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Short communication

Dinitrophenol modulates gene expression levels of angiogenic, cell survival and cardiomyogenic factors in bone marrow derived mesenchymal stem cells

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ABSTRACT

Various preconditioning strategies influence regeneration properties of stem cells. Preconditioned stem cells generally show better cell survival, increased differentiation, enhanced paracrine effects, and improved homing to the injury site by regulating the expression of tissue-protective cytokines and growth factors. In this study, we analyzed gene expression pattern of growth factors through RT-PCR after treatment of mesenchymal stem cells (MSCs) with a metabolic inhibitor, 2,4 dinitrophenol (DNP) and subsequent re-oxygenation for periods of 2, 6, 12 and 24 h. These growth factors play important roles in cardiomyogenesis, angiogenesis and cell survival. Mixed pattern of gene expression was observed depending on the period of re-oxygenation. Of the 13 genes analyzed, ankyrin repeat domain 1 (Ankrd1) and GATA6 were downregulated after DNP treatment and subsequent re-oxygenations. Ankrd1 expression was, however, increased after 24 h of re-oxygenation. Placental growth factor (Pgf), endoglin (Eng), neuropilin (Nrp1) and jagged 1 (Jag1) were up-regulated after DNP treatment. Gradual increase was observed as re-oxygenation advances and by the end of the re-oxygenation period the expression started to decrease and ultimately regained normal values. Epiregulin (Ereg) was not expressed in normal MSCs but its expression increased gradually from 2 to 24 h after re-oxygenation. No change was observed in the expression level of connective tissue growth factor (Ctgf) at any time period after re-oxygenation. Kindlin3, kinase insert domain receptor (Kdr), myogenin (Myog), Tbx20 and endothelial tyrosine kinase (Tek) were not expressed either in normal cells or cells treated with DNP. It can be concluded from the present study that MSCs adjust their gene expression levels under the influence of DNP induced metabolic stress. Their levels of expression vary with varying re-oxygenation periods. Preconditioning of MSCs with DNP can be used for enhancing the potential of these cells for better regeneration.

1. Introduction

Stem cell therapy for the repair of damaged tissues has become the most prominent part of the current research in the field of regenerative medicine. Bone marrow derived mesenchymal stem cells (MSCs) are multipotent in nature and hence, have the potential to differentiate into various cell types including muscle, bone, neurons and endothelial cells along with the mesenchymal cell lineage (Salem and Thiemermann, 2010). The use of stem cell therapy has now become a promising option for the treatment of a variety of diseases including those of the cardiovascular system (Krishnan et al., 2006).

The mechanism of stem cell differentiation is still not fully understood. One way to understand the mechanism of stem cell mediated cellular regeneration is to identify the gene expression patterns of those factors that are involved in cell growth and differentiation process during normal development as well as during tissue repair. A number of research groups have analyzed the expression levels of different growth factors in MSCs under various cellular conditions. Gnecchi et al. (2008) have analyzed a number of growth factors which are important for survival and maintenance of pluripotency and those that take part in the process of cell adhesion, homing and proliferation during the process of trans-differentiation.

The poor viability of engrafted stem cells is a major concern for stem cell researchers. Stem cell death can be avoided by utilizing preconditioning strategies in which cells are exposed to non-cytotoxic stress or shock before transplantation into harsh ischemic microenvironment of the heart (Ma et al., 2009; Rosova et al., 2008). It has been

Abbreviations: MSCs, mesenchymal stem cells; DNP, 2,4 dinitrophenol; Ankrd1, ankyrin repeat domain 1; Pgf, placental growth factor; Eng, endoglin; Nrp1, neuropilin; Jag1, jagged 1; Ereg, epiregulin; Ctgf, connective tissue growth factor; Kdr, kinase insert domain receptor; Myog, myogenin; Tek, endothelial tyrosine kinase.

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Table 1
Details of genes and their primers used in the study.

No.	Genes	Functions	Primer sequences	Annealing Temp. (°C)	Product sizes
1	Ankyrin repeat domain 1 (Ankrd1)	Cardio-regulator	F: AGCGGAGCAACCAGCTATAA R: AAGTCTTGCTCCCCCAAAT	51	214
2	Connective tissue growth factor (Ctgf)	Tissue growth	F: TAGCAAGAGCTGGGTGTGTG R: TTCACTTGCCACAAGCTGTC	58	156
3	Endoglin (Eng)	Endothelial growth	F: TGCAGAAAGAGTCGGTTGTG R: TCTCAGTGCCATTTTGCTTG	58	203
4	Epiregulin (Ereg)	Epidermal growth	F: TCTGACATGGACGGCTACTG R: TCACGGTCAATGCAACGTAT	59	163
5	GATA binding factor 6 (Gata6)	Cardiomyogenesis	F: AACTGTGGCTCCATCCAGAC R: CATATAAAGCCCGCAAGCAT	53	243
6	Jagged 1 (Jag1)	Angiogenesis	F: AATGGGTGGAAAGGGAAAAC R: TGCAGACACAGGTGAAGGAG	56	229
7	Fermitin family member 3 (Kindlin3)	Cell adhesion	F: TGACCCAGCTGTATGAGCAG R: ATGGTGGTGAGGCTATCCAG	59	244
8	Kinase insert domain receptor (Kdr)	Angiogenesis	F: CCAAGCTCAGCACACAAAAA R: CCAACCACTCTGGGAACTGT	58	190
9	Myogenin (Myog)	Muscle development	F: TTTTTCATGCGACTCACAGC R: CTGTGGGAAAGAGTGGGTGT	62	211
10	Neuropilin (Nrp1)	Cell survival/angiogenesis	F: GGAGCTACTGGGCTGTGAAG R: ACCGTATGTCGGGAACTCTG	58	208
11	Placental growth factor (Pgf)	Growth/angiogenesis	F: AGGGTCATTGGACACCTGAG R: GGTCCTTCAAGGCAAAATCA	59	230
12	T-box 20 (Tbx20)	Early heart development	F: TCTGCAGAGGAGATCCGATT R: CCGGGAACTCAAAACTGTGT	59	181
13	Tyrosine kinase, endothelial (Tek)	Angiogenesis	F: GTGGGAAGTGGCAAAGTTGT R: TTCGGCATCAGACACAAGAG	58	206

GAPDH was used as internal standard.
Primers: F: GAAAAGCTGTGGCGTGATGG and R: GTAGGCCATGAGGTCCACCA; annealing temperature: 60 °C and product size: 414 bp.

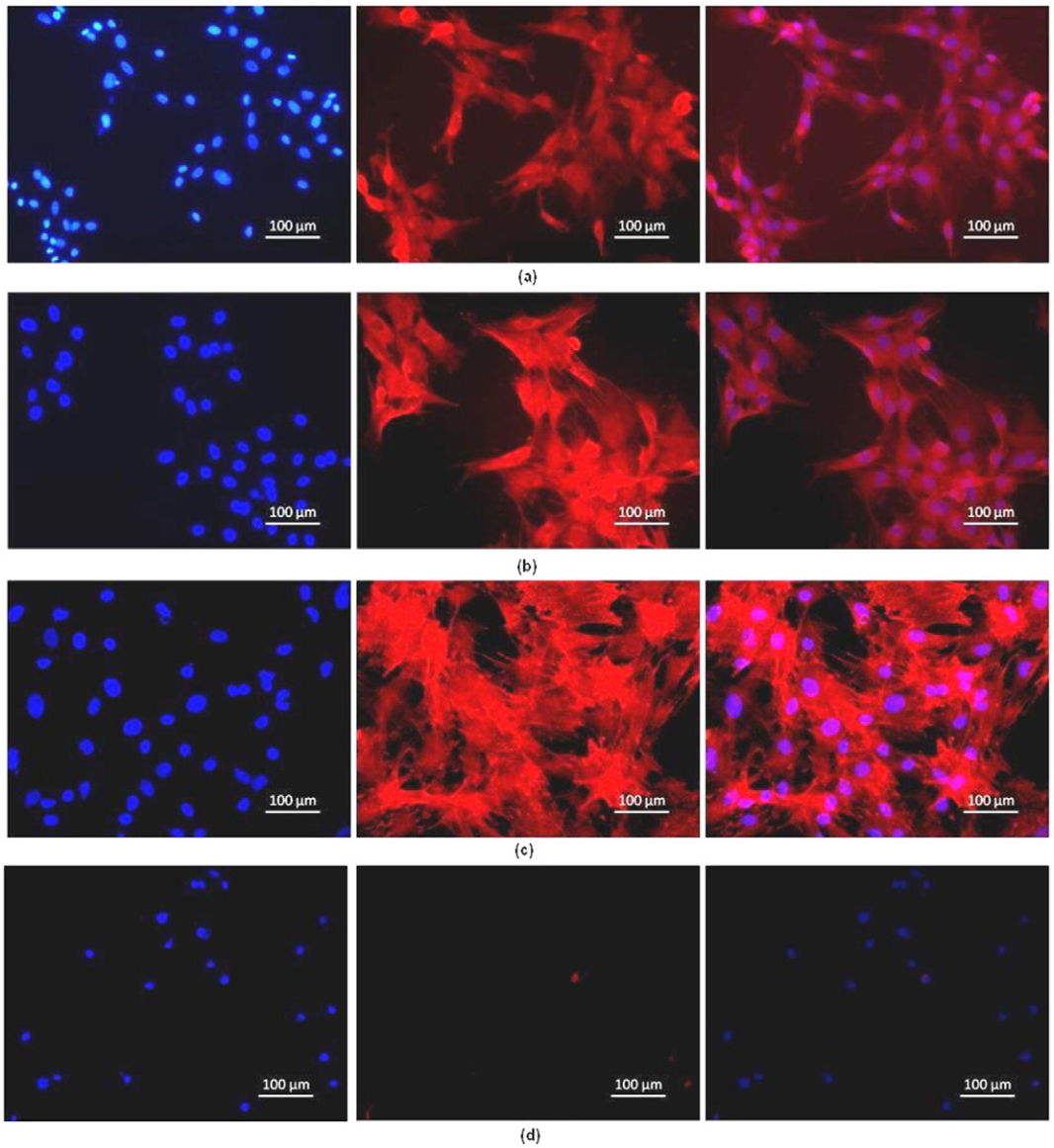

Fig. 1. Immuno-cytochemical analysis of mesenchymal stem cells (MSCs) for the presence of (a) c-kit, (b) CD44, (c) CD90 and (d) CD34 cell surface antigens. Alexa fluor 546 goat anti-mouse antibody was used as the secondary antibody. Nuclei were stained with DAPI. MSCs have shown positive expression of c-kit, CD44, and CD90 while CD34 which is a hematopoietic marker was not expressed in these cells.