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Low oxygen tension modulates the osteogenic differentiation of mouse embryonic stem cells

Seong Yeong An, Jung Sun Heo*

Department of Maxillofacial Biomedical Engineering and Institute of Oral Biology, School of Dentistry, Kyung Hee University, 26 Kyunghee-daero, Dongdaemun-gu, Seoul 130-701, South Korea

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ABSTRACT

This study examined the effects of low oxygen tension on the osteogenic differentiation of embryonic stem cells (ESCs) in a three-dimensional culture system. The high expression levels of hypoxia-related proteins hypoxia-inducible factor-1α and vascular endothelial growth factor were first validated in ESCs subjected to hypoxic conditions compared with normoxic controls. The osteogenic differentiation of hypoxic ESCs with either osteogenic or osteogenic factor-free media was subsequently evaluated by measuring alkaline phosphatase activity, intracellular calcium levels, matrix mineralization, and the protein levels of osteogenic markers Runt-related transcription factor 2 and osterix. We confirmed that hypoxia significantly stimulated ESC osteogenic activity; the strongest stimulation of ESC osteogenesis was exerted when cells were grown in osteogenic media. To identify differentially expressed genes associated with hypoxia-induced ESC differentiation, we performed microarray analysis of ESCs cultured in osteogenic media under normoxic and hypoxic conditions. This study demonstrated that differences in oxygen tension induced the differential expression of genes known to play roles in such processes as skeletal system development and signaling pathways for bone morphogenetic protein, Wnt, Notch, mitogen-activated protein kinase, and integrin. These findings reveal the effects of low oxygen tension on osteogenic progression in ESCs and provide insight into the molecular pathways that regulate ESC differentiation following exposure to hypoxia.

1. Introduction

Embryonic stem cells (ESCs) have the potential for prolonged self-renewal and differentiation into various cell lineages under controlled conditions, which makes them valuable cell sources for tissue repair and regeneration (Bratt-Leal et al., 2009). A number of studies have shown the differentiation capability of ESCs in vitro, but promising and efficient protocols to regulate cellular differentiation are still required (Lotfinia et al., 2016; Sim et al., 2016; Kidwai et al., 2016). Stem cells generate various types of tissues through proliferation and differentiation, which are controlled by their microenvironments; these microenvironments contribute to the various signals that maintain their stem cell properties. Among these physiological signals, oxygen tension is a critical factor that is known to regulate the biological functions of both embryonic and adult stem cells (Binh et al., 2014; Emara et al., 2014). Increasing evidence has demonstrated that oxygen is not only a metabolic product but also serves as a significant signaling molecule that governs stem cell proliferation and differentiation (Närvä et al., 2013; Mathieu et al., 2013). Specifically, the physiological significance of low oxygen tension in the expansion and maintenance of ESCs has been demonstrated: Recent studies have shown the regulatory effects of hypoxic conditions on ESC pluripotency (Forristal et al., 2013; Abaci et al., 2010; Bauwens et al., 2005). Thus, manipulating oxygen content in the cellular microenvironment can be an effective methodology to optimize in vitro tissue development. Among oxygen action as biochemical and environmental cues, the present study focused on oxygen as a signaling molecule and the mechanism by which it regulates the differentiation of ESCs into osteogenic lineages in vitro.

In addition to oxygen tension, stem cell fate can be impacted by culture dimensionality. Because two-dimensional substrates differ from the endogenous physiological environment, culturing cells in a three-dimensional (3D) system that functions as a natural extracellular matrix (ECM) is preferred to study the regulation of stem cell behavior (Meng et al., 2014; Yamamoto et al., 2014). A previous study found that a specific culture geometry was able to induce profound changes in stem cell differentiation (Ruiz and Chen, 2008). To investigate these effects in more detail, the present study employed hypoxic conditions as a stimulus to evaluate whether low oxygen tension could promote the

* Corresponding author.
E-mail address: heojs@khu.ac.kr (J.S. Heo).

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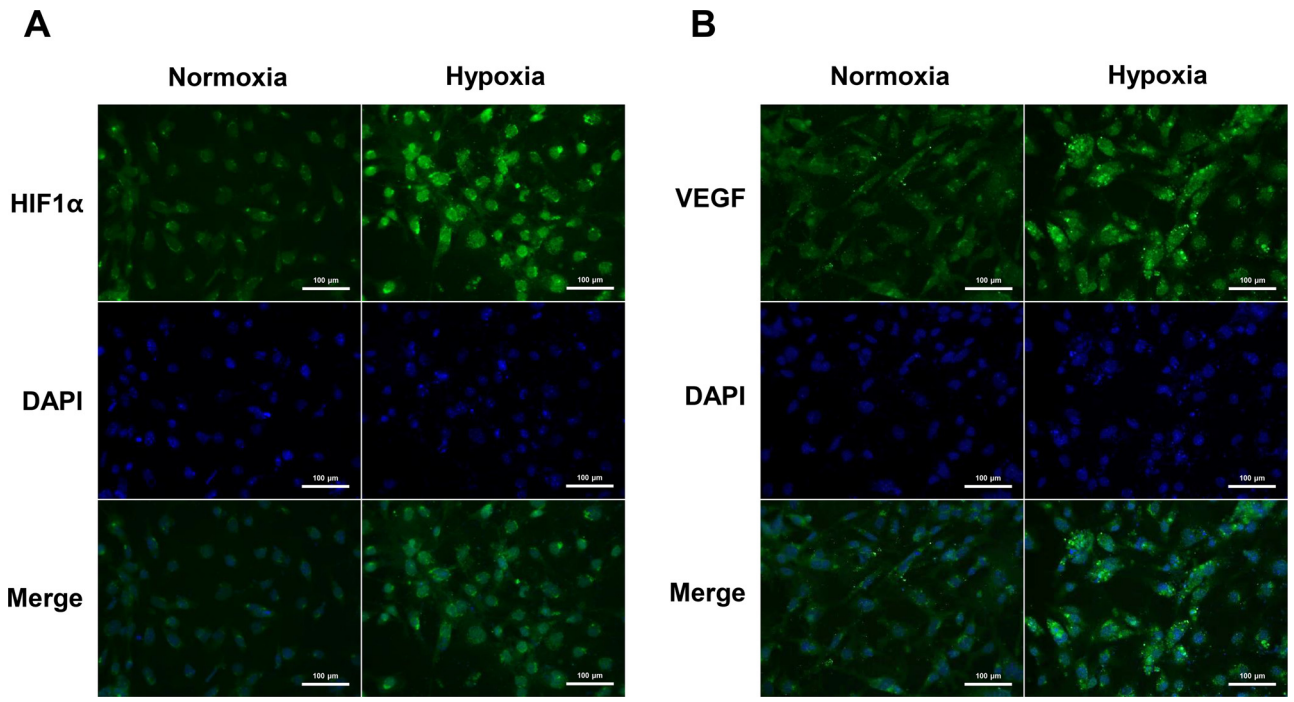

Fig. 1. Immunofluorescence staining for HIF-1α and VEGF proteins. Cells were incubated in growth media under either normoxic or hypoxic conditions for 7 days. HIF1-α (1:100, Santa Cruz, USA) and VEGF (1:100, Santa Cruz, USA) were detected by immunofluorescence staining. The nuclei were stained with DAPI. All images are displayed at a 400× magnification.

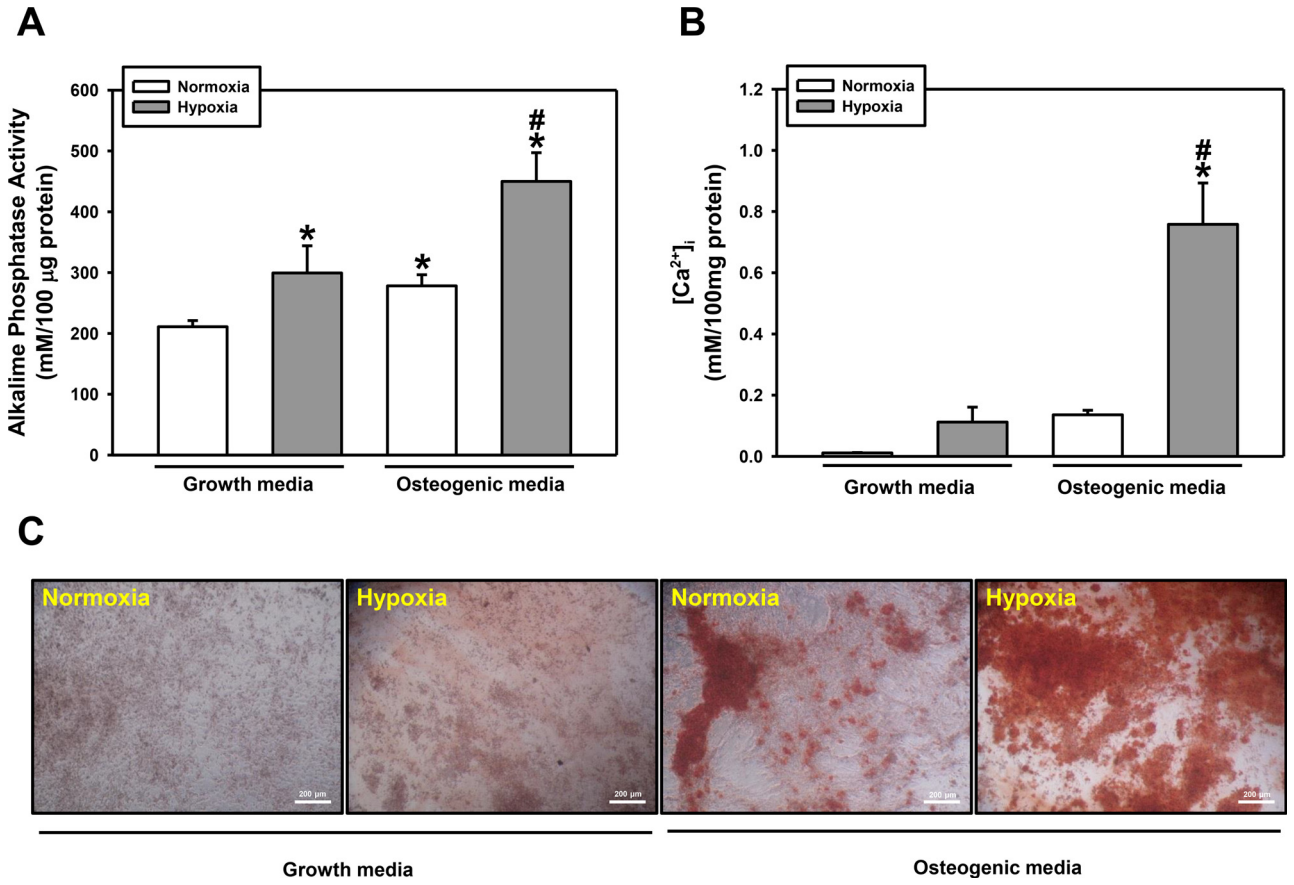

Fig. 2. Effects of hypoxia on the osteogenic differentiation of ESCs. Cells were incubated in growth (osteogenic factor-free) or osteogenic media under different oxygen tensions. Cultures were assayed for (A) ALP activity or (B) $[Ca^{2+}]_i$ on day 7. (C) Calcium nodules were visualized by Alizarin Red staining on day 21. Each microscopic image is shown as a representative of five separate experiments (magnification: 40×). The values reported are the means ± S.D. of four independent experiments. $^{*}P < 0.05$ versus the normoxia control with growth media and $^{\#}P < 0.05$ versus the normoxia control with osteogenic media.