

# HYPOXIA ENHANCES SELF-RENEWAL OF MULTIPOTENTIAL STROMAL CELLS THROUGH HIF-DEPENDENT AND HIF-INDEPENDENT MECHANISMS

Tamama, Kenichi  
M.D., Ph.D.  
The Ohio State University Medical Center  
Department of Pathology  
320 W 10th Ave, M364C Starling Loving Hall,  
Columbus, OH 43210  
Tel: 614-366-8527 Fax: 614-366-1183  
Kenichi.Tamama@osumc.edu

Kawasaki, Haruhisa  
Ph.D.  
Columbus, OH, 43210

Ganju, Ramesh K.  
Ph.D.  
Columbus, OH, 43210

Sen, Chandan K.  
Ph.D.  
Columbus, OH, 43210

Cell therapy with bone marrow multipotential stromal cells (MSCs) represents a promising approach to promote wound healing and tissue regeneration. MSCs could be expanded in vitro; however, both differentiation and therapeutic potentials of MSCs are gradually lost during in vitro expansion. Ambient O<sub>2</sub> (20% as opposed to 2-9% in vivo) has been recognized as an excessive O<sub>2</sub> condition that limits the life span of cultured primary cells. Hypoxia promotes MSC self-renewal through preserving early progenitors and maintaining undifferentiated phenotypes; however, key signaling pathways remain unknown.

Hypoxia inducible factor (HIF) pathway is a crucial signaling pathway activated in hypoxic condition. We hypothesize that HIF plays a pivotal role to enhance MSC self-renewal in hypoxic condition. Immunoblot analysis suggested that HIF-2 $\alpha$  was stabilized whereas that of HIF-1 $\alpha$  was unaltered under hypoxic condition; however, immunofluorescent images demonstrated that both HIF-1 $\alpha$  and HIF-2 $\alpha$  were translocated to the nucleus only under hypoxic condition. Furthermore, our studies revealed that hypoxic condition enhanced MSC colony formation. It was neither reversed by HIF-1 $\beta$  shRNA nor reproduced by introduction of stable HIF-1 $\alpha$  and HIF-2 $\alpha$ , suggesting that hypoxia increases MSC colony formation in a HIF-independent manner. Both stable HIF-1 $\alpha$  and HIF-2 $\alpha$  increased cell cycle arrest. Osteogenic differentiation, default differentiation pathway for MSCs, was reversibly inhibited by hypoxic exposure. This inhibition was further reversed by HIF-1 $\beta$  shRNA and reproduced by stable HIF-2 $\alpha$ , indicating that hypoxia inhibits osteogenic differentiation in a HIF-dependent manner.

Overall, these data demonstrate that hypoxic condition enhances MSC self-renewal through HIF-independent as well as HIF-dependent mechanisms.