Cancer Stem Cells and Their Niche

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1. Introduction

Stem cells within many tissues are thought to reside within a niche formed by a group of surrounding cells and their extracellular matrices, which provide an optimal microenvironment for the stem cells to function. In general, the niche is thought to consist of a highly organized microenvironment in which various factors, such as signals coming from secreted cytokines, extracellular matrix interactions, and intercellular adhesion, are thought to work cooperatively to maintain the undifferentiated stem cell phenotype. Among stem cells, adult stem cells are often localized into specific niches where they utilize many, but not necessarily all, of the external and intrinsic factors used by the embryonic counterparts in selecting a specific fate. Within the niche, stem cells are able to maintain their ability for self-renewal as well as their potential so that, consequently, detachment from the niche compartment induces stem cell differentiation and loss of self-renewal. Thus, when a stem cell begins to divide, it is thought that one daughter cell remains into niche to replace the original stem cell whereas other daughter cell is expelled out of niche and starts its process of differentiation. In this process, a cell retains self-renewal and differentiation inhibitory factors, so that keep being stem cell, whereas another daughter cell is destined to proliferate during a certain number of divisions for finally differentiate along a particular lineage. This latter daughter cell will receive too few stemness factors to maintain as stem cell, and/or inherit proliferation and/or differentiation factors that can overcome its stem cell phenotype. To maintain tissue homeostasis and correct functioning of organism, the number of daughter cells that retain stem cell identity must be strictly controlled such that differentiated cells can be generated in response to any injury. Likewise, the rate of division of stem cells into niche must be tightly controlled since an overproduction of daughter cells destined to be differentiated may be harmful because may result in cancer generation. In the present chapter, we speculate cancer stem cell niche for as well as the mechanisms that influence on the generation of daughter cells.

2. General concept

The concept of a stem cell niche was first proposed in 1978 by Schofield (Schofield, 2004), as a specific microenvironment in which adult stems cells reside in their tissue of origin.

Within the niche, stem cells are able to maintain their ability for self-renewal, as well as their multipotentially, and consequently, detachment from the niche compartment induces stem cell differentiation and loss of self-renewal.

The ability of stem cells to reside within niches is an evolutionarily conserved phenomenon. Adult stem cells are often localized to specific niches where they utilize many, but not necessarily all, of the external and intrinsic cues used by the embryonic counterparts in selecting a specific fate.

The regulation of the stem cell niche can therefore directly dictate the characteristic of an organ, and it is common that the regulation of the stem cell niche has a major influence on the function and morphology of an organ. This flexible regulation of the stem cell niche could have been a relatively easy way to acquire radically different stem cells types during evolution.

Stem cells within many tissues are thought to reside within a niche formed by a group of surrounding cells and their extracellular matrices, which provide an optimal microenvironment for the stem cells to function. In general, the niche is thought to consist of a highly organized microenvironment in which various factors, such as secreted cytokines, extracellular matrix interactions, and intercellular adhesion, are thought to work cooperatively to maintain the undifferentiated stem cell phenotype (Conti et al., 2005).

The identification of a niche within any tissue involves knowledge of the location of the stem cells. According to literature reported, to prove that a niche is present, the stem cell must be removed and subsequently replaced while the niche persists, providing support to the remaining exogenous cells (Sprandling et al., 2001).

Conceptually, a stem cell niche is a recess in a supporting medium that provides protection and nourishment to an individual, yet exclusion from molecules that may cause differentiation or mutation. Then, where the niche is well defined, the stem cells are virtually enveloped by differentiated cells, specialized to house and interact with the stem cells (Tulina & Matrevis, 2001; Morrison et al., 1997). The protective niches are composed not only of stem cells but also a diverse gathering of neighbouring differentiated cell types which secrete and organize a rich milieu of extracellular matrix and other factors that allow stem cells to manifest their unique intrinsic properties, including the ability to self renew, while keeping their pack-set of differentiation programs on hold. It is the combination of the intrinsic characteristics of stem cells and their microenvironment that shapes their properties and defines their potential.

Various lines of evidence suggest that once a stem cell niche is formed in a tissue, stem cells take up long-term residence there. Inside the niche, stem cells are often quiescent; outside the niche, stem cells must either possess sufficient intrinsic factors to overcome differentiation or succumb too much of fate. Direct physical interactions between stem cells and their non stem cell neighbours in the niche are critical in keeping stem cells in this specialized compartment and in maintaining stem cell character.

The niche is critical in maintaining the intrinsic self-renewing; undifferentiated character of the resident stem cells and the niche's microenvironments is both proliferation- and differentiation-inhibitory. The normal microenvironment, established by signals from the various other cells (stroma) that normally surround the niche seen to be important in maintaining the slow-cycling properties of labelled-retaining cells (LRCs) and keeping them in reserve. When stem cells cannot be identified or isolated in a particular organ, their existence may be inferred from kinetics studies of 5´-bromo-2´-deoxyuridine (BrdU) incorporation. Because stem cells are believed to be slowly dividing, the presence of labelled-retaining cells can identify the anatomical location of a stem cell niche.

Niche function

The protective niches are composed not only of stem cells but also a diverse gathering of neighbouring differentiated cells types which secrete and organize a rich milieu of extracellular matrix and other factors that allow stem cells to manifest their unique intrinsic properties, including the ability to self renew, while keeping their repertoire of differentiation programs on hold.

Without the appropriate microenvironment of specific intracellular interactions and cellular organization, the stem cell can become an undesirable beast; it is the combination of the intrinsic characteristics of stem cells and their microenvironment that shapes their properties and defines their potential. Direct physical interactions between stem cells and their non stem cell neighbours in the niche are critical in keeping stem cells in this specialized compartment and in maintaining stem cell character.

Regulating stem cell self-renewal is an essential feature of the niche. In the niche, regulating the balance between symmetric and asymmetric stem cell divisions becomes critical in maintaining proper stem cell number within the niche and in meeting the demand for differentiated cells within its surrounding tissue.

For a daughter to be a stem cell, it must retain self-renewal ad differentiation inhibitory factors. For a daughter destined to proliferate and differentiate along a particular lineage, this progeny cell must either receive too few stemness factors to maintain this state, and/or inherit proliferation and/or differentiation factors that can overcome this state.

To maintain tissue homeostasis, the number of daughter cells that retain stem cell identity must be strictly controlled such that differentiated cells can be generated in response to, for example wounding while the stem cell pool is simultaneously replenished but not expanded. The stem cells physically attach to the niche and, when they divide, orient their mitotic spindles with respect to the niche, so that one daughter inherits the attachment and stays in the niche, whereas the other daughter is displaced away from the niche and activates expression of genes that launch this cell along the differentiation pathway (Chen & McKearin, 2003; Kiger et al., 2000; Xie & Spradling, 2000; Yamashita et al., 2003).

The regulatory mechanisms of stem cell division within the niche to produce, on average, one stem cell and one cell committed to differentiate is as yet unknown, although there is no shortage of potential models (Loeffler & Roeder, 2002). When a stem cell divides, the possible outcomes are that two stem cells (A) are produced, that two daughter cells destined
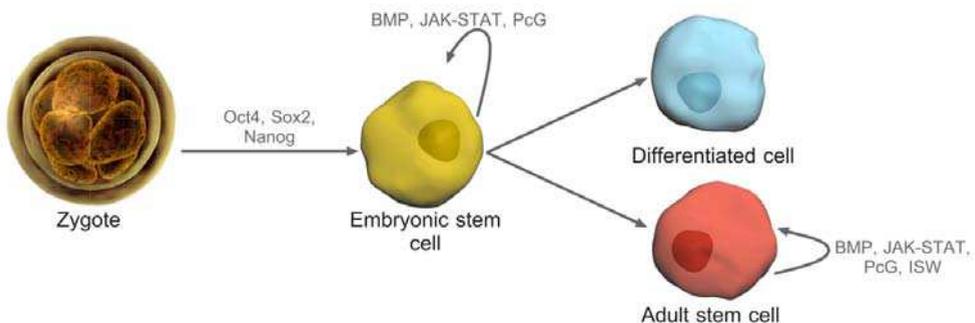

Fig. 1. Stem cell division.

to differentiate (B) cells are produced, or that there could be an asymmetric division resulting in one A and one B cell.

The process by which stem cells give rise to terminally differentiated cells occurs through a variety of committed progenitor cells (or transient amplifying cells), often overlapping in their differentiation capacity. During commitment, stem cells can undergo extensive proliferation and sequential differentiation, accompanied by a decrease in self-renewal capability to produce mature cells. The primary function of this transit population is to increase the number of mature cells produced by each stem cell division.

3. Structural architecture of the niche

Known niches are turning out to contain a high level of structural and regulatory complexity both in number and diversity cells. The association of stem cells with niches is also dynamic, and the same type of stem cell can use different niches at different times or under different physiological conditions.

The nature of the niche in terms of its composition and in the aspects of stem cell microenvironment is still not understood. The environment of the stem cells, the stem cell niche, defines the properties of the stem cell as much as the stem cell itself. The niche can be identified as the environment that sustains the stem cell population and is instructive in the differentiation and proliferation of the progeny.

Emerging evidence indicates that a specialized microenvironment, the stem cell niche, is one of the factors regulating normal stem cell maintenance and self-renewal. The stem cell niche controls stem cell maintenance and the crucial choice between self-renewal and the initiation of differentiation (Sprandling et al., 2001). Thus stem cells appear to require paracrine signals from the cellular niche in which they reside to maintain their identity and self-renewal capacity. As a result, the number of stem cells within a particular tissue can be regulated by controlling the number or size of available niches.

There is ample evidence that the maintenance of a functional tissue (i.e. epithelium) results from extensive regulation by and interaction with components of the extracellular matrix (ECM). Retention and loss of stem cells from the niche may be best achieved by regulating their adhesion to the ECM. No unequivocal molecular determinant of the stem cell niche has yet been identified, but there is an enormous potential for cross-talk between niche stem cells and the ECM. Mesenchymal matrix, subepithelial fibroblast, and myofibroblast may play a crucial role in defining the stem cell niche.

The molecular glue that anchors stem cells (SCs) to their niches is at least in part E-cadherin, which along with its partner, β-catenin in vertebrates, concentrates at stem cell niche borders. Cadherins and catenins participate in the formation of specialized intercellular junctions, called adherent junctions, which can be remodelled by virtue of their association with the actin cytoskeleton.

N-cadherin is expressed by putative stem/progenitor cells in the epithelial stem cell niche. N-cadherin is a member of the classic cadherin family that mediates cell-to-cell adhesion (Takeichi, 1991). N-cadherin may be a critical cell-to-cell adhesion molecule between epithelial stem/progenitor cells and their corresponding niche cells in the epithelium.

Other putative players in establishing stem cell relation are the integrins, which mediate adhesion of cells to a basal lamina composed of extracellular matrix (ECM). Elevated levels of integrins are often characteristic of stem cells, and loss function studies (in mice) reveal that both integrins and adherent functions play a critical roles in maintaining the location,

adhesiveness , and proliferative status of epithelial cells within tissues (Watt & Hohar, 2000). β_1-integrins, specially ($\alpha_4\beta_1$, $\alpha_5\beta_1$) have been reported to play a vital role in the early interaction of hematopoietic progenitor cells (HPCs) with the bone marrow (BM) niche (Voura et al., 1997; Papayannopoulou et al., 2001).

Adhesion between SCs and the surrounding support cells is important for holding stem cells within the niche, close to self-renewal signals and away from differentiation cues. Clusters of adherent junction are observed between stem cells and adjacent cells.

Gap junction intercellular communication via transfer of small molecules may also be involved in the survival and differentiation of early stem cells. The presence of *gap junctions* between SCs and adjacent support cells, coupled with the eventual loss of SCs, suggest that signaling via *gap junctions* may play a role in stem cell maintenance or may help physically maintain SCs in their niche.

Niche is in essence different, although there might be similarities in their structural architecture.

Microenvironment

The development of the most organs in vertebrates depends on a complex set of inductive interactions between epithelium and mesenchyme. These sequential and reciprocal interactions lead to the determination of stem cell fate and the organization of cells into tissues and organs. In the development, changes in gene expression patterns of several growths factors, transition factors, cell surface molecules, and structural molecules of the extracellular matrix have been implicated during the progressive determination of epithelial and mesenchymal cells. Similarly, in stem cell biology the niche describes the specialized microenvironment that supports stem cell maintenance and actively regulates cell function and proliferation (Li & Neaves, 2006; Yin & Li, 2006; Zhang & Li, 2008). A similar model has been suggested to delineate the interactions of malignant cells with their microenvironment at the primary tumor and at metastatic sites (Scadden, 2006; Sneddon & Werb, 2007; Psaila et al., 2006).

This microenvironment comprises supportive (non-malignant) stromal cells, soluble factors, vascular networks, nutrients and metabolic components, and the structural extracellular matrix (ECM) architecture (Folkman, 2002; Weigelt & Bissell, 2008; Joyce & Hanahan, 2004). A tumor-permissive immunological of inflammatory microenvironment is also required (Mantovani et al., 2008). Similar to stem cells, cancer cells seem to reside within highly distinct microenvironments, supported by uniquely specialized carcinoma-associated fibroblasts (Kalluri & Zeisberg , 2006). Epithelial-mesenchymal transition requires loss of cell-cell contacts and gain of cell motility.

Stroma

The stromal cells are the most important constituent of the niche structure, and they play important roles in both structural and functional maintenance and promotion for subsequent development as a matter of basic physiological need. The shaping of the niche structure is under continuous dynamics, most possibly due to regeneration oriented need of the constituent factors within the niche entity. Indeed as early as in 1978 (Schofield, 1978) has discussed about the stem cell niche where it was proposed that adult stem cells reside within a complex microenvironment of different cell types and extra-cellular matrix molecules that dictate stem cell self-renewal and progeny production in vivo (Schofield, 1978; Owen, 1998). Subsequent to the these first works it was propounded that the stromal

cells should be the following criteria: they are found in the extravascular compartment, they participate in providing physical and functional support for the stem cells, they are not of stem cell lineage, and they are numbers of stromal system (Scadden, 2006; Deans & Moseley, 2000; Blau et al., 2001).

The stromal cells are now known to constitute a group of cells that act as the supportive "mattress" on which the maturing precursor stem and the progenitor cells rest directly (Bianco & Riminucci, 1998). The stromal cells exert their effect on stem cell via direct cell-cell interaction as well as by releasing soluble factors (Ryan et al., 1991; Dittel et al., 1993; Watt, 2000). It is also presumed that normal cells in turn also might receive signals provided stem cells. Stromal cells provide extrinsic signals that maintain the stem cell niche and regulate the repopulation of stem cells. However, very little is known about the structural microcompartments as well as the factors that govern the growth, maintenance and localization of stromal cells. The formulation of stromal structure engraved in the form of a matrix and their role in constituting microenvironment nest the niche (Law & Chaudhuri, 2007), but the crosstalk between stromal cells for the generation of healthy stem cells are yet to explore (Rattis et al., 2004).

The presence nearly the niche of cell types termed the stromal cells, including fibroblast, macrophages, the reticular cells and adipocytes are all known to exhibit phagocytic activity under the event of emergency. They can act as the scavenger cells to clear up the niche structure and provide potential protective machinery against the foreign invasion.
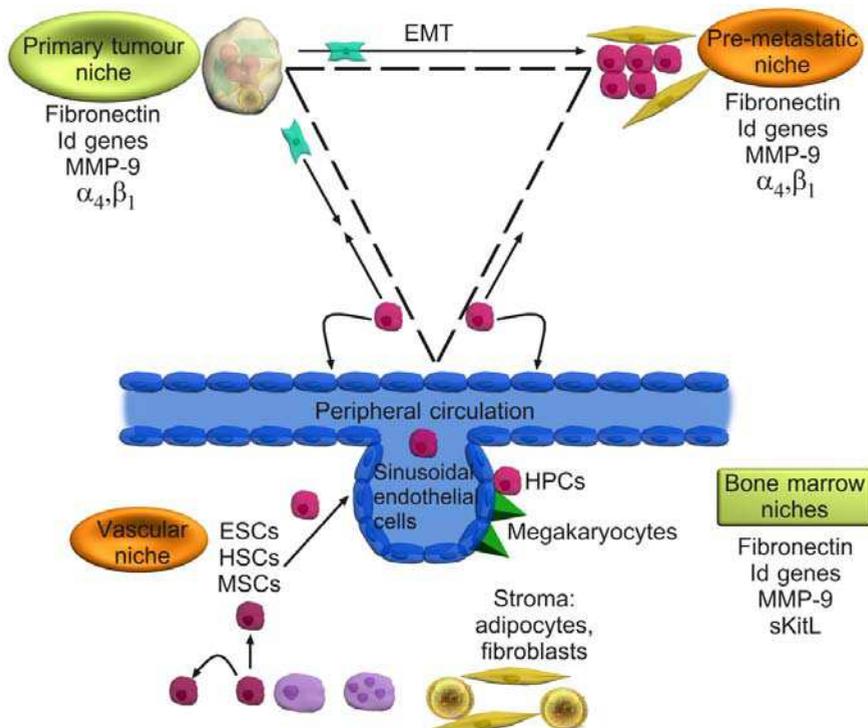

Fig. 2. Microenvironment and stroma.

Presumably, they form an immunological barrier surrounding the in vivo niche (Scadden, 2006). Stromal cell exhibit cytotoxic and phagocytic activity, and constitute the machinery of antigen presenting cell (APC) along with the stromal cell association as is found with the macrophages and the dendritic cells (Sujata & Chauduri, 2008).

Cancer cells and their associated stromal cells secrete a multitude of chemokines that direct the migration, proliferation, and differentiation of the vascular cell network to support the primary tumor and metastatic environment. A growing body of evidence recognizes the multiple signal transduction pathways, the details of the epithelial-to-mesenchymal transition, and the contribution of cell-to-cell and cell-to-matrix interaction as essential elements of the complex multistep process of metastasis. Molecular cross-talk between tumor-stromal as well as stromal-stromal components may enable synergy in the promotion of tumor progression (Burger & Kipps, 2006; Orimo et al., 2005). Also there are demonstrated that SDF-1 gradients mediate HSC retention within bone marrow niches, and growing evidence suggests that CXCR4-expressing cancer cells home to bone is a similar fashion, where they may lodge in the pre-existing supportive stromal microenvironment (Muller et al., 2001; Kaifi et al., 200). Stromal derived factor (SDF)-1, as is the case in bone marrow stroma, was highly expressed mediating recruitment and adherence of CSCR4^{+} expressing tumor cells (Kucia et al., 2005).

Inflammatory cells

The intriguing association between tumor and inflammation has long been a subject of research (Cousens & Werb, 2002). To date, little is known about the pro-inflammatory secreted factors that mediate the crosstalk inside the niche. Recently there are demonstrated that primary tumor cells secrete TGFβ, and TNFα, inducing the expression of the
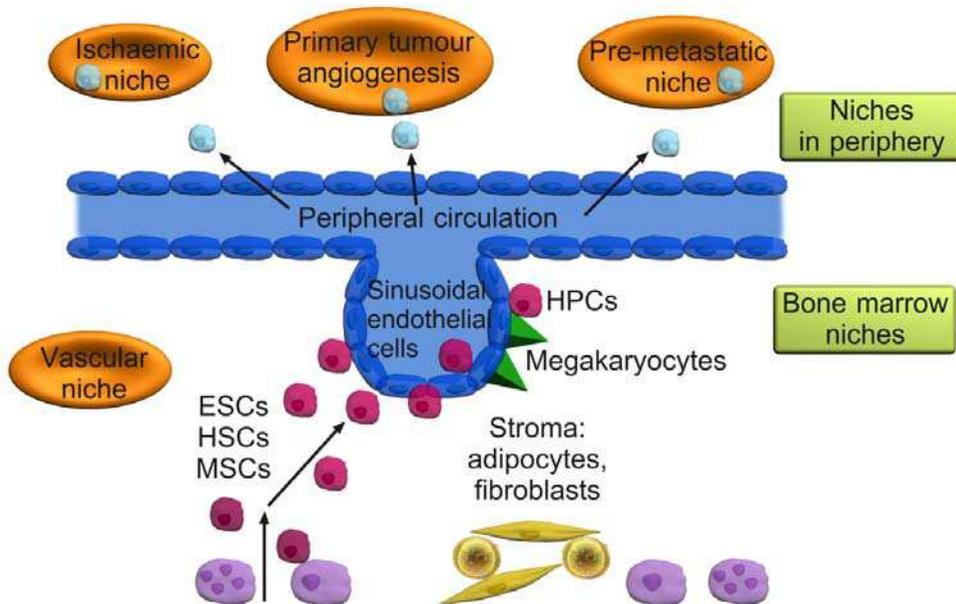

Fig. 3. Representative image of microenvironment.

proinflammatory chemokines S100A8 and S100A9, in the premetastatic microenvironment (Kucia et al., 2005). These chemoattractants increase the homing and engraftment of macrophage antigen 1 (Mac1)-expressing myeloid cells to the premetastatic niches. Activation of NF-kB signaling in macrophages in a serum amyloid A3 (SAA3) dependent fashion was also demonstrated (Hiratsuka et al., 2008). SAA3, a protein implicated in phagocyte chemoattraction, is up regulated in premetastatic niche by the inflammatory chemoattractant S100A8 and S100A9 (Hiratsuka et al., 2008). This finding raises the hypothesis that NF-kB in the premetastatic niche could be working to prepare a metastatic-like environment for primary tumor cells (Peinado et al., 2008). Nuclear factor kappa B (NF-kB) is a transcription factor that plays a pivotal role in connecting inflammation and cancer (Naugler & Karin, 2008).

Cell adhesion molecules

Individual cells in their particular environment adhere to the extracellular matrix (ECM) and their neighbours via integrin-containing and cadherin-containing complexes, respectively. Integrin-mediated adhesion to the ECM and cadherin-mediated adhesion between cells within developmental and physiological compartments are dynamically regulated.

A basic function of the niche is to anchor stem cells in the appropriate microenvironment. This function is mediated by adhesion molecules, including and adherent complex composed of cadherin and catenin. It has been reported that different forms of β-catenin interact with different protein complex. That is, the heterodimeric form of β-catenin/α-catenin interacts with membrane-bound cadherin, and the monomer form interacts with Tcf in nuclei (Gottardi & Gumbioner, 2004). It is, therefore, reasonable to proposed that β-catenin is a key molecule bridging two states of stem cells (Fuchs et al., 2004): the arrested state when stem cells are attached to the niche through the cadherin-β-catenin adhesion interaction (Zhang et al., 2003; Song & Xie, 2002) and the activated state in which β-catenin is nuclearly localized (Lowry et al., 2005; He et al., 2004).

A complex interplay of cytokines, chemokines, proteolytic enzymes and adhesion molecules maintain SC anchorage to the niche infrastructure.

4. Stem cell division and cancer

The processes which make possible that a cell gives rise to two daughter cells are defined as cell division cycle. These processes involve specific regulatory networks that impinge so that is strictly controlled both in time and space. Progression through the cell division cycle requires duplication of the genetic material and the delivery of the newly duplicated genomes to the two daughter cells during mitosis which represent one of the key processes in living organisms. This genetic duplication occurs in coordination with an increase in cellular components and changes in cell architecture. Balance between stem cell division and differentiation implies a fine coupling of cell division control, cell cycle arrest and reactivation, replication and differentiation.

In principle, stem cells can rely either completely on symmetric divisions or on a combination of symmetric and asymmetric divisions. The evidence for symmetric stem-cell divisions is strong, but the idea are that most stem cells can divide by either symmetric or asymmetric modes of division according to the fates of its daughter cells and the balance between these two modes is controlled by developmental and environmental signals.

Normally, SC divide asymmetrically (Cleevers, 2005) as a result of the asymmetric localization of cortical cell polarity determinants, such as Partner of Inscuteable (PINS) and atypical protein kinase C (aPKC), and cell fate determinants i.e Numb and Prospero, and regulated alignment of the mitotic spindle. For example when the machinery that regulates asymmetric divisions is disrupted, neuroblasts begin dividing symmetrically and form tumors (Lee et al., 2006; Albertson& Doe, 2003; Caussinus & Gonzalez, 2005). Cell clones lacking PINS are tumorigenic (Lee et al., 2006; Caussinus & Gonzalez, 2005), and cell clones lacking the cell fate determinants Numb or Prospero are also tumorigenic (Caussinus & Gonzalez, 2005). On the other hand it's known that the machinery that promotes asymmetric cell divisions has an evolutionary conserved role in tumor suppression (Cleevers, 2005).

Most stem cells have the ability to switch between asymmetric and symmetric modes of division, and that the balance between these two modes of division is defective in cancer disease. The adenomatous polyposis coli (APC) gene that regulates asymmetric division by stem cells in the intestinal epithelium is an important tumor suppressor in the mammalian colonic mucosa (Joslyn et al., 1991; Groden et al., 1991; Kinzler et al., 1991). Consistent with this tumorigenic potential, aPKC has been also identified as an oncogene in human lung cancers (Regala et al., 2005a; Regala et al., 2005b), and loss of Numb may be involved in the hyperactivation of Notch pathway signaling observed in breast cancers (Pece et al., 2004; Stylianou et al., 2006). In summary it is speculated that asymmetric division may suppress carcinogenesis, in addition to its role in maintaining a balance between stem cells and differentiated progeny.

Symmetric versus asymmetric division

Cell split in two at the end step of each division cycle. This division normally bisects through the middle of the cell and generates two equal daughters. When stem cells (SC) divide, their daughters either maintain SC identity or initiate differentiation. Conceptually, there are only three potential outcomes for SC after division: 1) a symmetrical division leading to net expansion of SC; 2) a symmetrical division that leads to the production of differentiated cells; and 3) an asymmetrical division leading to the maintenance of the SC population (Morrison & Kimble, 2006; Knoblich, 2008; Gonczy & DiNardo, 1996).

One SC can divide asymmetrically, producing one differentiating cell to maintain the tissue in a homeostatic state, or symmetrically, producing other SC; some mammalian SC populations may undergo both asymmetric and symmetric divisions depending on their circumstances (Chenn & McConnell, 1995). In summary, two main types of mechanism govern asymmetric cell division: the first, named intrinsic, relies on the asymmetric partitioning of cell components that determine cell fate; and the second, known as extrinsic, involves the asymmetric placement of daughter cells relative to external cues (Morrison & Kimble, 2006).

The relative proportion of symmetric divisions depending on their circumstances (Takahashi et al., 1996); the relative proportion of symmetric divisions appears to change over time, with symmetric divisions predominating at early time points when the SC pool would be expected to be expanding (Chenn & McConnell, 1995; Horvitz & Herskowitz, 1992). Whether this indicates that a single cell can switch from a symmetric to an asymmetric mode of cell division is not clear.

Asymmetry can manifest itself in two ways, namely by the unequal partitioning of cell-fate determinants and by the generation of daughter cells of different sizes. The mitotic spindle

is a key regulator of both of these events. First, its orientation controls the axis of cell division and can determine whether localized cell-fate determinants are segregated symmetrically or asymmetrically (Rappaport, 1996; Strome, 1993). Second, the position of the spindle within the dividing cell is thought to determine the relative size of two daughter cells (Rappaport, 1986; Albertson, 1984). The asymmetric segregation of cell-fate determinants and the generation of daughter cells of different sizes rely on the correct orientation and position of the mitotic spindle. The simple switch between symmetric and asymmetric segregation is achieved by changing the orientation of cell division: in vivo labelling mitotic spindles images reveals that the asymmetric spindle is formed in the same plane as symmetric spindle, but rotates before cell division. The direction of rotation usually correlates with the position of the centrosome at interphase: the spindle rotates in an anticlockwise direction when the centrosome is basal and clockwise when it is apical. Second, the cleavage furrow is not positioned equidistant between the spindle poles. As apical microtubules elongate and basal microtubules shorten, the midbody moves basally until it is positioned asymmetrically between the two spindle poles, at the site of the cleavage furrow, and the consequence are the generation of daughter cells of different sizes. The dogma indicates that the cleavage furrow always forms and generated two daughter cells of identical sizes equidistant from the spindle pole.

We have known that the asymmetric stem cell division is dictated by the spindle itself becoming asymmetric at anaphase. Microtubules on the apical side of the cell elongate, while those on the basal side become shorter. As the astral microtubules become longer, and seemingly more abundant, the apical aster enlarges, and the basal aster is concomitantly reduced in size (Kaltschmidt et al., 2000). Astral microtubules have been proposed to be involved in specifying the site of the cleavage furrow at cytokinesis (Rappaport, 1990; Oegema K & Mitchison, 1997).
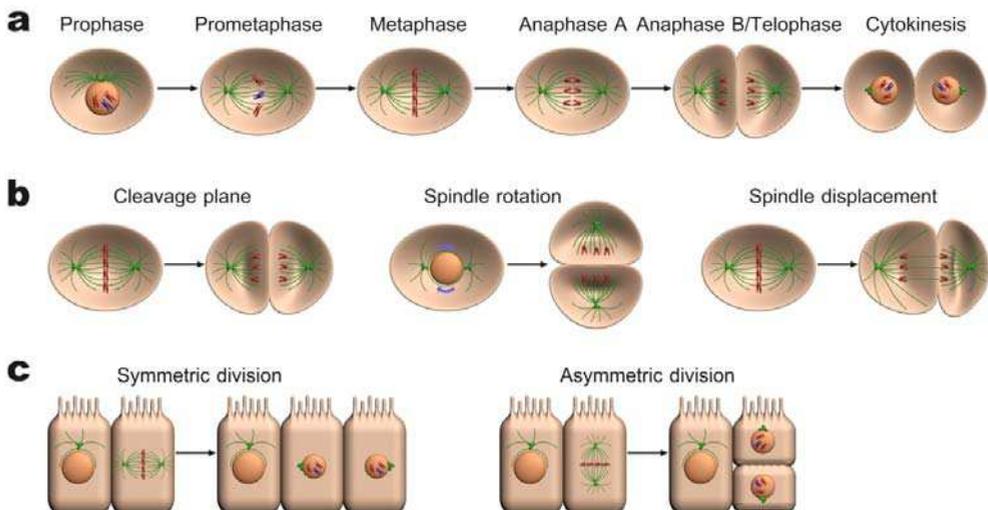

Fig. 4. A, B- Different phases of cell division and spindle rotation. C- Symmetric versus asymmetric stem cell division.

The stem cell niche functions to house regulate symmetric and asymmetric mitosis of stem cells; this regulation is affected through the action of various signalling pathways such as Wnt, Hh, Notch, Bmp, and probably others. Niche-forming cells are stimulated by growth factors and in turn, produce ligands (i.e. Delta), that act on stem cell receptors (i.e. Notch) to initiate stem cells mitosis or specify differentiation. Niche cells, the microenvironment they create, including the space between them, are features of a niche that allow it to maintain the stem cells, while preventing its differentiation and directing tissue growth and renewal through its daughters (Kiger et al., 2001).

5. Cell cycle in normal and tumor stem cells

Adult stem cells are often relatively slow-cycling cells able to respond to specific environmental signals and generate new stem cells or select a particular differentiation.

Exactly when and how most somatic stem cell niches develop is still a mystery, and in the world of stem cell niches, there are considerable variations in niche design. Some stem cells of adult mammals don't seem to have a specified niche within their respective tissue (i.e. skeletal muscle). In other cases, however, a stem cell compartment is established within a developing tissue, and cells within this niche are then activated in response to specific environment cues (i.e. skin, hair follicles, epidermis, mammary gland, lung, brain).

Stem cell repopulation is hierarchically organized and is intrinsically controlled by the intracellular cell cycle machinery. Their function appears to be highly associated with the differentiation stage in stem/progenitor pools. The negative regulation is important for maintaining homeostasis, especially at the stem cell level under physiological cues or pathological insults. By contrary disruption of cell cycle inhibition may contribute to the formation of the so-called cancer stem cells (CSCs) that are currently hypothesized to be partially responsible for tumorigenesis and recurrence of cancer. While a complex array of extracellular signals and intracellular transduction pathways certainly participate in the distinct response, the cell cycle machinery, as a final step, must communicate with the specific regulatory cues (Steinman & Nussenzweig, 2002) and cell cycle regulators must play key roles in this process.

The slow cycling feature seems to be a common behaviour in most adult stem cell types if not al, and their relative quiescence of stem cells may prevent their premature exhaustion lifespan, but it has been considered to be one of the hurdles in the context of the cancer recurrence and metastases propagation.

Stem cell (SC) quiescence is maintained by the balance between positive and negative proliferative factors: A variety of cell-cycle regulatory proteins, transcription factors, and cell-signaling molecules have been shown to regulate the quiescence of primitive stem/progenitor cells. The slow cycling feature seems to be a common feature in most adult stem cell types if not all (Potten, 1997; Bonfanti et al., 2001; Palmer TD et al., 2001). The relative quiescence of stem cells may prevent their premature exhaustation in vivo, but it has been considered to be one of the hurdles in the context of the in vivo cancer recurrence and/or metastasis.

The molecular principles of cell cycle regulation have been defined largely, and a number of surveillance checkpoints monitor the cell cycle and halt its progression. In mammalian cells, the cell cycle machinery that determines whether cells will continue proliferating or will cease dividing and differentiate appears to operate mainly in the G1 phase. Cell cycle progression is regulated by the sequential activation and inactivation of CDKs (Sherr, 1994;

Sherr & Roberts, 1995). In somatic cells, movement through Gi and into the S phase is driven by the active form of the cyclin D1, 2, 3/CDK4, 6 complex and the subsequent phosphorylation retinoblastoma (Rb) protein (Classon & Harlow, 2002). In parallel, the c-Myc pathway also directly contributes to the G1/S transition by elevating the transcription for cyclin E and cdc25A (Bartek & Lukas, 2001).

Several cell-cycle regulators have been shown to play critical roles in SC and/or progenitor cells proliferation, including p21, p27, p57, p16, p18 , and also the D-type cyclins (cyclin D1, D2, and D3) and their catalytic partners Cdk4 and Cdk6 . SC cell fate decisions are also regulated by several transcription factors (gfi-1, Pbx-1, MEF/ELF4, c-myc). Interestingly, many studies indicate that tumor-suppressor genes, including PTEN, p53, retinoblastoma (Rb), PML, APC, and FBw7, may play critical roles in maintaining SCs in a quiescent state. p18, a strong inhibitor for stem cell self-renewal has been suggested to be involved in the symmetric division of precursor cells in developing mouse brain (Tschan et al., 1999) and HSC self-renewal (Cheng et al., 2000; Yuan et al., 2004). The absence of p18 causes enhanced stem cell renewal, leading to an increased stem cell pool. The regulation for p18 gene and protein in stem cells is unclear at this moment. Given the striking outcome of p18 absence on stem cell renewal, it would be of great appeal to specifically look for the link of p18 with the several major signaling pathways controlling stem cell self-renewal.

p21, a gatekeeper for quiescent stem cells, is reduced in progenitor populations while is abundant in quiescent human HSCs (Stier et al., 2003; Dcos et al., 2000). Therefore, p21
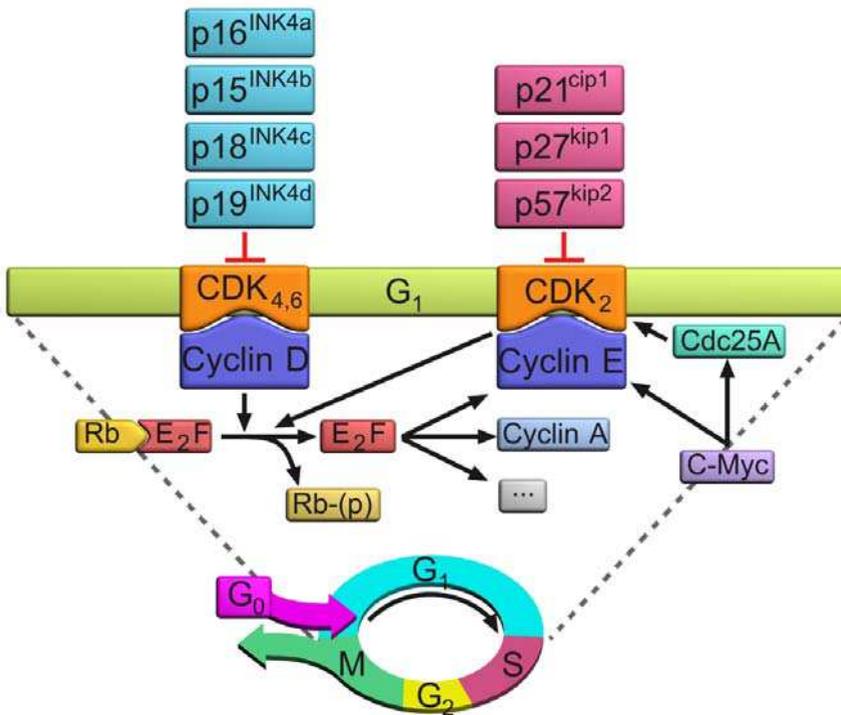

Fig. 5. Cell cycle regulators.

governs cell cycle entry of stem cells, and its absence leads to increased proliferation of the primitive cells (Cheng et al., 2000) , suggesting that restricted cell cycling is crucial to prevent premature stem cell depletion and death under conditions of stress.

p27, a progenitor-specific inhibitor to the repopulation efficiency, appears to accumulate at points in which signals for mitosis affect cell cycle regulators, and it has been shown to serve as an important regulator at a restriction point of mitogenic signals in many cell types (Coast et al., 1996). As progenitor cells are highly responsive to growth factors, though in a tissue-specific fashion, p27 must be a critical cell cycle mediator of many cytokines in progenitor cells (Polyak et al., 1995; Cashman et al., 1999). Thus, modulating p27 expression in a small number of stem cells without necessarily expanding the cells may translate into effects on the majority of mature cells.

The p16-Rb, of the family of "pocket proteins" that also includes p107 and p130, plays an important role in regulating the G1 checkpoint, cellular differentiation, apoptotic cell death, permanent cell-cycle arrest, and chromosomal stability (Sherr & Roberts, 1995; Classon & Harlow, 2002). pRb is likely to participate in the regulation of quiescence because its acute somatic inactivation is sufficient for Go-arrested cells to re-enter the cell cycle. Similarly, formation of p130/E2F4 complexes is thought to be a characteristic of Go and during the transition of cells from G1 to Go, p130 undergoes a specific phosphorylation event leading to its association with E2F4 (Sherr & Roberts, 1995).

6. Signaling from a support cell niche

One of the critical questions in the adult stem cell field concerns the mechanisms that regulate the decision between self-renewal and differentiation. Adult stem cells have two fundamental properties: a long-term capacity to divide and the ability to produce daughter cells that either retain stem cell identity or initiate differentiation along the appropriate lineage(s). The balance between self-renewal and initiation of differentiation is crucial. If too many daughter cells initiate differentiation, the stem cell population may be depleted. Conversely, if too many daughter cells maintain stem cell identity, the stem cell population may expand out of proportion, providing a pool of proliferative, incompletely differentiated cells that could mutate and became tumorigenic.

Physical attachment to the niche may be a feature of many adult stem cell systems, with the kind of functional complex depending on the nature of the niche; stem cells attach directly to somatic niche by adherent junctions. A general picture of how the stem cells niche mechanisms might work to control stem cell number and maintain the correct balance between self-renewal and differentiation is emerging. This process involves complex crosstalk between intercellular and intracellular mechanisms. First, the size, or number of stem cell niches defines the correct number of stem cells by sending short-range signal(s) for self-renewal or maintenance to the neighbouring stem cells. Second, cell-cell adhesion between supporting niche cells and stem cells enables stem cells to remain tightly associated with the niche. Third, stem cells are polarized with respect to the niche. Finally, stem cells polarized through contact with the niche can orient their mitotic spindles to ensure the normally asymmetric outcome of stem cell divisions by reliable placing one daughter cell firmly within the niche.

Within their niche apical-basal location determines stem cell self-renewal and/or differentiation. Theoretically, sister cells can either be in a planar orientation where both cells remain in direct contact with the basal lamina and host cells, or in an apical-basal

orientation where one daughter cell is pushed toward the basal lamina and the other cell apically toward the host cell. Taken together, this behaviour demonstrates that niche plays an important role in the maintenance of stem cell identity of newly divided daughter cells. The daughter cell attached to the basal lamina remains pluripotential, whereas the daughter that loses contact with the basal lamina up-regulates stem cell marker of differentiation and becomes a committed adult cell.

Stem cells are usually located adjacent to support cells that secrete factors, required for maintaining stem cell identity. Cell-cell adhesion between stem cells and niche cells is required for stem cell maintenance, physically maintaining stem cells within the niche and ensuring that stem cells are held close to self-renewal signals emanating from the microenvironment. Recent advances have provided important insights into the role played by the microenvironment in regulating stem cell identity and the asymmetric generation of committed daughter cells (Fuchs et al., 2004; Knoblich, 2001; Moore KA & Lemischka, 2006). Within the stem cell niche, signaling pathways such as Notch, Wnt, BMP/TGFβ, and STAT and proteins such as Num, PRA, PKCζ, LGL, and NUMA have implicated in the regulation of asymmetric cell division (Fuchs et al., 2004; Knoblich, 2001; Moore KA & Lemischka, 2006, Betschinger & Knoblich, 2004; Knoblich et al., 1995; Rhyu et al., 1995).

Hypoxia support the niche

Hypoxic microenvironments also occur during embryogenesis and in the adult, where one consequence may be the creation of niches that maintain pluripotential cells.

Stem cells reside in tissue regions, the niche that are low in vasculature and that are thought to provide a low-oxygen environment (Cipolleschi et al., 1993; Suda et al., 2005; Nilsson et al., 2001). Stem cells are harboured in vivo in a low-oxygen environment, and with the consequent hypothesis that self-renewal potential of stem cells is strictly linked to the capacity of these cells to survey in a hypoxic environment. The control of stem cell survival and the regulation of hypoxia response are intimately coupled and they share common control gene/pathways (Sansone et al., 2005). Recent data indicate that the stem cell regulatory Notch pathway share in an interplay with the hypoxia response modulator HIF-1α to promote the onset of a stem/undifferentiated phenotype (Gustafson et al., 2005).

There is evidence that hypoxia affects stem cell function and survival (Cejudo-Martin & Johnson, 2005; Covello et al., 2006). In vitro, hypoxia actively maintains a stem cell immature phenotype, induces a loss of differentiation markers, and blocks differentiation. In vivo, stem cells express higher levels of hypoxia-regulated genes than do the more mature progeny, as well as high levels of glycolitic enzymes.

In hematopoietic stem cells niche, Notch signalling induces/regulates diverse cell fate decisions during development (Singh et al., 2000). Also, as an intracellular second messenger, nitric oxide (NO) is implicated in the trafficking of hematopoietic progenitors (Zhang et al., 2007) and in the recruitment of stem/progenitor cells (Aicher et al., 2003; Ihle et al., 1998).

Many works has revealed that active niche that supports self-renewal of stem cells via activation of the Janus-kinase (JAK)-signal transducer and activator of transcription (STAT) pathway within the adjacent stem cells. JAKs are non-receptor tyrosine kinases that mediate signaling downstream of many mammalian cytokines and growth factors receptors, in part by phosphorylation and activation of STAT (Ihle et al., 1998). The signal for stem cell self-renewal is transduced from the activated JAL via STAT.

Some of the effects of hypoxia on stem cells correlate with the effects of Notch signaling on these cells. Notch is able to both maintain the pluripotential state of some cells and induce specific cell fates. Notch also influences proliferation and survival.

Hypoxia is a pathophysiological component of many disorders, including cancer (Semenza, 2001). Hypoxia controls many important aspects of cellular life, and a recently discovered function of hypoxia is to regulate differentiation in stem/precursor cells. In addition to their influences on proliferation and differentiation of various stem/progenitor cells populations, hypoxia altering cellular energy metabolism and angiogenesis. Recent studies suggest the existence of an intimate and functionally important interaction between Notch and hypoxia-inducible factor (HIF)-1α, a transcription factor that regulates many genes involved in the response to hypoxia, including factors that promote angiogenesis (Gordan & Simon, 2007).

Hypoxia activates Notch-responsive promoters and increases of Notch direct downstream genes. The Notch intracellular domain interacts with HIF-1α, a global regulator of oxygen homeostasis, and HIF-1α is recruited to Notch-responsive promoters upon Notch activation under hypoxic conditions.

The link between Notch signaling and hypoxia represents a novel facet of the hypoxic response. In the canonical hypoxic response, hypoxia acts by altering the stability and activity of HIF-1α leading to binding of HIF-1α to HRE-containing regulatory elements in specific target genes and activation of such genes e.g. VEGF, PGK, EPO, PDGF, and GLUT1. The difference between the canonical hypoxic response and the transfer of hypoxic information into the Notch signaling pathway results in the activation Notch response genes.

Regulatory pathways

Genetic studies of stem cell regulation have indeed revealed the operation of multiple regulatory circuits in many stem cell niches. Now, there are to consider two types of regulatory pathways in stem cells: those that active intrinsically with stem cells themselves (Oct4, Sox2, Nanog); and those that mediate interactions with their neighbours (Notch, Hh, Wnt, BMP, JAK/STAT).

1-Notch signaling

Notch encodes a transmembrane receptor that is cleaved to release an intracellular domain (Nicd) that is directly involved in transcriptional control and many components of the Notch pathway are expressed in the precursor cell compartment of the developing vertebrate (Artavanis- Tsakonas, 2002; Andromtsellis-Theotokis et al., 2006).

Notch receptor activation induces the expression of the specific target genes and enhancer of split 3 (Hes3) and Sonic Hedgehog (SHh) through rapid activation of cytoplasm signals, including the serine/threonine kinase Akt, the transcription factor STAT3 and mammalian target of rapamycin, and thereby promotes the survival of somatic stem cells.

The rapid effect of Delta4 (Dll4) on stem cells survival suggested that cytoplasm survival signals were induced in addition to slower transcriptional responses traditionally attributed to Notch activation.

Downstream of Akt, mammalian target of rapamycin (mTOR) is a key regulator of cell growth. Jag1 caused transit phosphorylation of mTOR. Like DAPT, the mTOR inhibitor rapamycin blocked the survival effect of Dll4. Jag1 induced phosphorylation of MSK1 and LKB1 kinases, which have been intensively studied as drug targets in diabetes and cancer (Alessi et al., 1998). The PDK1 and p70 ribosomal S6 kinase components of the insulin signaling pathways are known to limit mTOR activation.

The p38 mitogen-activated protein kinase is also a potential inhibitor of survival because it acts downstream of JAK and antagonizes growth of many cell types by activating MSK (Deak et al., 1998; Lavoie et al., 1996). JAK and p39 inhibitors increased survival in stem cells. Combined JAK abd p38 inhibition neither did nor substantially improves survival, further indicating that JAK may act through p38 to antagonize the survival pathway in stem cell niche. These data suggest that Notch acting through STAT3 promotes, and that p38 antagonizes, survival.

2- JAK/STAT

Although the surrounding microenvironment or niche influences stem cell fate decisions, few signals that emanate from the niche specify stem cells self-renewal.

A number of search have revealed that active niche supports self-renewal of stem cells (SCs) via activation of the Janus-Kinase (JAK)-signal transducer and activator of transcription (STAT) pathway within the adjacent SCs (Tulina & Matrevis, 2001; Kiger et al., 2000). JAKs are non-receptor tyrosine kinases that mediate signaling downstream of many cytokine and growth factor receptors of mammalians, in part by phosphorylation and activation of STAT (Ihle et al., 1998).

JAKs mediate signaling downstream of many mammalian cytokine and growth factor receptors, often by phosphorylation and activation of STAT proteins; STAT was required autonomously for stem cell maintenance.

Mutations on the JAK-STAT pathway resulted in stem cell loss, whereas JAK-STAT activation by cell loss ectopic expression caused unrestricted stem cell self-renewal. The signal transducers and activators of transcription (STAT) (Jove, 2000) family consist of seven members that are genetically localized to three chromosomal regions (Copeland et al., 1995).

3-NO

The capacity to generate new cells from stem cells niche is preserved along span life. Quiescent SC of the adult niches become activated and generates rapidly dividing transit-amplifying (TA) fells.

Nitric oxide (NO) an intercellular messenger, exerts antiproliferative effects on several cells and facilitate cell differentiation. However it is not clear if the actions are due to direct cytostatic action of NO on the stem cell niche precursors or whether they are an indirect consequence of changes in niche blood flow or cell-to-cell contact activity produced by NOS inhibition. The mechanism involved in the NO stemness action is also unknown at present. Based on previous finding that NO decreases stem cell proliferation in the subventricular zone (SVZ) we hypothesized that NO may participate in the control of stem cell niche proliferation and differentiation.

NO, is a physiological inhibitor of stem cell proliferation/differentiation in adult stem cell niches that exert a direct, 6-GMP-independent antiproliferative effect on stem cell progenitor without affecting cell survival. NO prevent the EGF-induced transphosphorylation of AKT, which are required for multipotent progenitor self-renewal, and NOS inhibition enhanced stem cell niche phosphor-AKT and reduced nuclear p27[Kip]. It was demonstrated that AKT phosphorylates the CDK inhibitor p27[Kip1] and prevents its translocation to the nucleus thus allowing cell cycle progression. Given that p27[Kip1] has been identified as a key regulator of the cell cycle specifically in transit-amplifying C cells this is probably that the mechanism by which NO-induced inhibition of AKT results in decreased multipotent precursor's proliferation. It is interesting to note a probably dissimilar distribution of p27[Kip1] in stem cell

niche, with a scared patron in the highly proliferative stem cell niche zone and abundant in the peripheral zone, where precursor that migrates arrest proliferation and differentiate.

Soluble factors

Under steady-state conditions, most stem cells are in contact with basal membrane and stromal cells, and are maintained in G0 phase of cell cycle (Cheng et al., 2000), while a small fraction is in S or G2/M phase of the cell cycle. The equilibrium between these two compartments is dictated by the bioavailability of stem cell-active cytokines, which are bound to the extracellular matrix or tethered to the membrane of stromal cells.

Local secretion of proteases may alter the stem cell-stromal cell interaction. The proteolytic cleavage of vascular cell adhesion molecule-1, expressed by stromal cells will be an essential step contributing to the mobilization of stem/progenitor cells. On the other way matrix metalloproteinase (MMPs) promote the release of extracellular matrix-bound or cell-surface-bound cytokines (Vu & Werb, 2000), such as vascular endothelial growth factor (VEGF), and can contribute to the release of stem cell-active cytokines following stress that shifts stem/progenitor cells from a quiescent to a proliferative niche.

7. miRNAs and stem cell

MicroRNAs (miRNAs) are a covered family of small regulatory molecules that function by modulating protein production. Each miRNA may regulate hundreds of different protein-coding genes. Each miRNA gene encodes a mature miRNA between 21-25 nucleotide (nt) long (Kim & Nam, 2006), non-coding RNAs that inhibit gene expression at the post-transcriptional level. They are transcribed as parts of longer molecules, up to several kilobases in length (pri-miRNA), that are processed in the nucleus into hairpin RNAs of 70-100 nt by the double-stranded RNA-specific ribonuclease, Drosha (Cullen, 2004; He & Hannon, 2004; Nakahara & Carthew, 2004; Bartel & Bartel, 2003; Ambros, 2001).The hairpin pre-miRNA are then transported to the cytoplasm by exportin 5 where they undergo final processing by a second, double-strand specific ribonuclease, known as Dicer. In animals, single-stranded miRNAs are incorporated into RNA induced silencing complexes (RISC) that bind primarily to specific messenger RNA (mRNA) at specific sequence motifs within the 3´untranslated region (3´UTR) of the transcript, which are significantly, although not completely, complementary to the miRNA.

Most characterized miRNAs from animals repress gene expression by blocking the translation of complementary messenger RNAs into protein; they interact with their targets by imperfect base-pairing, to mRNA sequences within the 3´UTR (He & Hannon, 2004).

Experimental evidence has suggested that small RNAs regulate stem cell character in animals (Bernstein E, et al., 2003; Carmell et al., 2002), and moreover, some miRNAs are differentially expressed in stem cells, suggesting a specialized role in stem cell regulation (Suh et al., 2004; Houbaviy et al., 2003).

Recently, the stem cell and miRNA fields have converged with the identification of stem-cell-specific miRNAs (Houbaviy et al., 2003). In addition to canonical miRNAs, mirtrons and shRNA-derived miRNAs have also been identified in mouse embryonic stem (ES) cells. It is now clear that miRNAs provide a new dimension to the regulation of stem cell functions. Based on their function in translational attenuation, miRNAs seem to regulate stem cell fate and behaviour by fine-tuning the protein levels of various factors that are required for stem

cell or niche cell functions. One important function of miRNAs in ES cells is to regulate cell cycle progression during stem cell differentiation.

The overall function of the miRNA pathway in EC cell has been evaluated in humans and mice by analysing the phenotypes of two proteins that have crucial roles in the production of mature miRNAs: DGCR8 and Dicer mutants (Bernstein et al., 2003).

Stem cells have distinct miRNA signatures, and their assessment have been done by cloning sequencing of miRNA from stem cells. Deep sequencing of miRNAs from stem cells has revealed the identity of the specific miRNAs that are expressed in stem cells and might function in self-renewal and differentiation of stem cells.

In addition, different molecules may regulate postnatal stem cell niches (Palma et al., 2005; Shi et al., 2005). Dicer-1 (Dcr-1) is essential for processing miRNAs, whereas Dicer-2 (Dcr-2) is required for siRNAs; loss of Dcr-1 completely disrupts the miRNA pathway and only has a weak effect on the siRNA pathway. Thus Dcr-1 is required for cell autonomously in the stem cell niche for cell divisions that developing more differentiated cells.

Regulatory role of miRNAs

Transcription factors are essentials players in stem cell self-renewal and differentiation (Pevny & Placzek, 2005; Ross et al., 2003). However, post-transcriptional gene regulation is emerging as another essential and, until recently, unexpected regulator of development. Many different classes of small non-coding RNAs are present in stem cells, with diverse roles including RNA modification and chromatin remodelling (Mattick & Makunin, 2005).

Recently there are identified a large family of small non-coding miRNAs, which are likely key post-transcriptional players in stem cells and their differentiated progeny (Bartel, 2004). The cloning and sequencing of small RNAs using conventional methods revealed that the miR-290-295 cluster and miR-296 are specific to stem cells and that their levels decreases as the stem cells differentiate. Simply the miR-290-295 cluster has specific role in maintaining pluripotency (Singh et al., 2008): the real role of miR-290-295 is to induce differentiation. In contrast miR-21 and miR-22 increase substancially follow the induction of differentiation: these miRNAs might have important roles in stem cell differentiation (Kim & Nam, 2006; Singh et al., 2008).

miRNAs are especially attractive candidates for regulation stem cell self-renewal and cell fate decisions, as their ability to simultaneously regulate many targets provides a means for coordinated control of concerted gene action.

miRNAs are 21-25 nt, non-coding RNAs that are expressed in a tissue-specific and developmentally regulated manner and comprise approximately 1% of the total genes in the animal genome (Bartel, 2004). Although direct evidence for a functional role of miRNAs in stem cell biology is just emerging, hints regarding their involvement based on expression patterns, predicted targets, and over-expression studies suggest that they will be key regulators.

miRNA are likely important regulators for stem cell self-renewal: distinct sets of miRNAs are specifically expressed in pluripotent ES cells but not in differentiated embryonic bodies or in adult tissues, suggesting a role for miRNAs in stem cell self-renewal (Kim & Nam, 2006). Loss of Dicer1 causes embryonic lethality and loss of stem cell populations (Nakahara & Carthew, 2004; Wienholds et al., 2003), and in the other way, Argonaute family members are required for maintaining germline stem cells in differentiated organisms (Carmell et al., 2002).

As stem cells differentiate, they down-regulate stem cell maintenance genes and activate lineage-specific genes. These transitions require a rapid switch in gene expression profiles.

Although the transcription factor pool is replaced, remaining transcripts that were highly expressed in the previous stage need to be silenced. miRNAs are uniquely poised to rapidly effect such changes through simultaneous repression of many targets of any remaining transcripts. This would predict that miRNAs are also transcriptional regulated in different cell types such that there is extensive crosstalk between transcription and post-transcriptional regulation and that distinct miRNAs are active in particular lineages (Kanellopoulou et al., 2005; He & Hannon, 2004).

Since then, miRNAs have been implicated in a wide variety of developmental and metabolic pathways in both invertebrates and vertebrates, including cell differentiation, proliferation, programmed cell death, the number of functional miRNAs target pairs identified to date is minimal (He & Hannon, 2004).
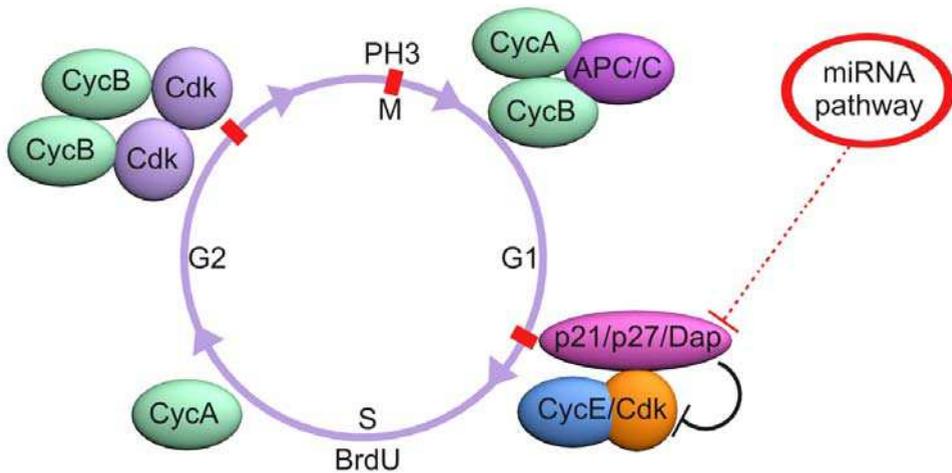

Fig. 6. miRNA pathway and stem cells cell cycle.

miRNA function in embryonic and adult stem cells

The functions of miRNAs in somatic tissue stem cells have also been identified, and their mechanisms of action are to regulate adult stem cell proliferation and differentiation. Evidence for this activity comes from experiments demonstrating that ES cells that were deficient in miRNA processing enzymes exhibited defects in their capacity for differentiation and self-renewal (Murchison et al., 2005; Wang et al., 2007). In addition, Dicer deficiency is embryonic lethal, and Dicer deficient embryos exhibit greatly reduced expression of Oct4 suggesting a stem cell defect (Kloosterman & Plasterk, 2006). The pluripotent property of ES cells is subject to regulation by the homeobox transcription factors, Oct4 and Nanog, which are essential regulators of early development and ES identity (Chambers et al., 2003; Mitsui et al., 2003; Nichols et al., 1998): it has been suggested that Oct4 initiates pluripotency state whereas Nanog maintains it (Chambers et al., 2007).

Little is known with respect to mechanisms by which miRNA function in controlling the developmental potential of ES cells, and it is largely unknown how ES cell-specific transcription factors and miRNA work together. The three stem factors (Oct4, Sox2, and Nanog) were found to occupy the promoters on many transcription factors and of 14 miRNAs (Boyer et al., 2005).

The actions of miRNA have been shown to regulate several developmental and physiological processes including stem cell differentiation, haematopoiesis, cardiac and skeletal muscle development, neurogenesis, etc... (Tay et al., 2008).

8. Tumor stem cell concentric niche model

Current investigations on primary cultures of solid tumors are generally conducted on random portions (i.e regionally undetermined) of surgically resected tumor or metastatic samples. It has been reported the existence of two types of cancer stem cells (CSCs) primary cancer stem cells (pCSCs) and/or metastatic cancer stem cells (mCSCs). But at intratumoral areas it can demonstrated the existence of two types of cancer stem cells (CSCs) within different regions of the same human tumor in relation to the pO2 gradient: the tumor mass characterized by a phenotypically immature anoxic core surrounded by a proliferating hypoxic layer, the more vascularised and more oxygenated peripheral area characterized by the presence of more differentiated cell types, with cells expressing pro-angiogenic signaling.

This model describes intratumoral areas in order to define potential phenotypic heterogeneity and differential expression of molecular signaling pathways in correlation to the oxygen tension gradient within the tumor mass. Thus there are identified three layers: the internal core, the intermediate, and the peripheral layers, based on the distance from the anoxic central core, to define their molecular and phenotypic features in correlation to the hypoxic concentric gradient. The three concentric layers bear quite diverse cell phenotypes.

The inner, highly hypoxic/anoxic core, characterized by stem cells with low proliferation index, and intermediate, mildly hypoxic layer, lining the anoxic core, with immature and proliferating tumor precursor cells, and the peripheral, more predominantly committed/differentiated cells.

Immunohistological analyses revealed that both the core and the intermediate layer were characterized by high level of HIF-1α expression which is over-expressed with VEGF. The expression of both Glut1 and CAIX was higher in the core, progressively undetectable at the periphery of the tumor.

Analysis of cell cycle marker Ki67 indicated that the inner core and, particularly, the intermediate-hypoxic area had the highest proliferation rate, whereas in the peripheral area, Ki67 expression was very low.

The intermediate portion is a thin transition area between the partially necrotic core and the peripheral area, which is defined by the presence of tumor angiogenesis. Nevertheless, VEGF highly expressing cells, characterized by poor HIF-1α expression, were found in the peripheral and more vascularised layer of the tumor mass. The expression of CD34, antigen constitutively expressed on endothelial cells, is found at the peripheral layer, the area highly enriched in CD34+ vessels.

Tumor cells derived from the intermediate area tended to form spheroids in vitro and displayed the highest proliferation rate, confirmed also by Ki67 expression, compared with cells from the core and from the peripheral area. Conversely, cells from the peripheral areas appeared more morphologically differentiated.

Moreover, cells recovered from the intermediate layer resulted to form the highest number of big size spheroids, whereas cells from the inner core formed small size spheroids; oppositely, cells derived from the peripheral area did not generate spheroids but rapidly differentiated. These behaviour support the assumption that stem cells, which are found to

be mainly located within the inner core, are characterized by a lower proliferation rate compared with committed precursors.

It has been shown that malignant tumors are characterized by a hypoxic microenvironment, which correlates with tumor aggressiveness (Azuma et al., 2003; Helczynska et al., 2003; Jogi et al., 2002), and over-activity of hypoxia inducible factor-1α (HIF-1α), the best described low oxygen sensor, is implicated in tumor progression (Smith et al., 2005). Recent data suggest that HIF-1α and multiple HIF-regulated genes are preferentially expressed in cancer stem cells in comparison with non-stem tumor cells and normal cell progenitors.

Importantly, hypoxia is also implicated in the regulation of several developmental critical signaling pathways, such as Notch (Gustafson et al., 2005), and, as were reported, bone morphogenic proteins (BMPs) (Pistollato et al., 2009) and Akt/mTOR pathways (170). Also HIF-2α has been described as a proto-oncogene.

Moreover, we speculate that the hypoxic signature is crucial in determining the epigenetic activation (HIF-1α, Glut1, and CAIX) and/or inhibition (BMP, Akt/mTOR/Stat3) of signaling pathways involved in the maintenance of the stem cell pool.

9. The pre-metastatic niche

Metastasis is know as a cascade of molecular/cellular events involving tumor cell intravasation, transport and immune evasion within the circulatory systems, arrest a secondary site, extravasations and finally colonization and growth (Chambers et al., 2002). Dissemination of tumor cells is a prerequisite for metastasis, but the two processes are not synonymous. Less than 1% of cancer cells entering the blood circulation successfully generate metastatic foci (Fidler, 1970; Fidler et al., 1977; Liotta et al., 1978; Varani et al., 1980; Mehlen & Puisieux, 2006).

Certain characteristics distinguish those cells able to colonize secondary tissues from other circulating tumor cells. The genetic and phenotypic make-up of a tumor is a major determinant of metastatic efficiency, but a receptive microenvironment is a requisite for establishing primary/secondary tumor growth. Gene-expression *signatures* that correlate with overall tumor metastatic efficiency (van der Vijver et al., 2002), and also those that can predict metastasis to a random organ have been described (Chang et al., 2004). The poor prognosis *signatures* encode not only genes important for intrinsic tumor cell cycle regulation, but also cell surface receptors and proteins expressed by the tissue stroma, such as matrix metalloproteinases, highlighting the importance of tumor cell-stroma interaction (Chang et al., 2008). Additionally, a transcriptional *signature* of fibroblast serum response has been shown to predict cancer progression (Kang et al., 2003). However, the factors underlying metastatic *dormancy*, and the dichotomy between tumor dissemination and metastatic establishement, remain enigmatic.

Bone marrow-derived hematopoietic progenitors cells (HPCs) recently emerged as key in initiating the early changes in metastatic cascade, creating a receptive microenvironment at designated sites for distant tumor growth and establishing the *pre-metastatic niche* (Kaplan et al., 2005).

Seminal research works demonstrated a key role for bone marrow-derived HPCs in *priming* distant tissues for tumor cell implantation and proliferation. BM-derived VEGFR-1^{+} cells preceded the arrival of tumor cells and VEGFR-2^{+} endothelial progenitor cells (EPCs), which migrate to established VEGFR-1^{+} clusters. The pre-metastatic niches may function as physiological niches, and allow the VEGFR-1^{+} cells to maintain expression of primitive cell

surface markers. It is possible that VEGFR1 activation, which leads to increased activity of epithelial-to-mesenchymal transition-associated transcription factors Snail, Twist, and Slug in the primary tumors, may also regulate VEGFR-1^{+} HPCs in the *pre-metastatic niche* (Yang et al., 2006).

Proangiogenic cytokines such VEGF induce homing of endothelial progenitor cells (EPCs), expressing VEGFR-2, to the tumor site, along with HPCs expressing VEGFR1.

These VEGFR-1^{+} HPCs are essential for stability and growth of the neovasculature (Lyden et al., 2001; Raffi et al., 2002; Okamoto et al., 2005; Carmeliet et al., 2001; Li et al., 2006). A tumors´s chemokine profile can greatly influence the contribution of the stromal microenvironment, such that those tumors co-expressing both VEGF and its family member placental growth factor (PIG), which exclusively signals through VEGFR-1, have a more aggressive metastatic phenotype (Marcellini et al., 2006).

10. References

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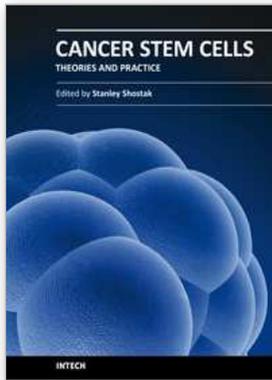
Cancer Stem Cells Theories and Practice
Edited by Prof. Stanley Shostak

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