

NF-E2-related factor 2 over-expression in mesenchymal stem cells to improve cellular cardiomyoplastyMohammad Mohammadzadeh-Vardin¹, Mehryar Habibi Roudkenar^{2*}

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Abstract:

Myocardial infarction (MI) is the leading cause of death worldwide. Various therapeutic strategies have been introduced for MI treatment. In recent years, interest in utilizing mesenchymal stem cells (MSCs) for MI therapy has increased. In fact, the use of MSCs for MI treatment, known as cellular cardiomyoplasty, is in the clinical trial stage. However, despite promising results, most MSCs die after transplantation as a result of exposure to various stresses. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a well-known cytoprotective transcription factor, protects MSCs against some stresses. Over-expression of Nrf2 in MSCs decreases their apoptosis in vitro without any adverse effects on their differentiation capacity. Therefore, we hypothesized that over-expression of Nrf2 in MSCs can improve cellular cardiomyoplasty.

Keywords: NF-E2-Related Factor 2 (Nrf2); Mesenchymal Stromal Cells; Myocardial Infarction; Cardiomyoplasty

Additional Information for citing this article:

Title of Journal: Electronic physician; Abbreviated title of journal: Electron. Physician

doi: 10.14661/2014.808-813

Editorial information:

Type of article: original

Received: December.10.2013

Revised: 1st Revision: February.09.2014; 2nd Revision: May.04.2014, 3rd Revision: May.07.2014

Accepted: May.07.2014

Published: May.10. 2014

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1. Introduction**1.1. Background**

Myocardial infarction (MI) remains a leading cause of morbidity and mortality, resulting in 12.8% of deaths worldwide (1, 2). Many therapeutic options, ranging from pharmaceutical therapeutics to mechanical procedures, are available for acute myocardial infarction (3). Reactive oxygen species (ROS) play an important role in the pathogenesis of cardiovascular diseases. Many studies have reported an association between such diseases and oxidative damage to cardiac cells. This association might be caused by an increased rate of free radical formation and/or weakening of the antioxidant defense system (4,5). When the antioxidant defense system does not fully neutralize the effects of ROS, ROS can react with various cellular components, including phospholipids and proteins, resulting in lipid peroxidation and the oxidation of thiol groups. These oxidation reactions impair the normal functions of cell membranes and various cellular proteins, resulting in cardiac injury (due to inflammation, apoptosis, and cell death) (6-8). Hence, ROS are the main focus of many studies pertaining to MI pathology.

Cellular cardiomyoplasty is a new potential therapeutic approach that uses exogenous cells to repair regions of damaged myocardium. Improved heart function following the transplantation of mesenchymal stem cells (MSCs) has been reported in animal models of acute MI as well as in clinical studies on patients with heart failure (9). Various favorable characteristics, such as multilineage differentiation potential, ability to evade the host immune system, immunomodulatory capacities, and ease of proliferation in vitro, make MSCs particularly attractive for cell therapy (10). It has been well established that MSC infusion improves the function of infarcted myocardium (11).

Several mechanisms have been proposed to explain the ability of MSCs to revive ischemic tissues. These mechanisms include the following: 1) secretion of antioxidant chemicals and free radical scavengers at the site of ischemia, 2) secretion of multiple angiogenic growth factors (e.g., vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF)) with the potential to induce endothelial growth, migration, and tube formation, and 3) differentiation of transplanted MSCs into myocytes, smooth muscle cells, and endothelial cells (12).

1.2. Statement of the problem

Despite the several advantages of MSCs, they have not shown satisfactory outcomes in many investigations, mostly due to their poor survival rate after transplantation (13-17). In fact, more than 99% of transplanted MSCs die within one day after transplantation, there are no well-defined reasons for this low survival rate. However, it is worth noting that, during the isolation of MSCs from their natural niche, they are inevitably exposed to harmful conditions, such as serum deprivation, hypoxia, and oxidative stress (18). However, due to radiotherapy, chemotherapy, inflammation, and expression of pro-apoptotic factors, the microenvironment of the damaged tissues of recipients is not favorable for the survival of transplanted MSCs (19). Hence, to develop an effective therapeutic modality, it is necessary to strengthen MSCs so they can withstand such stresses (20). It seems that higher survival rates of MSCs can improve infarcted tissues through the secretion of protective factors and/or differentiation of the MSCs. Novel strategies are being developed to improve the biological and functional properties of MSCs, such as preparation of the cells in special bioscaffolds (21), preconditioning of the cells in cultures (14, 15), and genetic transformation (10). Nuclear factor E2-related factor 2 (Nrf2) is a potent transcription factor that is critical for the protection of cells against oxidative stresses. Activation of Nrf2 as a redox-sensor under stress conditions up-regulates the transcription of phase II detoxification enzymes and antioxidant proteins, such as NAD (P)H:quinone oxidoreductase (NQO1), glutathione S-transferases (GSTs), glutamate-cysteine ligase, heme oxygenase-1 (HO-1), thioredoxin, and ferritin (22, 23). Up-regulation of these factors in transplanted cells protects the cells against the ROS produced in MI tissue. We recently showed that adenoviral-mediated Nrf2 over-expression in MSCs reduces oxidative stress-induced apoptosis and cytotoxicity. Also, we showed that over-expression of Nrf2 had no deleterious effect on the differentiation capability of MSCs. In addition, Nrf2 enhanced the activity of SOD and HO-1 (10).

2. Hypothesis and its evaluation

We hypothesized that over-expression of Nrf2 in MSCs (Nrf2-MSCs), can effectively improve the efficiency of the transplantation of MSCs, thereby enhancing cellular cardiomyoplasty. Nrf2 over-expression can protect MSCs against oxidative stresses encountered in the microenvironment of infarcted tissue. Furthermore, Nrf2-MSCs produce many antioxidants that protect them against stress conditions.

Activation of the Nrf2 signaling pathway is an important mechanism for protecting cells against oxidative stresses (24). Under non-stimulated conditions, Nrf2 is retained in cytoplasm via interaction with the anchor protein, Kelch-like ECH-associated protein-1 (Keap1), where it is maintained at a very low level by the Keap1-dependent ubiquitination and proteasomal degradation systems (25-28). This interaction sequesters most of the Nrf2 molecules in the cytoskeleton and keeps them away from the nucleus. The cysteine-rich surface of Keap1 is oxidized in cases of oxidative and nitrosative stresses. This apparently produces a conformational change in Keap1, resulting in dissociation of Nrf2 from Keap1. Then, the released Nrf2 is translocated to the nucleus, heterodimerizes with small Maf proteins, and then binds to the antioxidant response element (ARE), a common regulatory element found in the 5'-flanking regions of the antioxidant and detoxification enzymes (Fig. 1) (29-32). Further activation of Nrf2 is mediated through phosphorylation of Nrf2 by mitogen-activated protein kinases (MAPKs), protein kinase C (atypical isoform), and phosphoinositol-3-kinase (PI3K). The exact mechanism associated with these activation pathways is unknown. Nrf2 effector genes that bear antioxidant response elements include a majority of the antioxidant proteins (e.g., GCL, HO-1, and thioredoxin) and phase II detoxifying enzymes (e.g., NQO1 and GSTs) (26).

Theoretically, the survival rate of the MSCs will increase in stressful, oxidative environment of the infarcted myocardium by over-expression of Nrf2, resulting in higher potential of the MSCs to repair the ischemic tissue. As mentioned earlier, we previously showed that over-expression of Nrf2 in MSCs protects them against various stresses. In addition, these engineered cells produce higher levels of antioxidants, such as SOD and HO-1. Also, the Nrf2-MSCs showed less apoptosis when exposed to oxidative stresses (10). Furthermore, over-expression of Nrf2 probably confers some additional benefits to MSCs after they are transplanted. It has been shown that Nrf2 has a role in the formation of blood vessels through the induction of the expression of VEGF and HIF-1 α target genes (33). The progression of angiogenesis is highly advantageous in the treatment of ischemic myocardium (34). We

could find no study that had been performed to evaluate the effect of Nrf2 over-expression on differentiation of MSCs into myocytes, smooth muscle cells, and endothelial cells. Because of the importance of Nrf2 signaling in anti-oxidative pathways, various studies have focused on the activation of Nrf2 for cardio-protection. They have shown that the application of various factors, such as carbon monoxide, red wine, fumarate, hydrogen sulfide, and acute exercise, mediates cardio-protection through promotion of antioxidant mechanisms by activation of the Nrf2 antioxidant pathway (35-40).

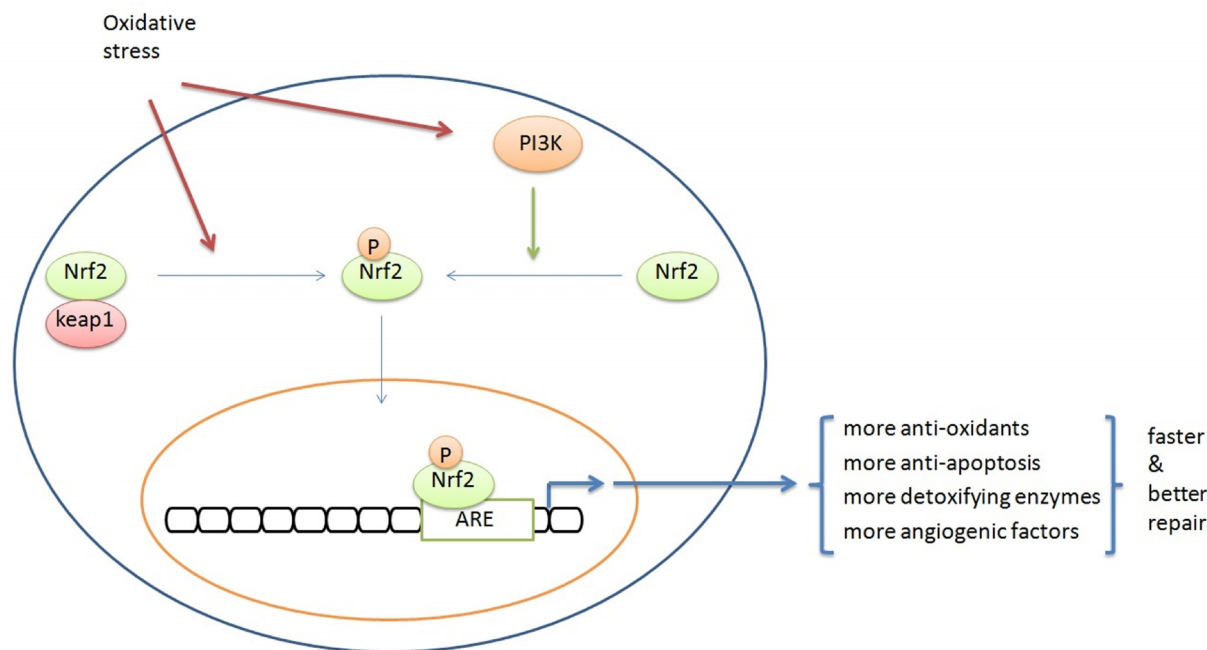


Figure 1. Predicted molecular pathways of Nrf2 activation in MSCs leading to more effective repair of infarcted myocardium

To evaluate these hypotheses, first, MSCs must be isolated from bone marrow and characterized. Next, Nrf2 must be over-expressed in the authenticated MSCs, and it is suggested that this be performed with the adenoviral expression system for transient over-expression of Nrf2, as described in our previous study (10). Then, after the evaluation of the safety concerns related to the Nrf2 over-expression in MSCs has been conducted, the MSCs must be transplanted to the infarcted myocardium of MI patients via intramyocardial injection. Then, biochemical and histological analyses must be performed to evaluate the effects of Nrf2 over-expression in MSCs on cardiac function.

3. Expected results

It is expected that the over-expression of Nrf2 in MSCs will protect the MSCs against the unfavorable conditions of the infarcted tissue (such as oxidative stresses), increase their survival rate in the infarcted tissue, and improve their function in repairing the injured tissue.

Acknowledgements:

The authors thank all participants for their kind participation in the study. The article was edited by native English Editor at Mehr Publishing Group (<http://www.MehrPublishing.com>) and American Journal Experts (AJE: <http://www.aje.com>).

Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

Both authors contributed to this article equally. All authors read and approved the final manuscript.

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