

Mesenchymal Stem Cells and Osteogenic Differentiation

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ABSTRACT

The osteogenic differentiation of mesenchymal stem cells consists 1: in an early peak regulating the number of cells, 2: transcription and protein expression of alkaline phosphatase, 3: this is followed by high expression of osteocalcin and osteopontin. Perivascular mesenchymal stem cells and macrophages form niches that harbor dormant and self-renewing HSCs. Bone regeneration is obtained by culture-expanded human mesenchymal stem cells: osteogenic, chondrogenic, adipogenic and mature marrow stromal lineages. Two distinct niches supporting HSCs were identified: the osteoblastic and the vascular niches. They produce daughter cells. Asymmetric division means that they divide into 2 daughter cells; one daughter cell remaining in the niche as a stem cell, while the other leaves the niche to produce a large number of progeny. Symmetric division implies that stem cells divide into 2 identical daughter cells. Recent studies suggested a perivascular nature of the MSC niche, isolated from all tissue types tested. Localization of CD45⁻/CD31⁻/Sca-1⁺/Thy-1⁺ cells was found, using the markers Stro-1 and CD146, lining blood vessels in human bone marrow and dental pulp. The cells also expressed α SMA and 3G5, a pericyte associated cell-surface marker. The gene expression profiles of Oct4 and Nanog, Runx2, collagen type I, and bone sialoprotein demonstrate that the cells differentiated into osteoblasts and lost their self-renewal capability, as there was no expression of Oct4 and Nanog in the differentiated cells and in the ALP-enriched population, despite the expression of osteoblasts-related genes was enhanced.

KEYWORDS

Osteogenic Differentiation; Osteoblastic and Vascular Niches; α -Smooth Muscle Actin; Immunohistochemical Localization of CD45⁻/CD31⁻/Sca-1⁺/Thy-1⁺ cells.

INTRODUCTION

The osteogenic differentiation of mesenchymal stem cells (MSCs) may be divided into three stages (Huang et al., 2007). Between day one and four, a peak in the number of cells was found, followed by early cell differentiation from days 5 to 14, characterized by the transcription of alkaline phosphatase. After the initial peak, the level of ALP starts to decline. Thereafter, following this 3d stage, collagen type I matrix was expressed and the mineral deposited. From days 14 to 28 a high expression of osteocalcin and osteopontin was observed, followed by calcium and phosphate deposition. After the initial plating, about 0.001 to 0.01% fibroblastic cells grew into visible symmetric colonies (Pittenger et al., 1999, Bruder & Fox 1999).

Osteogenic differentiation of MSCs was characterized by ALP activity and mineralization, greater when the cells were co-cultured with osteocytes rather than osteoblasts (Birmingham et al., 2012). Perivascular mesenchymal stem cells and macrophages join the previously identified endothelial cells and cells of the osteoblastic lineage to form similar, but distinct niches that harbor dormant and self-renewing HSCs during homeostasis. They mediate stem cell mobilization in response to granulocyte colony-stimulating factor (Ehninger & Trumpp, 2018).

Adipose-derived stem cells (ASCs) were found in abundant quantities. They differentiate along multiple cell lineage pathways in a reproducible manner and were safely and effectively

transplanted to an autologous or allogenic host (Brunnell et al., 2008). Stem cells persist in adult tissues, although they represent a rare population localized in niches and have the capacity to differentiate into cells of mesodermal, endodermal and ectodermal origins (Paduano et al., 2017).

Dental follicle cells (DFCs) were evaluated by immunocytochemistry. Using embryonic stem cells (OCT4 and SOX2), mesenchymal stem cells (MSCs) (Notch1, STRO-1, CD44, HLA-ABC, CD90), neural stem cells (nestin and β -III-tubulin), neural crest stem cells (NCSCs) and a glial cells (GFAP) markers. RT-PCR allowed identifying the expression of OCT4 and NANOG in DFCs and dental follicle tissue (Lima et al., 2017). Immunocytochemistry and RT-PCR analysis revealed that a significant proportion of the DFCs expressed human embryonic stem cells marker (OCT4) whereas NANOG was weakly expressed. A considerable amount of MSCs (90%) expressed Notch1, STRO-1, CD44, and HLA-ABC, but were weakly positive for CD90. DFCs were positive for p75 (50%), HNK1 (<10%) and a small proportion (<20%) of cells. These studies report the presence of NCSCs and glial-like cells in the dental follicle. The Human dental follicle contains heterogeneous populations of stem cells derived from mesoderm and ectoderm.

Bone regeneration may be obtained by culture-expanded human mesenchymal stem cells. Alkaline phosphatase was transiently increased whereas Type I collagen was down-regulated during the late phase of osteogenesis. Osteopontin and Bone Sialoprotein (BSP) were up-regulated late in the differentiation cascade while osteonectin was constitutively expressed. The stem cell niche in bone plays an essential role in regulating self-renewal and differentiation (Yin & Li, 2006).

In adults, stem cells are closely associated with the bone marrow. The niche controls the cell number interacting not only with osteoblasts but also with stromal cells, including endothelial cells. The vascular niches in bone marrow maintain a quiescent HSC microenvironment and regulate proliferation and differentiation. They were implicated in the action of osteoblastic and vascular niches, revealing a role for numerous signaling and adhesion molecules.

Bone Marrow-derived Mesenchymal Stem Cells (BMMSCs)

A stem cell niche is a specific site in adult tissues. Structurally, the niche is formed by supporting cells that provide a microenvironment for stem cells as well as the signals emanating from the supporting cells.

Two distinct niches supporting HSCs have been identified: the **osteoblastic** and the **vascular niches**. They have the capacity to produce daughter cells of two types: enterocytes and enteroendocrine cells. They seem to be able to self-renew and

provide a long-lived ability to generate more mature offspring in a clonal manner. Notably, these cells do not appear to be in direct contact with a heterologous cell type. They have focal localization of the β -catenin paralogue, at the interface between the stem cell and its descendant daughter cell (the enteroblast). This is distinct from the cells of different lineages composing most previously defined niches. In this case, the stem cells are associated with a basement membrane. It is suggested that the basement membrane itself might participate in the specialized microenvironment, possibly providing an opportunity for shifting. These data challenge the expectation that heterologous cells must necessarily be within the niche and suggest that stem cells might have a niche composed of an extracellular matrix and other non-cellular constituents regulating their control (Scadden, 2006)

The biology of adult mesenchymal cells (Kolf et al. 2007)

Osteogenesis: BMPs, in particular, BMP-2 and BMP-6, strongly promote osteogenesis in MSCs. BMP-2 induces the p300-mediated acetylation of Runx2, which results in enhanced Runx2 transactivating capability. Interestingly, the cytokine TNF- α associated with inflammation-mediated bone degradation downregulates Runx2 protein level. Transgenic TNF- α mice showed increased levels of Smurf1 and Smurf2, concurrent with decreased Runx2 protein levels.

The stem cell niche (Ohlstein et al., 2004):

Stromal microenvironments are likely to act as niches. They have been associated with a wide and diverse set of stem cells. Simple stromal microenvironments are defined as a specific location acting as niches. They have been associated with a diverse set of stem cells that can reside for an indefinite period of time and produce progeny cells. Complex niche and storage niches may contain quiescent stem cells.

Historically, "niche" is generally used to describe the stem cell location (Li & Xie, 2005). A primary function of the niche is to anchor stem cells. In addition to N-cadherin, other types of adhesion molecules, including integrins play an important role in the microenvironment/stem cell interaction.

1. The stem cell niche is composed of a group of cells in a special tissue location. The niche's overall structure is variable, and different cell types can provide a niche environment. For example, N-cadherin-positive osteoblastic lining cells in the trabecular bone form the niche for HSCs, whereas endothelial cells form the NSC cell niche.

2. The niche functions as a physical anchor for stem cells. E-cadherin-mediated cell adhesion is required for anchoring GSCs and SSCs, and N-cadherin may be important for anchor-

ing HSC in the bone marrow niche. Other adhesion molecules, such as integrins, may help anchor stem cells to extracellular matrixes.

3. The niche generates extrinsic factors that control stem cell fate and number. Many signal molecules have been shown to be involved in the regulation of stem cell behavior. Among these, the BMP and Wnt signal pathways have emerged as common pathways for controlling stem cell self-renewal and lineage fate. Several pathways can be utilized to control self-renewal of one stem cell type, whereas one growth factor can regulate several different stem cell types. The presence of signaling components of multiple developmental pathways supports the ideas that stem cells retain the ability to respond to these embryonic regulatory signals and these signals are essential for proper regulation of stem cell self-renewal and lineage commitment.

4. The stem cell niche exhibits an asymmetric structure. Upon division, one daughter cell is maintained in the niche as a stem cell (self-renewal); while the other daughter cell leaves the niche to proliferate and differentiate, eventually becoming a functionally mature cell. Asymmetric stem cell division leads to the retention of one daughter cell in the niche and to the other daughter cell leaving the niche to become committed.

Cellular components of MSC niche

Recently, multipotent cells have also been isolated from many other tissue types of non-mesodermal origin. Plastic-adherent MSC-like colonies are derived from the brain, spleen, liver, kidney, lung, bone marrow, and pancreas of mice. Murine MSCs are obtained from freshly isolated cells of the heart, liver, kidney, thymus, ovary, dermis, and lung on the basis of a CD45-/CD31 /Sca1+/Thy-1+ phenotype. Is there an MSC niche that is common to all of these tissues, or does MSCs function autonomously, in a manner that is independent of their environment?

Programming daughter cells

Niches with active stem cells contain routes for progeny cells to exit. For example, HSC daughters move away from the osteoblasts of the trabecular bone and toward the center of the marrow. A cell has left the niche when it reaches a location that cannot itself support a stem cell because one or more critical adhesive or signaling factors is no longer present. Niches contain specific structural features and mechanisms designed to ensure appropriate daughter cell movement and initiate cell terminal differentiation.

In each stem cell lineage, specific mechanisms are probably required to stabilize the early steps of differentiation. One mechanism ensures this balance. It is the control of asym-

metric/ symmetric stem cell division. The adhesion molecules are important in niche function that includes N-cadherin/ β -catenin, VCAM/integrin, and osteopontin/ β 1 integrin (OPN/ β 1 integrin). Switching between symmetric and asymmetric division can occur in multiple stem cells that occupy the same niche. Osteoblasts are heterogeneous and include the spindle-shaped endosteal lining cells and the oval-shaped cells that are the direct precursors of osteocytes. Among the osteoblastic lining cells, only a subset of osteoblasts interacts with HSCs.

Two studies suggested a perivascular nature of the MSC niche, on the basis of the expression of α -smooth muscle actin (α SMA) in MSCs isolated from all tissue types tested and the immunohistochemical localization of CD45-/CD31-/Sca1+/Thy-1+ cells to perivascular sites. MSCs were found, with the use of the markers Stro-1 and CD146, lining blood vessels in human bone marrow and dental pulp. These cells also expressed α SMA and some even expressed 3G5, a pericyte associated cell-surface marker. It has been hypothesized that pericytes are in fact MSCs because they can differentiate into osteoblasts, chondrocytes, and adipocytes. Localization of MSCs to perivascular niches gives them easy access to all tissues and lends credence to the notion that MSCs are integral to the healing of many different tissues. The transmembrane cell adhesion proteins function in cell-cell adhesion, migration, differentiation, and polarity.

Their role in the MSC niche is an unexplored territory and is crucial to an understanding of the molecular basis of the interactions between the MSC and its neighbors. The gene expression profiles of Oct4 and Nanog, Runx2, collagen type I, and bone sialoprotein demonstrate that the cells in osteogenic medium differentiated into osteoblasts and lost their self-renewal capability, as there was no expression of Oct4 and Nanog in the differentiated cells and the ALP-enriched population. In contrast, the expression of osteoblasts-related genes was enhanced (Arpornmaeklong et al., et al., 2009)

CONFLICTS OF INTEREST

The author has no conflict of interest.

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