Stem Cell Rev and Rep (2009) 5:319–327
DOI 10.1007/s12015-009-9096-7

Building a Framework for Embryonic Microenvironments and Cancer Stem Cells

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Published online: 10 November 2009

Abstract The putative existence of a cancer stem cell niche consisting of bi-directional stromal and stem cell secreting factors that trigger cancer stem cell growth and proliferation has been hypothesized in the nervous and hematopoietic systems. In light of this theory, it has been proposed that embryonic stem cell microenvironments, upon interactions with cancer stem cells, may reprogram cancer cells resulting in a substantial inhibition of tumor cell properties. Here, we discuss emerging data that support this novel concept of cancer inhibitory factors produced in the context of embryonic microenvironments as well as by embryonic stem cells (ESCs).

Keywords Embryonic stem cells · Cancer stem cell · Niches · Microenvironment · Reprogramming

Stem Cells & Their Niches

Stem cells are fundamental players in cell biology allowing tissues to be replenished from freshly created cells throughout their life-time. Teleologically, the gold standard of a stem cell is the fertilized egg, which generates a complete set of specialized somatic diploid cell types, together with the haploid germ line that will be responsible for the transmission of the characters to the next generation. As the embryo develops, an outer protective membrane of trophoectoderm encases a mass of pluripotent stem cells conforming the inner cell mass (ICM) [1], thus forming one of the first local stem cell microenvironments during development. Embryonic stem cells (ESCs) are artificially created after the separation of the ICM from its niche and cultured in specific conditions preserving an especific phenotype (Table 1).

Niches are protective local microenvironments composed of stem cells and neighboring differentiated cell types, which secrete and organize the extracellular matrix allowing stem cells to maintain their unique property of undifferentiation and self-renewal through asymmetric division [2]. The injection of ESCs into the ICM of a recipient mouse blastocyst or in a blastocyst without ICM induces the incorporation in the new niche and contributes to generating cells from all tissues in healthy chimera offspring [3, 4]. Interestingly, subcutaneous transplantation of ESCs or induced pluripotent stem cells (iPS) into immunodeficient nude mice form typical multicellular tumors, known as teratomas [5–7]. These paradoxical results clearly indicate that a combination of intrinsic factors in ESCs and their microenvironment define the stem cell fate.

Somatic stem cells (SSCs) remain dormant usually at the G_0 phase in the tissue and proliferate through asymmetric cell division, giving rise to one daughter stem cell and one transit amplifying cell [8]. Activation occurs during particular periods of time or after external injury, and their regulation is strictly controlled in their niches [9] (Table 2).

The interactions with their niches are crucial to this process, SSCs are often quiescent and exhibit low cell cycle entry indicating that the niche's microenvironment is a proliferation/differentiation-inhibitory zone. The niche becomes occupied over time, and SSCs are displaced from

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Table 1 Markers of human embryonic stem cells

Protein name or gene name	Cellular location	Author and journal
OCT4	Nucleus	Nichols et al., Cell, 1998
NANOG	Nucleus	Chambers et al., Cell, 2003
SOX2	Nucleus	Avilion et al., Genes Dev, 2003
FOXD3	Nucleus	Hanna et al., Genes Dev, 2002
UTF1	Nucleus	Okuda et al., EMBO J, 1998
REX1	Nucleus	Henderson et al., Stem Cells, 2002
SSEA3	Plasmamembrane	Draper et al., J. Anat, 2002
SSEA4	Plasmamembrane	Draper et al., J. Anat, 2002
TRA-1-60	Plasmamembrane	Draper et al., J. Anat, 2002
TRA-1-81	Plasmamembrane	Draper et al., J. Anat, 2002
THY1	Plasmamembrane	Draper et al., J. Anat, 2002
LCTM2	Plasmamembrane	Pera et al., Differentiation, 1988

the niche leading to cell differentiation. This hypothesis was demonstrated by the labeling of SSCs with 5-bromo-2′ deoxyuridine (BrdU) that binds to DNA. Typically, after a sufficiently long pulse of BrdU, quiescent cells remain BrdU-positive, whereas the rapidly dividing progeny dilute out the BrdU dye [10, 11].

The hair follicle represents a remarkable example where the niche receives a continuous stimulus from specialized mesenchymal cells (known as dermal papilla). During a given hair cycle, a growth period is followed by degeneration of the bulge, this unique cellular process seems to depend on alterations in the niche that normally activate BrdU-retaining cells, which divide and terminally differentiate to recolonize the follicle producing a new hair [12]. The example outlined above raises the question about the signaling pathways required for maintaining the balance among quiescence, self-renewal, and cell fate commitment.

An important family of niche signaling molecules are the bone morphogenetic proteins (BMPs) and the transforming growth factor beta (TGF-beta) superfamily. Several examples in invertebrates and vertebrates illustrate the role of these signaling pathways in the niche compartment. In the fruit fly *Drosophila* ovary, *Dpp* (a TGF-beta family member) triggers activation of several receptors in germ stem cells (GSCs), resulting in phosphorylation and subsequent activation of the transcriptional co-repressors (I-Smads), which silences the differentiation gene *Bam* [13]. In the mammalian testis, *Bmp8b* is essential for the initiation and maintenance of GSCs [14]. Moreover, BMPs/TGF-beta signaling pathways seem to be obligatory for maintaining stemness althought they are not sufficient by themselves. Genetic analysis in oocytes from *Drosophila Melanogaster* indicates that the JAK-STAT pathway is required for oogenesis [15]. This pathway may also

Table 2 Examples of well-characterized mouse stem cell niches

Stem cell	Location	Supporting cells	Major signaling pathways	Author and journal
Satellite muscle cells	Under basal lamina on myofiber	Myofiber	WNT; NOTCH; HGF; CXCL 12	Dhawan and Rando, Trends Cell Biol, 2005
Haematopoietic stem cells (HSC_s)	Endosteal, penvascular	Osteoblasts, osteoclasts, mesenchymal progenitors, reticular cells	CXCL 12; SCF; TPO; SHH; ANG1	Adams and Scadden, Nat. Immunol, 2006
Lateral ventricle subventricular zone (SVZ) stem cells	SVZ	Endothelial	SHH; NOTCH; WNT; TGF-alpha; FGF; VEGF	Doetsch, Curr. Opin. Genet. Dev, 2003
Intestinal epithelium	Base of crypt	Fibroblasts	WNT; NOTCH; BMP	Barker et al., Nature, 2007
Hair follicle bulge	Bulge	Vascular	WNT; BMP; TGF-beta	Blanpain and Fuchs, Annu. Rev. Cell. Dev. Biol, 2006
Interfollicular epidermis	Basal layer	Dermis	WNT; NOTCH	Clayton et al., Nature, 2007
Spermatogonial	Basal layer, Seminiferous tubules	Loydig, sertoli cells	BMP4; BMP8b; SCF; FGF; GDNF	Yoshida et al., Science, 2007

function in the maintenance of vertebrate stem cells as well. In fact, STAT3 activation appears to maintain cultured mouse ESCs in an undifferentiated state [16].

Another important signaling pathway integrated by the WNT family members has been proposed to be crucial in regulating SSCs in the skin epithelium [17], the fruit fly *Drosophila* ovary [18], the mammalian intestinal crypt [19] as well as in the hematopoietic system [20]. Therefore, WNT, TGF-beta and STAT pathways, seem to be important niche crossroads critical in regulating the balance between self-renewal versus differentiation.

Cancer Stem Cells & Their Niches

The "embryonal rest" theory of cancer was initially proposed by Rudolf Virchow [21] and further extended by Cohnheim and Durante [22]. This theory proposed that cancer arises from dormant embryonic-like cells that maintain the potential to become cancerous. This theory is similar to the current cancer stem cell theory, which indicates that cancer cells arise from a subpopulation of stem cells that present typical cancer hallmarks, such as acquisition of oncogenes (Fig. 1) and chromosomal instability, as well as maintaining the capacity to initiate and support tumor growth [23, 24]. In 1994, John Dick and colleagues published a pioneering paper in which it is shown that human acute myeloid leukemia is hierarchically organized originating from primitive haematopoietic cells, thus supporting the cancer stem cell theory [25].

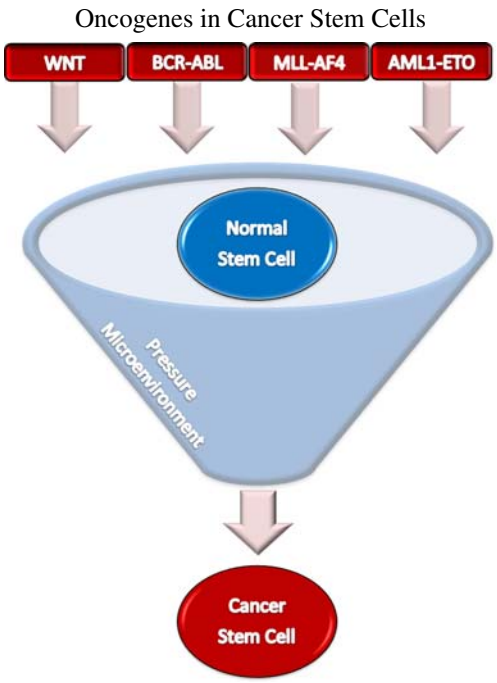

Fig. 1 Oncogenic hits in cancer stem cells. Multiple oncogenes have been demonstrated to induce cancer stem cells from normal stem cells including the fusion proteins BCR-ABL, MLL-AF4 and AML1-ETO, as well as components of the WNT signaling pathway such as beta-catenin

It has been demonstrated that there is a small subpopulation of cells in the tumor that are responsible for generating long-lasting and unlimited dividing tumor cells termed cancer stem cells (CSCs) [20]. By consensus definition, a cancer stem cell is a cell within the tumor that possesses the capacity to self-renew and to produce the heterogeneous lineages of cancer cells that comprise the tumor [26]. Moreover, CSCs also present common characteristics and properties such as 1) CSCs are a subset of cancer cells within each tumor that show tumorigenic capacity when transplanted into immuno-deficient mice; 2) each CSC type is characterized by a distinctive profile of surface markers (such as CD133, CD44, Sca1 or Thy1 [26]) as well as non-surface markers (such as aldehyde dehydrogenase activity [27]), that can be differentially and reproducibly used for isolation of CSCs; 3) tumors grown from CSCs contain mixed populations of both tumorigenic and non-tumorigenic cancer cells, thus recreating the full phenotypic heterogeneity of the parent tumor.

Cancer stem cells have been described in leukemia [28], brain tumors [29], breast cancer [30], colon cancer [31] or, even more recently, in ovarian cancer [32] (Table 3). Recent studies by Guo et al. used a mouse model in which deletion of the *Pten* tumor suppressor gene in hematopoietic stem cells resulted in a myeloproliferative disorder follow by acute T-lymphoblastic leukemia [33]. By using this model, it was demonstrated that by limiting dilution transplantation a rare population of leukemia stem cells (LSCs) was responsible for the leukemia development. In solid breast cancer tumors, it was also demonstrated that a rare population of $CD44^{+}/CD24^{-}/lineage^{-}$ was responsible for cancer after transplantation into immunocompromised non-obese diabetic-severe combined immunodeficient (NOD-SCID) mice [30]. In the case of solid brain tumors, a tumor-initiating cell population was purified by FACS analysis using the cell surface marker CD133 [34]. Other examples come from the analysis of transplantation of p53 null cells and MMTV-WNT1 transgenic cells, after limiting dilution transplantation of these cells a rare population of CSCs was found to be responsible for tumor cell growth [35, 36]. Alternatively, CSCs may arise from more differentiated progenitor cells found in certain tissues. Recent studies in leukemia have shown that expression of MOZ-TIF2 oncogene in committed hematopoietic progenitors leads to the reactivation of stem cell properties, showing thus the first evidence that stem cell properties can be acquired in differentiated committed hematopoietic progenitors to induce leukemia [37].

Table 3 Cell surface markers associated with cancer stem cells

Tumor type	Cell surface markers	Author and journal
Acute myeloid leukemia	CD34+; CD38-	Bonnel et al., Nat. Med, 2003
Breast cancer	CD44+; CD24-; CD326+	Al-Hajj et al., Proc. Natl. Acad. Sci. USA, 2003
Brain tumor	CD133+	Singh et al., Cancer Res., 2003; Taylor et al., Cancer Cell, 2005
Colon cancer	CD133+	O'Brien et al., Nature, 2007
Colorectal cancer	CD326+; CD44+; CD166+	Dalerba et al., Proc. Natl. Acad. Sci, USA, 2007
Head and neck cancer	CD44+	Prince et al., Proc. Natl. Acad. Sci. USA, 2007
Hepatocellular carcinoma cells	CD133+	Suetsugu et al., Bioch. and Biophys. Res. Commun., 2006
Lung adenocarcinoma	SCA1+; CD45-; CD31+; CD34+	Kim et al., Cell, 2005
Metastatic melanoma	CD20+	Fang et al., Cancer Res., 2005
Pancreatic cancer	CD24+; CD44+; CD326+	Li et al., Cancer Res., 2007
Prostate cancer	CD133+	Collins et al., Cancer Res., 2005
Renal cancer	CD133+	Florek et al., Cell Tissue Res., 2005

The tumor microenvironment is composed of extracellular matrix (ECM) components including laminin and collagen, growth factors such as the vascular endothelial growth factor (VEGF), nutrients such as glucose as well as lower concentrations of oxygen. Together, all these elements supply growth and survival signaling to cancer cells [38]. In the recent years it has been shown that tumorigenic properties are deeply influenced by the surrounding tissue microenvironment at both primary and metastatic sites [39, 40]. Tumor progression is also linked with an extensive remodeling of surrounding tissues to support a microenvironment for cancer cell proliferation, migration and tumor vascularization required for cancer cell growth [41, 42]. Efforts have been conducted to understand the role of proteases, heparanases, and many other enzymes expressed by cancer cells or by surrounding stromal cells that are necessary for the degradation of extracellular matrix components thus allowing the release of cytokines and growth factors which induce angiogenesis, or just support the growth of cancer cells [43].

Over the past several years, it has become clear that the initiation and growth of at least some cancers is driven by CSCs, specifically malignant cancer cells that are more tumorogenic than others [44]. It implies that many cancer cells are organized hierarchically with rare stem cells at the top of the hierarchy that self-renew to form more cancer stem cells, which present phenotypic and functional characteristics similar to normal stem cells [45, 46]. The concept that a specific subpopulation of tumor cells possesses distinct stem cell properties implies that CSCs arise as an intrinsic property for tumor biology and development. However, the surrounding microenvironment (stromal fibroblasts, adipocytes, endothelial cells as well as the extracellular matrix) and the immune system are known to play important roles in cancer progression [47, 48]. Therefore, one caveat to the current cancer stem cell theory, which is based on transplantation of human cancer stem cells into mouse models of xenografts (immunodeficient mice) is the lack of an appropiate microenvironment due to the differences between the mouse and the human, and the lack of a complete immune system which has been shown to play a role in tumor progression. Thus, when evaluating the tumor-initiating capacity of the human cancer cells is possible that the subpopulation that exhibited non-tumorgenic properties might actually be tumorgenic in the presence of the appropiated human microenvironment or immune system, and therefore tumor cells might be functionally homogeneous (stochastic model), instead of tumor cells organized as hierarchy (hierarchical model), in which CSCs are placed at the top of the pyramid [49]. This raises the question of whether cancer stem cells depend upon a specialized microenvironment for their maintenance, just like any other stem cells.

Recent studies have served in illuminating this issue. A vascular niche (defined as a local microenvironment enriched in blood vessels) is necessary for the growth of neural stem cells (NSCs). These niches, which are formed by endothelial cells, are thought to block NSCs from apoptosis by maintaining a proper balance between self-renewal and differentiation [50]. In support of this notion, the tumor growth of transplanted NSCs into immunodeficient mice was accelerated when NSCs were injected together with endothelial cells [24]. This evidence indicates that cells surrounding and infiltrating tumors typically secrete factors that promote the growth and progression of cancer cells [51].

A significant example comes from the haematopoietic system where myeloproliferative disease can arise as a result of mutations that mainly affect the bone marrow microenvironment, but not the hyperproliferative haematopoietic cells themselves [52, 53]. Interestingly, the relationship between CSCs and their microenvironment may be

bi-directional since CSCs may also contribute to the maintenance of their niche. In this regard, it has been demonstrated that stem cell-like glioma cells (SCLGCs) induced the secretion of high levels of vascular endothelial growth factor (VEGF) potentially affecting surrounding endothelial cells and inducing neovascularization [54] (Fig. 2). Moreover, it has been shown that bone marrow-derived haematopoietic progenitor cells that express vascular endothelial growth factor receptor 1 (VEGFR1; also known as Flt1) are mobilized by fibronectin, which is induced by primary tumors, to organ-specific pre-metastatic sites forming thus cellular clusters before the arrival of $CXCR4^+$ metastatic tumor cells. Elimination of $VEGFR1^+$ cells from bone marrow using anti-VEGFR1 antibodies abrogates the formation of the pre-metastatic clusters and abolished tumor metastasis [55]. These together results raise the therapeutical possibility of targeting the micro-environments in which cancer stem cells reside as an alternative to conventional treatment.

Embryonic Stem Cell Microenvironments & Cancer Stem Cells

Embryonic stem cells (ESCs) have the ability to divide and self-renew while maintaining an undifferentiated state as well as the potential to generate differentiated daughter cells [56]. Unlike CSCs, they present activation of specific signaling pathways that allow a controlled growth and regulation of their daughter cells [57]. ESCs under the regulation of the STAT-3, TGF-beta and SMAD signaling cascades express the proper genetic repertoire [58], leading to the establishment of an adequate epigenetic status [59], which is often missing in CSCs [60]. The gene-expression program of embryonic stem cells is the result of specific transcription factors, chromatin-modifying enzymes, regulatory RNA molecules and signal-transduction pathways that are responsible of the pluripotent state. Genetic studies have illustrated that the transcription factors Oct4, Nanog and Sox2 are essential regulators of early development and ESC identity [61], and occupy actively transcribed genes necessary to maintain the stemness such as *Hesx1*, *Zic3* and *Stat3* [61]. In addition, the three factors (Oct4, Nanog and Sox2) also occupy silent genes responsible of cell differentiation such as *Pax6*, *Meis1* and *HoxB1* (ectoderm); *Dlx5* and *Hand1* (mesoderm), as well as *Atbf1* (endoderm) [61]. Most of the trascriptionally silent developmental regulators targeted by Oct4, Sox2, and Nanog are also occupied by the Polycomb group (PcG) proteins, which are epigenetic regulators that falicitate pluripotency through gene silencing [61]. The PcG proteins form multiple polycomb repressive complexes (PRCs), PRC2 induces histone H3 lysine-27 (H3K27) methylation resulting in chromatin condensation and repression [61].

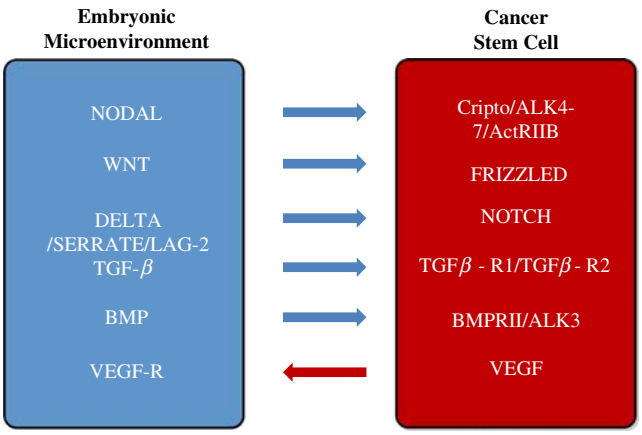

Fig. 2 Bi-directional comunication between cancer stem cells and embryonic microenvironments. Cells derived from embryonic microenvironments secrete multiple soluble factors that induce changes in the cell fate of cancer stem cells. Several molecular axes (defined as ligand-receptor) have been described between embryonic microenvironments and cancer stem cells including NODAL-CRIPTO; WNT-FRIZZLED; DELTA-NOTCH; TGF β–TGFβ-R1 and BMP-BMPRII. In addition, cancer stem cells secrete VEGF that induces neovascularization required for the growth of cancer stem cells. This dual effect between the embryonic microenvironment and cancer stem cells dictate the final outcome of cancer stem cells

Despite the most obvious differences between CSCs and ESCs [62], they share many signaling pathways and transcription factors in common leading to a crosstalk between CSCs and ESCs. Based on the indicated similarities, the concept of differentiation therapy infers that cancer is a problem of developmental biology and embryology. In fact, an unusual intersection between tumorogenesis and embryogenesis was recently revealed in human cleavage-stage embryos, in which a high frequency of chromosome instability involving complex patterns of segmental chromosomal imbalances has been detected, only comparable to human cancers [63]. These results also indicate that a putative self-correction molecular mechanism takes place in early embryos that accounts for normal development in adult organisms.

Along these lines, one initial hypothesis was that the embryo itself (as a structural stem cell system) might be strong enough for inhibiting the proliferation (cytostasis) of cancer stem cells, thus possessing a cancer-correction mechanism. Initial studies were performed by injecting several types of tumor cells including carcinomas, sarcomas, leukemia, neuroblastoma or even york sac carcinoma into mouse blastocyst, which resulted in an in vivo inhibition of tumor growth [64]. It has been proven that this cancer inhibitory effect is due to the proteins contained in the blastocyst. In fact, in a different species, one study revealed that zebrafish-embryo protein extracts induced

programmed cell death and growth inhibition in colorectal cancer cells [65].

The inhibitory effect of cancer proliferation by the blastocyst "niche" is not only achieved by an activation of programmed cell death programs, but also by epigenetic reprogramming of tumor cells as demonstrated when using human embryonic stem cell microenvironments [66]. Based on these results, it was postulated that a tumor phenotype might arise as a result of the absence of the proper repressive signals that are present in human ESCs cultures [67]. This last statement has been successfully demonstrated by the injection of human leukemia cells into day 3.5 mouse embryos that were transferred to the animal thereafter. These experiments illustrated that the mice born which were generated after injection were chimeras of normal mouse and human cells in all the tissues, with no generation of cancer [68]. These intriguing results suggest that human cancer cells not only lose their malignant fate, but are also reprogrammed towards pluripotent cells which conserve the ability to differentiate into all the germ layers in the embryo.

Normal stem cell (SC) and CSCs are also under the influence of bi-directional communications with their respective microenvironment raising the question of how stem cells might impact on tumor cell development and what is the contribution of stem cell microenvironments in tumor progression. One of the first studies in addressing these questions demonstrates the suppression of teratocarcinoma cell development using the blastocyst microenvironment [69]. The ability of embryonic microenvironment to negatively impact tumor progression was further tested on various cancer cell lines including melanoma cells [70]. Further studies have showed the inability of Rous sarcoma virus to induce sarcomas in avian embryos [71], suggesting the presence of anti-tumor factors in embryos (Table 4).

Therefore, an extraordinary relationship must exist between SC and their microenvironment that has a critical role in determining cancer cell fate. This notion is supported by the observation that mouse ESCs do not generate cancers in the chimeras formed, although iPS (generated from mouse fibroblasts by retroviral introduction of Oct-3/4, Sox-2, c-Myc and Klf-4) do form cancer in 20% of the offspring obtained through chimera formation due to the retroviral introduction of c-Myc [72]. A number of studies aim to identify the epigenetic role of the stem cell microenvironment over cancer cells involving interaction among cells and secreting factors [73]. In this regard, several groups have studied the capability of embryonic stem cell microenvironments to reprogram the phenotype of tumor cells [73]. Specifically, the exposure of the melanoma cells to the 3D matrices preconditioned by hESC induced melanoma cells to form spheroids similar to embryoid bodies. In addition, the expression of melanocyte-specific markers is dramatically reduced in aggressive melanoma tumor cells, for example; the expression of pigmentation pathways-related genes, such as melan-A (MLANA) and tyrosinase (which catalyses the conversion of tyrosine to the pigment melanin), are reduced about 20-fold and 35-fold, respectively, in aggressive melanoma after exposure with embryonic stem cell microenvironment [73]. The ability of highly aggressive tumor cells to undergo cellular plasticity defines thus their multipotent potential.

Moreover, a significant reduction in the invasive ability of melanoma cells has been shown after exposure to hESC microenvironment, thus indicating anti-tumor factors associated with the hESC microenvironment. In conclusion, these results suggest that hESCs are a promising source of cytostatic approaches, as well as an important tool for the molecular analysis of the signaling pathways that allow cancer cell reprogramming.

Future Directions

The unique ability of stem cells to replenish themselves through self-renewal and their potential to differentiate into different types of mature cells play essential roles in organogenesis during embryonic development and tissue regeneration. The stem cell niche is composed of a group of cell types that provide a microenvironment in a special tissue location for the maintenance and the physical anchor

Table 4 Bi-directional communications between cancer cells and stem cell-dependent microenvironments

Type of embryonic stem cell-dependent microenvironment	Type of cancer cells	Cellular response	Author and journal
Mouse blastocyst	Carcinoma, sarcoma, leukemia, neuroblastoma, york sac carcinoma	Cytostasis	Pierce et. al., Cancer Research, 1982
Avian embryo	Sarcoma	Cancer inhibitory effect	Dolberg et. al., Nature, 1984
Zebrafish embryo	Colorectal cancer cells	Programmed cell death	Cucina et. al., Apoptosis, 2006
Human embryonic stem cells	Melanoma	Epigenetic reprogramming	Hendrix et. al., Nat. Rev. Cancer, 2007

for stem cells through adhesion molecules. In this sense, the niche generates intrinsic factors that control the fate of stem cells. The identification of these signaling pathways from various niches is essential for proper regulation of stem cell self-renewal and lineage commitment.

Studies of the cross-talk between these signaling pathways, as well as the intrinsic factors required for self-renewal and maintenance of stem cells will provide further insight into the molecular mechanisms governing stem cell self-renewal and differentiation. We propose that the niche prevents tumors by controlling stem cells in the arrested state and thus maintaining the balance between self-renewal and differentiation. Therefore any change that leads stem cells to escape from the niche would result in tumor transformation. It is reasonable to hypothesize that one potential difference between normal stem cells and cancer stem cells is that cancer stem cells may not respond to the stem cell niche signaling responsible of the maintaining of the niche homeostasis. This could be cause by oncogenic mutations in stromal cells that would cause loss in niche homeostasis.

We hypothesize that emulating the stem cell niche in vitro will shed more light on the mechanisms that regulate stem cell fate as well as those potential mechanisms of cancer stem cell transformation. Finally, understanding the interaction between stem cells and their naturally nearby cells will substantially benefit therapeutic approaches to human cancer.

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