"Niche" concept and the hematopoietic stem cell niches

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ABSTRACT

A stem cell niche is defined as the most suitable tissue microenvironment where somatic cells (parent cells) or in some cases extracellular matrix elements and stem cells are present in an exclusive interaction. In recent years, promising data were published revealing the molecular and functional significance of cell-cell contacts in stem cell proliferation (self-renewal) and differentiation into mature cells. In this brief review, recent progress has been documented with special emphasis on the structural-functional relationship between stem cells and surrounding cells. Hematopoietic stem cells and their niches are among the most studied stem cell types and cell-cell interactions, which have specific as well as some common properties observed in other niche sites in the human body. Some of the signaling pathways, which are illustrated by original drawings herein, obviously play major roles in stem cell self-renewal capacity and differentiation status. Therefore, stem cell niches stand as the most critical biological sites in stem cell physiology and could also be considered as the new targets for the treatment of certain diseases.

Key Words: Hematopoietic stem cell, stem cell, Notch signaling, niche, Wnt signaling

ÖZET

"Niş" kavramı ve hematopoietik kök hücresi nişleri

Kök hücre nişi, kök hücreleriyle bulundukları dokudaki mikroçevrede yer alan yardımcı hücreleri veya bazı örneklerde olduğu gibi hücrelerarası ortamdaki bileşenlerin birbirleriyle olan ilişkilerini tanımlar. Son yıllarda, kök hücrelerle ilişkide bulundukları hücreler arasındaki temasların moleküler-işlevsel anlamını ortaya koyan önemli çalışmalar yayınlanmıştır. Bu kısa derlemede özellikle, bu iki hücre grubu arasındaki ilişkinin yapısal ve işlevsel önemini vurgulayan bulgular üzerinde durulmuştur. Hematopoietik kök hücreler ve nişleri, hücrelerarası

ilişkilerin kısmen kendine özgü yapısı, kısmen de vücutta bulunan diğer niş bölgeleriyle ortak özellikleri nedeniyle insan vücudunda en çok çalışılan kök hücreler olmuştur. Bu derlemede özgün çizimlerle desteklenerek üzerinde durulan bazı önemli sinyal yolakları, kök hücrelerin kendini yenileme ve farklanma süreçlerinde temel rol üstlenmektedirler. Sonuç olarak kök hücre nişleri, kök hücresi fizyolojisinde önemli biyolojik bölgeler olarak ve bunun da ötesinde bazı hastalıkların tedavisinde hedef seçilecek bölgeler olarak karşımıza çıkmaktadır.

Anahtar Sözcükler: Hematopoietik kök hücreleri, kök hücresi, niş, Notch sinyali, Wnt sinyali

INTRODUCTION

Stem cell niches are firstly defined as specific anatomic locations that regulate how stem cells participate in tissue generation, maintenance and repair [1]. The niche must have both anatomic and functional dimensions, specifically enabling stem cells to reproduce or self-renew. This becomes particularly important as new sites of stem-cell localization are defined.

The primary characteristic of a stem cell niche is the ability to maintain a compartment of stem cells in an undifferentiated state [2]. This involves not only the prevention or over-riding of commitment and differentiation promoting signals, but also maintenance of stem cell viability.

The niche saves stem cells from depletion, while protecting the host from over-exuberant stem-cell proliferation. It constitutes a basic unit of tissue physiology, integrating signals that mediate the balanced response of stem cells to the needs of organisms. Yet the niche may also induce pathologies by imposing aberrant function on stem cells or other targets. The interplay between stem cells and their niche creates the dynamic system necessary for sustaining tissues, and for the ultimate design of stem-cell therapeutics. Ultimately, the function of an effective stem cell niche is to provide the appropriate cellular milieu where proper numbers of undifferentiated stem cells are maintained in balance with those destined for active tissue homeostasis.

Adult or somatic stem cells generally have limited function without the niche. Hematopoietic stem cells (HSCs), for instance, regenerate the entire blood and immune system, and make copies of it [3]. HSCs circulate freely, but seem to have little function outside specific anatomic locations. Certain specific cues from specific sites would allow stem cells to persist, and change in number and fate. Its dynamic capability makes the "niche" concept particularly important and central to the realization of regenerative medicine.

Towards Understanding the Niche Concept

Historically, "niche" is generally used to describe the stem cell location. In our view, however, "niche" is composed of the cellular components of the microenvironment surrounding stem cells as well as the signals emanating from the support cells. However, recent reports regarding the niche function depict that acellular structures such as extracellular matrix (ECM) elements and glycoproteins could also serve as a niche [4-7]. The concept of the ECM regulating stem cells is longstanding and at least three examples now exist in mammalian stem-cell systems. The first is in the skin, where β-1 integrins are known to be differentially expressed on primitive cells and to participate in constrained localization of a stem-cell population through presumed interaction with matrix glycoprotein ligands [6,7]. Second, in the nervous system, absence of tenascin C alters neural stem-cell number and function in the subventricular zone [8]. Tenascin C seems to modulate stem-cell sensitivity to fibroblast growth factor 2 (FGF2) and bone morphogenetic protein 4 (BMP4), resulting in increased stem-cell propensity to generate glial offspring. In the hematopoietic system, tenascin C deletion has also been shown to af-

fect primitive cell populations, raising the possibility that it participates in several stem-cell niches [9]. Taken together, there are at least two types of niches regarding their structural composition: one is the cellular microenvironment and the other is the acellular microenvironment (Figure 1).

In 1978, Schofield proposed the "niche" hypothesis to describe the physiologically limited microenvironment that supports stem cells [1]. The niche hypothesis has been supported by a variety of co-culture experiments in vitro and by bone marrow transplantation, in which the niche is first "emptied" through irradiation or drug treatments [10-15]. However, these studies did not resolve the issue of the exact stem cell location and niche structure in vivo [16]. Although locating and further identifying stem cell niches in mammals has been difficult owing to their extremely complicated anatomic structures, studies regarding stem cells and their location/niche in other genetic model systems, including those of *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (round worm), have been successful.

In mammals, the epithelial stem cell location was successfully identified in the bulbi of hair follicles, and the intestinal stem cell location was identified near the crypt base. These were based on the adult stem cell's ability to retain the BrdU or 3H-thymidine labels [17,18]. Recently, there has been a significant progress

regarding stem cells and their surrounding microenvironments in a variety of mammalian models. In 2003, two independent, simultaneous studies using genetic mutant mouse models led to the identification of osteoblastic cells, primarily those lining the trabecular bone surface (see below), as the key component of the HSC niche [19,20]. In the neural system, the stem cell niche was found in endothelial cells located at the base of the subventricular zone and subgranular zone [21-23].

Maintaining the physical organization of a niche site is probably an active and important process; however, the signals governing it are not well defined. Indirect evidence suggests that ephrins, important mediators of structural boundaries in neural and vascular tissue, might participate in at least one stem-cell system. Graduated expression of ephrin B1 and, reciprocally, its transmembrane tyrosine kinase receptors, EphB2 and EphB3, has been shown to organize mouse intestinal epithelial cells [24]. Disrupted expression of these receptors leads to aberrant organization of crypt and villus cells, including cells at the interposition between them, which are defined as stem cells. The altered topographic orientation of primitive and maturing cells results in polyploid outgrowths. Therefore, it will be important to discern molecular events sustaining architectural organization of the niche, particularly in considering how niche elements might participate in dysregulated growth in tumors.
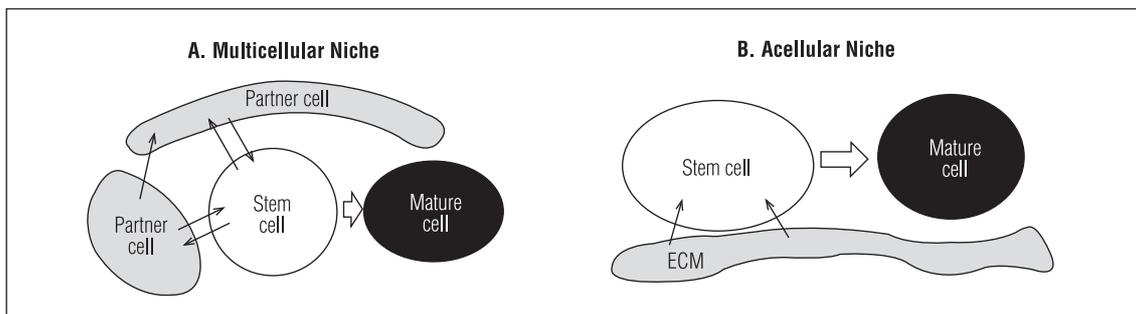
Figure 1. Two types of niches are defined depending on the existence of parent cells adjacent to stem cell(s). **A. Multicellular niche** is typically composed of two or more types of parent cells interrelated with each other and with stem cells (arrows). They build several intercellular adhesion sites and molecular signaling pathways providing a suitable niche environment. **B. Acellular (extracellular matrix) niche** is characterized as the expression of certain cell adhesion molecules on the stem cell surface which interact with specific extracellular matrix proteins (arrows). ECM: Extracellular matrix (original drawing by A. Can).

Key Regulators in the Hematopoietic Stem Cell Niche

Mammalian bone marrow is classically defined as a myeloreticular tissue in which two basic components, parenchyma and stroma, are present in an intermingling fashion. Table 1 summarizes the major cellular and extracellular elements of human bone marrow. Obviously, the HSC niche lies somewhere in this highly complicated microenvironment. Cumulative studies show that osteoblasts are one of the key cellular elements of the HSC niche, building an intimate contact with HSCs to help their self-renewing capability and to keep them in an undifferentiated state. Recent evidences clearly demonstrated that the control of stemness of HSCs is under the regulatory

Table 1. Human bone marrow cellular and intercellular elements are structurally and functionally divided into two main compartments, parenchyma and stroma, which are closely linked to each other. The HSC niche is the microenvironment milieu among those components basically considered as "endosteal" (adjacent to bone trabeculae) and "vascular" (adjacent to capillaries) niche compartments. HSCs are approximately 1:2.000 of all hematopoietic cells whereas MSCs are 1:10.000-1:100.000 of all stromal cells.

Parenchyma

Lymphoid progenitors and derived cells

Myeloid progenitors and derived cells

Hematopoietic stem cells (HSCs)

Stroma

Blood vessels (arterioles, sinusoidal capillaries, venules)

    Endothelial cells

    Arteriolar myocytes

    Pericytes

Adipocytes (red and yellow marrow depending on the number)

Stromal cells (reticulum cells and mesenchymal stem cells=MSCs)

Osteoblasts and osteoclasts

Intercellular fibers

    Reticulum fibers

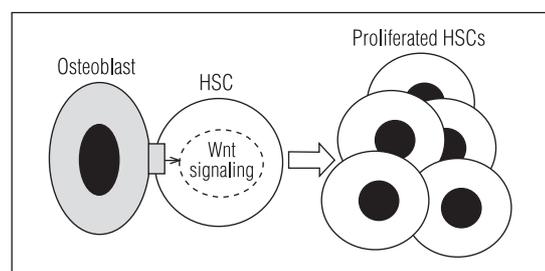   Type I collagen fibers

    Type III collagen fibers

Extracellular matrix proteins

    Fibronectin

    Laminin

    Proteoglycans

    Hemonectin

signals mostly emerging from the Jagged-1/N-cadherin cell adhesion sites between osteoblasts, also called SNO cells (Spindle shaped N-cadherin positive Osteoblasts) and inactive HSCs. Wnt/β-catenin signaling pathway seems to play a major role in the HSC self-renewal process as demonstrated by Reya et al. [25]. Overexpression of activated β-catenin expands the pool of HSCs in long-term cultures by both phenotype and function. Furthermore, HSCs in their normal microenvironment activate a LEF-1/TCF reporter, which indicates that HSCs respond to Wnt signaling in vivo. To demonstrate the physiological significance of this pathway for HSC proliferation, Reya et al. also showed that the ectopic expression of axin or a frizzled ligand-binding domain, inhibitors of the Wnt signaling pathway, leads to inhibition of HSC growth in vitro and reduced reconstitution in vivo [25]. Furthermore, activation of Wnt signaling in HSCs induces increased expression of HoxB4 and Notch1, genes previously implicated in the self-renewal of HSCs. Taken together, the Wnt signaling pathway stands as a critical prerequisite for normal HSC homeostasis in vitro and in vivo. The authors [25] provide insight into a potential molecular hierarchy of regulation of HSC development (Figure 2).

Another fundamental question in HSC biology is how cells are maintained in an undifferentiated state. Molecular regulation of two critical elements of self-renewal, inhibition of differentiation and induction of proliferation,

Figure 2. N-cadherin (+) cell adhesion sites (small rectangle between osteoblast and hematopoietic stem cell, HSC) stimulate the Wnt/β-catenin signaling pathway, which induces HSC proliferation while significantly inhibiting their differentiation, thereby resulting in functional self-renewal (Original drawing by A. Can).

could be under control of diverse mechanisms. Duncan et al. identified that Notch signaling is a key factor in inhibiting differentiation [26]. Using transgenic Notch reporter mice, they demonstrated that Notch signaling is active in HSCs in vivo and downregulates as HSCs differentiate. Inhibition of Notch signaling leads to accelerated differentiation of HSCs in vitro and depletion of HSCs in vivo. Furthermore, intact Notch signaling is required for Wnt-mediated maintenance of undifferentiated HSCs but not for survival or entry into the cell cycle in vitro. These data suggest that Notch signaling has a dominant function in inhibiting differentiation and provide a model for how HSCs may integrate multiple signals to maintain the stem cell state (Figure 3).

Endothelial cells in the vascular niche environment contacting HSCs could also provide maintenance signals on the HSC behavior [27]. Endothelial cells expressing vascular cell-adhesion molecule 1 (VCAM-1) associate closely with megakaryocytes and their progenitors through very late activation antigen 4 (VLA4) in response to chemotactic factors, stromal cell-derived factor-1 (SDF1) and fibroblast growth factor-4 (FGF4), and thus provide a niche for megakaryocyte maturation and platelet production [28,29]. The immediate juxtaposition of HSCs to endothelial cells also facilitates their rapid mobilization and entry into circulation in response to stress and, in the

case of megakaryocytes, release of platelets directly into the blood. HSCs and hematopoietic progenitor cells as well as megakaryocytes produce vascular endothelial growth factor (VEGF) and other angiogenic factors, which might act in a feedback loop to support endothelial cells in the bone marrow and in the periphery at sites of normal and pathologic angiogenesis [27]. Further study of the functional roles of endothelial cells in promoting adult organ maintenance, and stem-cell and progenitor-cell proliferation, seems certain to reveal more interesting functions for the vasculature than simply carrying the blood supply.

In addition to signaling pathways as described above, ECM components of the niche have also been shown to play a role in regulating the hematopoietic stem cell dynamics. A matrix glycoprotein, osteopontin (OPN), as a constraining factor on hematopoietic stem cells within the bone marrow microenvironment, is produced by osteoblasts in response to stimulation [30]. Using studies that combine OPN-deficient mice and exogenous OPN, Stier et al. [30] demonstrated that OPN modifies primitive hematopoietic cell number and function in a stem cell-non-autonomous manner. The OPN-null microenvironment is sufficient to increase the number of stem cells associated with increased stromal Jagged-1 and Angiopoietin-1 expression and reduced primitive hematopoietic cell apoptosis. The activation of the stem cell microenvironment with parathyroid hormone induces a super-physiologic increase in stem cells in the absence of OPN. Therefore, OPN is a negative regulatory element of the stem cell niche that limits the size of the stem cell pool and may provide a mechanism for restricting excess stem cell expansion under conditions of niche stimulation.

The production of OPN by osteoblasts is likely to be an essential requirement as shown by our recent work [31]. Osteogenically induced umbilical cord stromal cells express OPN during the first week of induction followed by a third week expression of another matricellular protein, bone sialoprotein-2 (BSP-2). In the following weeks, in conditioned media, diffe-
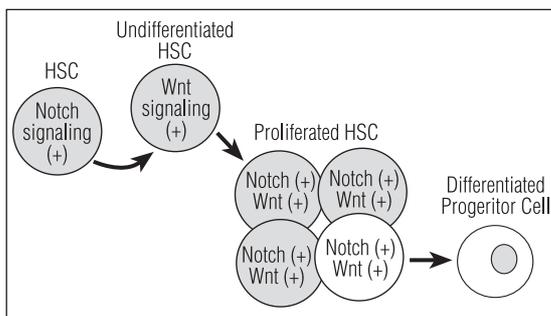
Figure 3. Notch signaling executed by a series of highly conserved receptors is a key factor in inhibiting differentiation. Intact Notch signaling is required for Wnt-mediated maintenance of undifferentiated HSCs. Notch signaling is active in HSCs and downregulated as HSCs differentiate. Inhibition of Notch signaling leads to accelerated differentiation of HSCs (Original drawing by A. Can).

rentiating osteoblasts express osteonectin and osteocalcin. It is possible to suggest that all of those proteins have roles in autocrine regulation of osteoblast maturation and thus might serve to determine the conditional status of the partner cell(s) in the hematopoietic niche microenvironment.

Concluding Remarks

The exact nature of the mechanisms responsible for the stem cell supporting properties of a niche is under investigation by several comprehensive studies. Since the nature of a niche comprises both intrinsic and extrinsic factors, a systematic examination of gene profiles as well as the candidate molecules is inevitable. It is reasonable to suggest that many niche sites share some common properties. Moreover, some properties of stem cells are also shared by cancer cells. For instance, it is becoming clear that signals are shared between stem cell renewal and cancer cell renewal [3,32]. Understanding how signals are functionally related to control stem cell renewal may also provide insight into how these signals coordinately drive oncogenic renewal. On the other hand, specific interactions confined to specific stem and parent cells seem to be more active in determining the specificity of niche structure and function.

When stem cells are taken out of their niches, considered as safe harbors, they quickly die or differentiate into distinct cell types. If we could better understand their "Neverland", we could also begin to understand the abnormal behavior of the niche microenvironment, possibly occurring in certain diseases, and therefore might discover an avenue to target certain molecules and cellular sites to combat that disease in a more specific manner.

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