analysis

The results were analyzed using t-test. The statistical significance was determined at $P<0.05$ by Graph Pad Prism version 6.01.

Results

Identification of BM resident C-kit$^+$-expressing stem cells

C-kit$^+$ cells were enriched from a total population of BM-MNCs, Using MACS technique. After cell counting, the purity of enriched C-kit$^+$ cells was investigated by FCM. Figure 1A shows the total cell population of enriched BM resident C-kit$^+$ cells. Figure 1B shows the shift of the C-kit$^+$ cells population (blue dots) from the isotype control (red blots). In other words, FCM analysis indicated that enriched BM-derived CD117+ cells by MACS had high levels of expression of CD117 (95.7%).

Cardiomyogenic differentiation confirmation of BM resident C-kit$^+$ cells by ICC

To assess the capability of C-kit$^+$ cells differentiation to cardiomyocyte, C-kit$^+$ cells were cultured in SCM102 cardiomyocyte differentiation medium for 14 days. Desmin expression is considered a sign of differentiation towards the cardiac cells and is confirmed by ICC. As shown in Figure 2, Desmin had been expressed by BM-resident C-kit$^+$ cells as the cardiomyogenic marker.

Effect of LC on GATA-4 protein expression in cardiac differentiated BM-resident C-kit$^+$ cells

To evaluate the effect of LC on the cardiomyogenic differentiation of BM-resident C-kit$^+$ cells, the GATA-4 protein expression was examined by western blotting. As shown in Figure 3, the protein expression level of GATA-4 was significantly increased by about 1.60-fold in the experimental group compared to the control group ($*P<0.05$).

Effect of LC on ERK protein expression in cardiac differentiated BM-resident C-kit$^+$ cells

FCM was done for evaluating the ERK signaling pathway involved in the effect of LC on the cardiac differentiated C-kit$^+$ cells. As shown in Figure 4, the protein expression of ERK was significantly increased by about 1.65% in the experimental group in comparison to the control group ($*P<0.05$).

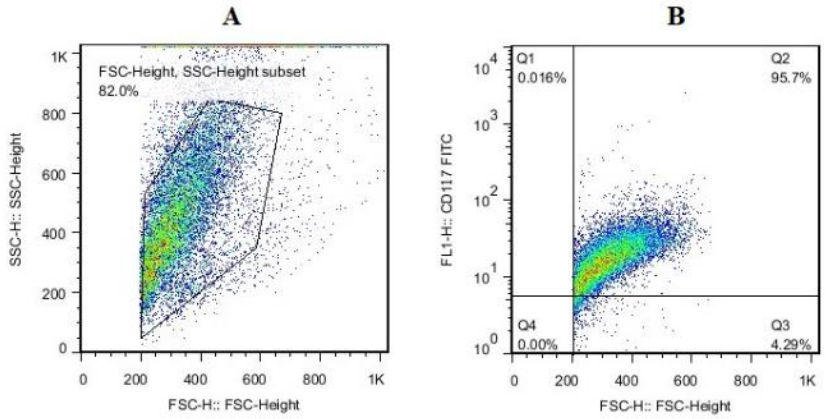

Figure 1. Characterization of isolated BM resident C-kit+-expressing progenitor cells by flow cytometry. (A) A total population of cells for C-kit evaluation; (B) Flow cytometry showed that 95.7% of cells were positive for C-kit marker

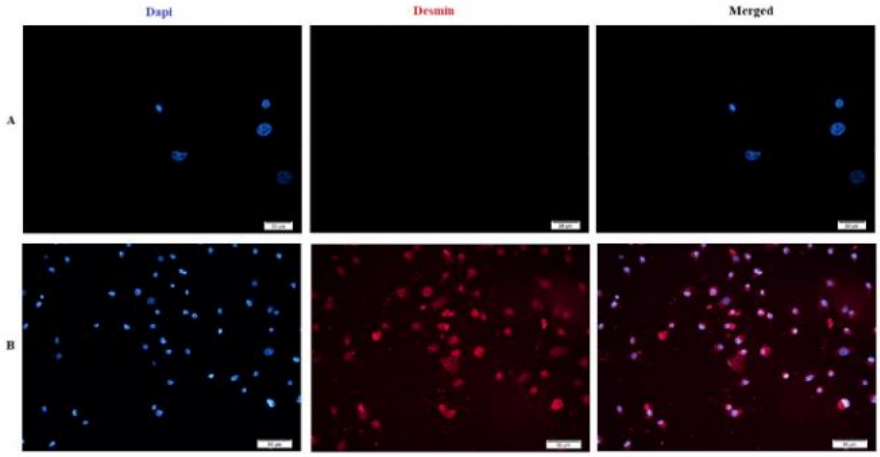

Figure 2. BM resident C-kit$^+$ cells differentiate into the cardiac lineage *in vitro*. (A) Negative control (C-kit$^+$ cells in the absence of cardiac differentiation culture medium), (B) Desmin (red)-positive cells expressed in the C-kit+ cardiac differentiated cells; Nuclei were stained by DAPI in blue

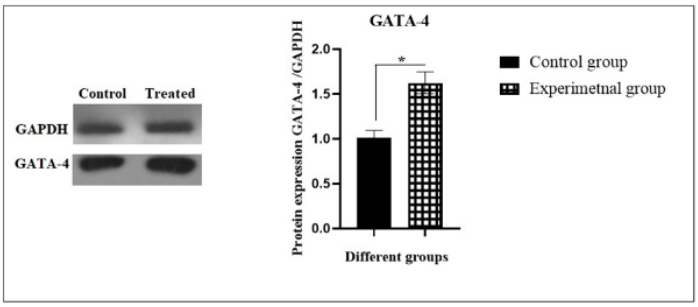

Figure 3. Effect of LC on GATA-4 protein expression in BM-resident C-kit+ cells. BM-resident C-kit+ cells were cultured in 6-well plates and exposed to western blotting. (A-C) Protein was extracted from cultured C-kit+ cells in group I (the control group without any LC treatment) and group II (an experimental group with LC treatment) as described in the methods section and was subjected to western blotting; mean±SEM; n=3; **P*<0.05

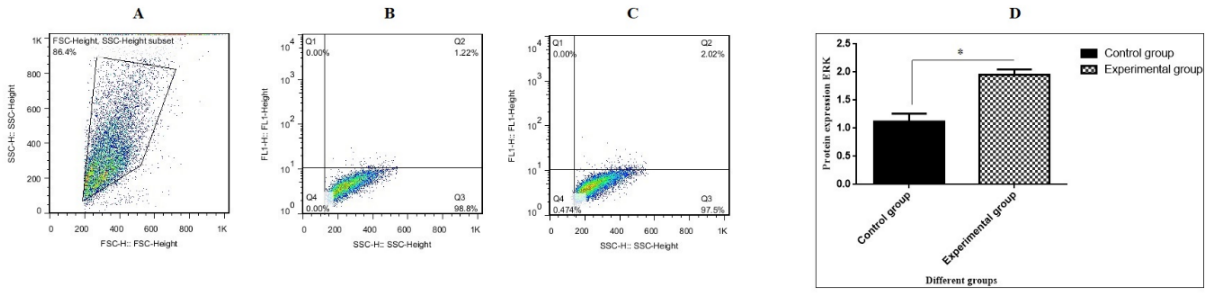

Figure 4. Investigation of ERK protein expression in cardiac differentiated BM-resident C-kit$^+$ cells in the presence and absence of LC. In this figure, A is the selected cell population, B is the control group, C is the experimental group, and D is a statistical analysis of ERK protein expression in control and experimental groups; Mean±SEM; n=3; **P*<0.05.

Discussion

In previous studies, the C-kit$^+$ hematopoietic stem cell markers treated with 5-azacytidine were transplanted into the heart and the injection region showed an increase in vessels. Correspondingly, they demonstrated that C-kit+ hematopoietic stem cells cooperate with cardiac contraction (22). In 2005, C-kit+ hematopoietic stem cells were cultured among the layers of beating cardiomyocytes. Moreover, cardiomyocyte surface markers have been expressed in C-kit+ cells, which were then detected by Immunofluorescence (23). In this study, BM-resident C-kit+ cells have been cultured in cardiomyogenic differentiation medium and C-kit+ differentiation has been discovered through Desmin cardiac cell marker using ICC. The results were in line with those of previous studies. Moreover, according to prior studies, senility causes abnormal C-kit+ cells' function in mice and this C-kit+ cells' abnormality consequently impairs angiogenesis after MI. Thus, BM–derived cells with C-kit+ receptors need to be activated to be mobilized in damaged parts of heart, in order to form the myofibroblast as repair tissue. As well, the transplantation of BM C-kit+ cells from young donors raises cardiac repair (24). In another study, it has been shown that C-kit+ cardiac progenitor cells co-cultured with NRVMs could be differentiated into cardiac myocytes with contractile proteins and sarcomeres. As a result, C-kit+ BMSCs have the trans-differentiating ability to the functional cardiomyocytes. This is in line with the present findings (25). Cardiac-resident C-kit+ cells' subset has been isolated from neonatal hearts and then co-cultured with fetal cardiomyocytes. As a result, cardiomyogenic phenotype appeared, which insisted on the conversion possibility of cardiac-resident C-kit+ cells from neonatal hearts to cardiac cells during co-culturing with fetal cardiomyocytes (5). There is evidence of phenotype attributes and similar performance of cardiac mesenchymal cells in C-kit+ cells (6,26). In 2011, intra-myocardial C-kit+ BM cells have been transferred after MI, which then caused an improvement in ventricular function. However, this effect was not observed with MSCs. It was shown that the C-kit+ cells could improve function through the activation of endogenous paracrine communications (27). Following the published reports, C-kit+ cells can be developed into cardiomyocytes in vivo. C-kit+ cells' ability in participating in heart integrity has been determined using lineage tracing techniques. Moreover, the role of C-kit+ lineage in the adult heart in cardiomyocyte regeneration has been detected by the mT/mG detection system. Notably, C-kit+ cells were residents in the BM in this study, and similar results were also obtained (28). Xue *et al.*, in their study aimed to show the differentiation potential of C-kit+ cardiac stem cells (CSCs) into the cardiac pacemaker cells by treating mouse C-kit+ CSCs with Ang II and growth factors. As a result, they had succeeded to increase the differentiation into sinus node-like cells. These results were in line with those of the present study (29). The existence of two carnitine uptake systems in human cultured muscle was confirmed in a study by Martinnzzi *et al.*, (30). This showed that LC increased the possibility of myocyte differentiation development. In this study, the effect of LC on increasing cardiomyocyte differentiation was also proved (30). Based on another research, 221 patients (aged up to 18 years old) with cardiomyopathy were evaluated in the two control (without LC treatment) and experimental groups (with oral LC treatment). The LC treatment group was better than the control group, but it was not significant (31). Additionally, it has been proved that propionyl-L-carnitine (PLC) and Acetyl-L-carnitine (ALC) could

affect cardiovascular pathologies. PLC showed more effective signs when it was combined with exercise training, pharmacologic programs, or pulsed muscular compressions (32). There is another research in this area showing that LC modified mitochondrial antioxidant activity and provoked several signaling pathways. These results are consistent with the results of the present study. In another study conducted by Fathi *et al*., it was revealed that LC with the concentration of 0.2 mM increased human telomerase reverse transcriptase (hTERT) gene expression in BM-resident C-kit+ cells differentiated to cardiomyocytes (17). Furthermore, they hypothesized that telomerase elongation is a factor to increase cell survival and decrease cell's aging. In addition, they found a considerable increase in the mRNA and protein expression of ERK1/2 using real time-PCR and western blot analysis, which has determined the role of the ERK1/2 signaling pathway (17). Furthermore, they reported the C-Kit receptor regenerative role in myocardial injury via MAPK signaling activation. LC (0.2 mM) developed cardiac markers' expressions of BM-resident C-kit+ cells due to cytokines' (TGF-b, IGF-1, IL-6, and VEGF) effects (33). MAPK signaling pathway (ERK and p38) was found to be relevant to stem cells' cardiomyogenesis, proliferation, and survival. MAPK has an important function in cell therapy for MI treatment (13). Of note, for the first time, Kempf *et al*., reported the MAPK regulation's details in human embryonic stem cells (hESC) (34). As well, three MAPK subsets (ERK, P38, and JNK) have had a severe reduction in phosphorylation during embryoid body formation. Therefore, the balance of MAPK pathway regulation is important in lineage-specific differentiation in hESC (34). Collectively, BM-resident C-kit+ cells have the ability of differentiating into functional cardiomyocytes. After the addition of LC to the heart differentiation medium, an increase was detected in differentiation by western blotting. ERK1/2 protein was expressed in C-kit+ differentiated cells and revealed on signaling pathways in cardiomyogenic differentiation. These results can be used for future studies and also in cell therapy. However, more studies are needed to use this cell line in clinical therapy.

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References

1. Iwanaga, Y. and S. Miyazaki, Heart failure, chronic kidney disease, and biomarkers. Circ J 2010;74:1274-82.
2. Mitra A, Basak, Datta TK, Naskar S, Sengupta S, Sarkar S. Role of α-crystallin B as a regulatory switch in modulating cardiomyocyte apoptosis by mitochondria or endoplasmic reticulum during cardiac hypertrophy and myocardial infarction. Cell Death Dis 2013;4:e582.
3. Shafei AE, Ali MA, Ghanem HG, Shehata AI, Abdelgawad AA, Handal HR, et al. Mesenchymal stem cell therapy: A promising cell- based therapy for treatment of myocardial infarction. J Gene Med 2017;19:e2995.
4. Bahit MC, Kochar A, Granger CB, Post-myocardial infarction heart failure. JACC Heart Fail 2018;6:179-86.
5. Zaruba MM, Soonpaa M, Mark Soonpaa, Field LJ. Cardiomyogenic potential of c-kit+ expressing cells derived from neonatal and adult mouse hearts. Circulation 2010;121:1992-2000.
6. Gambini E, Pompilio G, Biondi A, Alamanni F, Capogrossi MC, Agrifoglio M, et al. C-kit+ cardiac progenitors exhibit mesenchymal markers and preferential cardiovascular commitment. Cardiovasc Res 2011;89:362-73.
7. Pekala J, Patkowska-Sokoła B, Bodkowski R, Jamroz D, Nowakowski P, Lochyński S, et al. L-carnitine-metabolic functions and meaning in humans life. Curr Drug Metab 2011;12:667-78.
8. Karlic H, Lohninger A. Supplementation of L-carnitine in athletes: does it make sense? Nutrition 2004;20:709-15.
9. Wang ZY, Liu YY, Liu GH, Lu HB, Mao CY. l-Carnitine and heart disease. Life Sci 2018;194:88-97.
10. Muslin AJ. MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. Clin Sci (Lond) 2008;115:203-18.
11. Sun Y, Liu WW, Liu T, Feng X, Yang N, Zhou HF. Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. J Recept Signal Transduct Res 2015;35:600-4.
12. Hu B, Song JT, Ji XF, Liu ZQ, Cong ML, Liu DX. Sodium ferulate protects against angiotensin II-induced cardiac hypertrophy in mice by regulating the MAPK/ERK and JNK pathways. BioMed Res Int 2017;2017:3754942.
13. Hao T, Zhou J, Lü S, Yang B, Wang Y, Fang W, et al. Fullerene mediates proliferation and cardiomyogenic differentiation of adipose-derived stem cells via modulation of MAPK pathway and cardiac protein expression. Int J Nanomedicine 2016;11:269-83.
14. Liu P, Zhong TP. MAPK/ERK signalling is required for

zebrafish cardiac regeneration. Biotechnol Lett 2017;39:1069-77.
15. Khaksar M, Sayyari M, Rezaie J, Pouyafar A, Montazersaheb S, Rahbarghazi R. High glucose condition limited the angiogenic/cardiogenic capacity of murine cardiac progenitor cells in in vitro and in vivo milieu. Cell Biochem Funct 2018;36:346-56.
16. Farahzadi R, Fathi E, Vietor I. Mesenchymal stem cells could be considered as a candidate for further studies in cell-based therapy of Alzheimer's disease via targeting the signaling pathways. ACS Chem Neurosci 2020;11:1424-35.
17. Fathi E, Farahzadi R, Javanmardi S, Vietor I. L-carnitine Extends the Telomere Length of the Cardiac Differentiated CD117+-Expressing Stem Cells. Tissue Cell 2020;67:101429.
18. Tarhriz V, Wagner KD, Masoumi Z, Molavi O, Hejazi MS, Ghanbarian H. CDK9 regulates apoptosis of myoblast cells by modulation of microRNA- 1 expression. J Cell Biochem 2018;119:547-54.
19. Tarhriz V, Eyvazi S, Musavi M, Abasi M, Sharifi K, Ghanbarian H, et al. Transient induction of Cdk9 in the early stage of differentiation is critical for myogenesis. J Cell Biochem 2019;120:18854-61.
20. Mehdizadeh A, Somi MH, Darabi M, Farajnia S, Akbarzadeh A, Montazersaheb S, et al. Liposome-mediated RNA interference delivery against Erk1 and Erk2 does not equally promote chemosensitivity in human hepatocellular carcinoma cell line HepG2. Artif Cells Nanomed Biotechnol 2017;45:1612-9.
21. Fathi E, Valipour B, Farahzadi R. Targeting the Proliferation Inhibition of Chronic Myeloid Leukemia Cells by Bone Marrow Derived-Mesenchymal Stem Cells via ERK Pathway as a Therapeutic Strategy. Acta Med Iran 2020;58:199-206.
22. Loffredo FS, Steinhauser ML, Gannon J, Lee RT. Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. Cell Stem Cell 2011;8:389-98.
23. Lagostena L, Avitabile D, De Falco E, Orlandi A, Grassi F, Iachininoto MG, et al. Electrophysiological properties of mouse bone marrow c-kit+ cells co-cultured onto neonatal cardiac myocytes. Cardiovasc Res 2005;66:482-92.
24. Van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin SC, et al. C-kit+ cells minimally contribute cardiomyocytes to the heart. Nature 2014;509:337-41.
25. Matuszczak S, Czapla J, Jarosz-Biej M, Wiśniewska E, Cichoń T, Smolarczyk R, et al. Characteristic of c-Kit+ progenitor cells in explanted human hearts. Clin Res Cardiol 2014;103:711-8.
26. Fazel S, Cimini M, Chen L, Li S, Angoulvant D, Fedak P, et al. Cardioprotective c-kit+ cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. J Clin Invest 2006;116:1865-77.
27. Xue C, Zhang J, Lv Z, Liu H, Huang C, Yang J, et al., Angiotensin II promotes differentiation of mouse c-kit-positive cardiac stem cells into pacemaker-like cells. Mol Med Rep 2015;11:3249-58.
28. Gojo S, Gojo N, Takeda Y, Mori T, Abe H, Kyo S, et al. In vivo cardiovasculogenesis by direct injection of isolated adult mesenchymal stem cells. Exp Cell Res 2003;288:51-9.
29. Kubo H, Berretta RM, Jaleel N, Angert D, Houser SR. c-Kit+ Bone Marrow Stem Cells Differentiate into Functional Cardiac Myocytes. Clin Transl Sci 2009;2:26-32.
30. Martinnzzi A, Vergani L, Rosa M, Angelini C. L-Carnitine uptake in differentiating human cultured muscle. Biochim Biophys Acta 1991;1095:217-22.
31. Winter SC, Buist NR. Cardiomyopathy in childhood, mitochondrial dysfunction, and the role of L-carnitine. Am Heart J 2000;139:s63-9.
32. Mingorance C, Rodríguez-Rodríguez R, Justo ML, Alvarez de Sotomayor M, Herrera MD. Critical update for the clinical use of L-carnitine analogs in cardiometabolic disorders. Vasc Health Risk Manag 2011;7:169-76.
33. Fathi E, Farahzadi R, Vietor I, Javanmardi S. Cardiac differentiation of bone-marrow-resident c-kit+ stem cells by L-carnitine increases through secretion of VEGF, IL6, IGF-1, and TGF-β as clinical agents in cardiac regeneration. J Biosci 2020;45:92.
34. Kempf H, Lecina M, Ting S, Zweigerdt R, Oh S. Distinct regulation of mitogen-activated protein kinase activities is coupled with enhanced cardiac differentiation of human embryonic stem cells. Stem Cell Res 2011;7:198-209.