Effects of Hypoxia on Human Mesenchymal Stem Cells
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INTRODUCTION:
Mesenchymal stem cells (MSCs) can be obtained from a variety of human tissues1,2. These cells possess self-renewal capacity and can differentiate into mesodermal lineage progenies. Hypoxia (1-5% oxygen) enhanced the self-renewal of hematopoietic stem cells (HSCs) and murine embryonic stem cells (ESCs) in several previous studies. However, it was also reported that hypoxia induced differentiation of neuronal and osteoprogenitor cells.3,5 The purpose of the study is to investigate the effects of hypoxia on MSCs. We hypothesize that hypoxia participates in the intricate balance between cellular proliferation and commitment towards differentiation. In this study, we examined the proliferation ability of human MSCs under hypoxic condition. Clonally-derived human bone marrow-derived MSCs were cultured under hypoxia (1% oxygen) and normoxia.

METHODS:
Human MSCs were isolated from bone marrow as previously described1. The cells were incubated in hypoxic (1% O2, 5% CO2 and 90%N2) or normoxic conditions (5% CO2 and room air). The medium was replaced every 3-4 days. At different time points, the cells were detached by 0.25% trypsin and 1mM EDTA, RNA extraction was done by RNEasy (Qiagen, Stanford, Valencia, CA). Gene expression was analyzed by RT-PCR as previously described2.

RESULTS:
Hypoxia (1% O2) increased the number of MSCs after 5 days (Fig.1) and 7 days. Under in vitro culture, compared with normoxia, the cell density of MSCs under hypoxic condition was higher. Moreover, expression of HIF-1 and SDF-1, and the stemness gene Oct4 were enhanced under hypoxic condition (Fig.2).

Fig.1: Cell densities of MSCs cultured under hypoxic and normoxic conditions. Photos taken at 40X magnification D1= cultured 1 day; D3= cultured 3 days; D5= cultured 5 days; D7= cultured 7 days. Bar= 200μm.

Fig.2: Changes of SDF-1, HIF-1 and Oct4 expression in MSCs under hypoxia (NTC: no template control).

Under hypoxic condition, the proliferating cell nuclear antigen protein (PCNA; a replication and DNA-repair factor), and the cyclin D1, as well as Cdk2 were up-regulated at protein level. In contrast, the cyclin dependent kinase (Cdk) inhibitor p27 was down regulated under hypoxic condition (Fig.3 and Fig.4).

Hypoxia also enhanced the migration ability of MSCs as shown in the photomicrographs taken 24 and 36 hours after insert removal in which more cells migrated into the scratched area in the hypoxic group (Fig. 5a). Twenty-four hours post removal of the culture insert, the cell-covered area was almost completely full (90%±2.89) in the hypoxic group more than normoxic group (56%±4.3) (Fig.5b).

Fig.3&4: Western blot analysis of cell cycle checkpoint regulators.  Figure 5

Cytokine antibody array analysis showed that growth factors including IGFBP6, GMCSF, PLGF and EVGF were up-regulated after a 7-day hypoxic incubation compared to normoxic incubation. In addition, EGF, FGF4, IGF1, IGF2 were only expressed under hypoxic condition. (Fig.6)

Figure 6: Cytokine profiles of MSCs under normoxia and hypoxia treatment.

SIGNIFICANCE:
Our results show that hypoxia can provide a favorable condition for rapid proliferation and self-renewal of MSCs and this phenomenon may be caused by autocrine or paracrine actions exerted by MSCs. Other possible effects of hypoxia on MSC cell fate specification need to be further investigated.

REFERENCES:

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