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The Bed and the Bugs: Interactions between the Tumor Microenvironment and Cancer Stem Cells

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Abstract

Tumors have been increasingly recognized as organs with a complexity that approaches, and may even exceed, that of healthy tissues. When viewed from this perspective, the biology of a tumor can be understood only by studying tumor cell heterogeneity and the microenvironment that is constructed during the course of tumorigenesis and malignant progression. Recent work has revealed the existence of cancer stem cells, the “bugs”, with the capacity for self-renewal and tumor propagation. In addition, it is now recognized that the tumor microenvironment, the “bed”, plays a critical role in supporting cancer stem cells and also may promote neoplasia and malignant progression. The interdependence of the cell-intrinsic features of cancer, including the cancer stem cell “bugs” and the tumor microenvironment “bed”, is only beginning to be understood. In this review, we highlight the rapidly evolving concepts about the interactions between tumor stem cells and their microenvironment, the insights gained from studying their normal tissue counterparts, and the questions and controversies surrounding this area of research, with an emphasis on breast and lung cancer. Finally, we address evidence supporting the notion that eliminating the bed as well as the bugs should lead to more effective and personalized cancer treatments that improve patient outcome.

Keywords

stem cells; cancer stem cells; tumor microenvironment; breast cancer; lung cancer; tumor heterogeneity

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1. Tumor heterogeneity: the complexity of carcinomas and their microenvironment

Tumor heterogeneity has been appreciated since the first descriptions of cancer, most generally referring to the varied organs in which tumors present, and recently focusing on distinguishing features of cells within individual tumors. In addition to histopathological characterization, molecular analyses have revealed that within each organ, several tumor subtypes exist, each one with distinct gene expression characteristics [1, 2]. The tumor “onco-genotype”, which defines the collection of disease-related mutations and evolves over time due to inherent genomic instability, differs among patients so that nearly every tumor cell population is unique, thus adding to the clinical challenges.

Even within clonal tumor populations, it is believed that not all cells are equal in their ability to initiate or propagate disease, processes which, in the clinical sense, may refer to maintaining tumor mass, rebounding following chemotherapy, or establishing new tumors at distant sites. Such capabilities have been ascribed to cancer stem cells (CSCs). Generally stated, the cancer stem cell hypothesis predicts that each tumor contains a population of cells that is able to self-renew and divide asymmetrically, thus differentiating and giving rise to the phenotypically distinct cells found within the tumor population. This hypothesis, coupled with emerging hypotheses that tumor-initiating cells are relatively resistant to standard chemotherapies, has lead to the theory that identification of CSCs in each tumor would enable development of treatments to specifically target the cells responsible for recurrent and metastatic disease [3–5].

The concept of tumor heterogeneity applies not only to the tumor epithelial cells but also to the diverse microenvironment in which tumors arise and with which tumor cells interact. Elements of the tumor microenvironment (TME) have been shown to be instructive to tumor cells, making them more or less tumorigenic [6]. In keeping with our analogy, one model of tumor evolution can be proposed wherein “*the bed (TME) determines the bug fate*”. While this first model is undoubtedly in play during tumor progression, an equally probable and likely coexisting model of tumor evolution is one in which “*the bugs (CSCs) modify or make their own bed*”. Evolving evidence to support these two models in breast and lung cancers is reviewed in detail below.

Despite recent advances, how the microenvironment influences CSCs during tumor evolution is difficult to study because it is not clear which cells act as CSCs in which contexts. In fact, stem cell-like behavior and CSC potential are influenced not only by the methods used to isolate them, but also by the context in which the putative stem cells are assayed. A clear example of the role of the microenvironment was reported from studies of melanoma, in which the same tumor cell populations had 1000-fold different tumor initiation ability depending on the strain of mouse into which cells were injected, whether or not matrigel was used for the transplants, and the duration of the experiment [7]. Therefore, in order to understand how these two models contribute to breast and lung tumor evolution, we first review the current definitions of normal stem cells and normal and cancer stem cell niches.

2. Normal Tissue Stem Cells

Because of the challenges in studying CSCs, especially in the lung, hypotheses about interactions between CSCs and their microenvironment are often extrapolated from the study of normal tissue stem and progenitor cells and their niche.

Normal Breast Stem Cells

The mouse and human mammary glands share similar organization, including branching ducts that emanate from the nipple and end in alveolar bud structures. The basal, or myoepithelial, cells lie against the basement membrane, lining the ducts of the gland and serving as contractile cells to move milk through the ducts. The milk secreting epithelial cells line the lumens of the ducts and alveoli of the gland. During pregnancy, the mammary gland undergoes massive expansion, in part through proliferation of the mammary stem cells, which can establish committed progenitor cell pools as well as differentiate into cells of either the myoepithelial or the luminal lineage [8].

The cell surface phenotypes of stem and progenitor cells in both human and mouse mammary glands have been extensively characterized, partially due to the robust ability of transplanted mammary cells to grow and differentiate into mature ductal and alveolar structures in a cleared mouse mammary fat pad. Although mammary gland stem cells were postulated to exist in 1980 [9], enriching for these cells by cell surface antigens was not preformed until 2006. That year, two separate groups reported that human CD49^{thi}/CD24^{med} cells and mouse CD29^{hi}/CD24⁺ cells prepared from the mammary gland have the capacity to reconstitute mature mammary ductal trees in fat pad transplantation assays in mice [10, 11]. It was subsequently reported in 2007 that the murine CD49^{thi}/CD24^{lo}/Sca-1⁻ population was enriched for mammary stem cells [12]. The apparent discrepancies between stem cell enrichment within the CD24⁺, CD24^{med} and CD24^{lo} populations have been attributed to differences in reagents and staining protocols used to define them [13]. As research progressed to isolate and characterize the committed luminal mammary epithelial progenitors, it became clear that across all studies, although the mammary stem cell enriched population is CD24-positive, the level of expression is clearly lower in the stem cell population than in cells with luminal features, including luminal progenitors.

At present, there is general consensus that mammary stem cells in the mouse are CD49^{f+}/CD29⁺/CD24^{lo}, while those in the human are enriched within the CD49^{f+}/EpCAM^{-/lo} fraction, although this subpopulation also contains a majority of the myoepithelial population [10–12, 14]. Both luminal progenitors and mature luminal cells from mouse and human have also been characterized by growth *in vivo* as well as in matrigel culture and differentiation assays *in vitro* [14–16]. Human mammary stem cells are able to grow as non-adherent sphere cultures when cultured on low attachment plates in the presence of the cytokines FGF and/or EGF; however, in these cultures, the cells lack complete luminal differentiation and instead maintain a more undifferentiated phenotype [17]. These studies reiterate the point that the ability to recognize stem cells and their behavior is influenced by the methods used to study them and the microenvironmental cues that are provided to them.

Normal Lung Stem Cells

As in the breast, the mouse and human lung contain several distinct epithelial cell populations that are stratified by their anatomical position and specialized functions. Similar to the ductal architecture of the mammary gland, airways branch from the trachea out into the alveolar space where gas exchange takes place. Along the airway tract, secretory bronchiolar Clara cells line the basement membrane with more specialized ciliated and goblet cells interspersed. At the termini of the bronchiolar tracts are alveolar spaces that contain the specialized alveolar type II cells, which produce surfactants, and alveolar type I cells, which participate in gas exchange [18].

Stem and progenitor cells in the lung are thought to reside in several distinct locations, including at a basal location in the more proximal airways, near neuroendocrine bodies along the airway, at the bronchioalveolar duct junction, and within the alveolar space itself

[19]. However, it is unclear to which lineages each of these populations can contribute during lung homeostasis or after specific lung injuries. Complicating these studies, until recently, the lung stem cell field lacked a reproducible and rigorous transplant model that would enable one to examine functional properties of isolated cell populations. Within the past year, a renal capsule assay was reported that allows for formation of self-organizing lung epithelium, lending hope that further examination of lung stem cell properties *in vivo* will be possible [20]. Nevertheless, to date, much of the work in the lung field relies on *in vitro* growth assays and *in vivo* endogenous lung injury studies.

In 2005, Sca1⁺/CD34⁺ bronchioalveolar stem cells (BASCs) were isolated from distal mouse lung tissue. BASCs were shown to have extensive *in vitro* colony forming and differentiation abilities, and were characterized as the first cells to proliferate in response to naphthalene injury to the lung [21]. Furthermore, BASCs were shown to be uniquely positioned at the bronchioalveolar duct junction, arguably an ideal location to replace both damaged bronchiolar and alveolar tissue and a known niche for damage-resistant Clara cells [22]. However, soon thereafter, it was shown that the Sca1⁺ fraction of dissociated mouse lung tissue also contains mesenchymal cells, leading to the idea that perhaps the epithelial progenitors fall into a Sca1^{lo} fraction [23].

Further studies reconciled these findings, showing that mouse lung cells enriched by EpCAM⁺/CD49f⁺/CD24^{lo}/Sca1^{lo} markers have low, but not negative, expression of several different lineage markers, and have the potential to grow complex structures in three-dimensional (3D) matrigel cultures, thereby leading to their designation as epithelial progenitor colony forming units (CFUs) [24–26]. In the wake of these studies, it was confirmed that the Sca1^{high} fraction of lung tissue contains mesenchymal progenitor cells, and that these cells are instrumental in supporting growth of the epithelial progenitor CFUs in 3D cultures [25]. It is thought that the mesenchymal cells secrete factors that support epithelial growth, and this theory is supported by the observation that epithelial cultures can be grown in the absence of mesenchymal cell support if high concentrations of FGF10, HGF, or inhibitors of TGFβ are added to the medium [24, 25].

Much less is known about stem cells in the human lung, but one putative population of human lung stem cells is enriched by the marker c-Kit [27]. Similarly, clonogenic p63⁺ populations have been isolated from human nasal passages, proximal lung, and distal lung, and despite the phenotypic homogeneity of these cultures, the three anatomical locations give rise to distinct lineages of airway or alveolar cells in 3D cultures [28]. It was recently shown that these human cells also rely on mesenchymal support, as endothelial cells are critical in supporting human lung epithelial alveolarization, mainly through secretion of cytokines such as EGF [29].

In the proximal airway, the basal epithelial cells of the trachea are critical during the development and repair of injured lung tissue. These cells have been isolated from both mouse and human lung tissue based on their expression of the markers NGFR (nerve growth factor receptor) and CD49f [30]. Like the distal lung epithelial cells, the behavior of these basal cells is mostly studied by using 3D cultures, in which they form tracheospheres, and in proliferation assays after lung injury induced by agents such as SO₂. Recently, p63⁺/CK5⁺ cells were shown to migrate and proliferate in order to repair damaged alveolar lung tissue after H1N1 viral infection *in vivo* [28]. Given that basal cells also express p63 and CK5, it is possible that the migratory cells observed were basal cells, suggesting the possibility that basal cells can act as progenitors for distal lung tissue during certain regenerative processes. The ability of basal cells to form 3D tracheospheres apparently does not require supporting stromal cells; however, the growth medium used for these cultures contains EGF, FGF, and bovine pituitary extract, which may subvert the need for stromal cell components [30].

3. The bugs: Cancer Stem Cells

Since the first description of functional CSCs in acute myeloid leukemia, much research effort has centered on understanding the hierarchical relationships between cells within a tumor [31]. CSCs are identified by unique expression of cell surface markers and their ability to seed tumors in mouse transplant models after enrichment for these markers using cell separation techniques. Isolating cells based on stem cell markers followed by limiting dilution analyses has revealed populations of cells that are enriched for cancer stem cell behavior, particularly for human breast cancer [32–35]. While many other solid tumors appear to have distinct CSC populations, the phenotypic consistency of these cells has yet to be established.

It has been hypothesized that stem and progenitor cells are the only cells that have the lifespan required to accumulate sufficient genetic lesions to produce a tumor [31, 36]. The idea that stem cells are tumor ‘cells of origin’ is attractive because it would follow that CSCs possess inherent self-renewal capacity and do not need to reawaken self-renewal programs during neoplastic transformation. However, an equally plausible explanation for how a tumor cell with self-renewal capacity arises is that a more differentiated cell acquires self-renewal capacity through genetic and/or epigenetic mechanisms.

Currently in debate is whether tumors are more likely to arise from stem or progenitor populations, and if CSCs, when present, maintain phenotypic resemblance to the cell of origin or the normal stem/progenitor cells in the tissue. Experimental data from transgenic animal and human-in-mouse transplant studies suggest that stem and progenitor cells are primary targets for transformation in both the mammary gland and the lung [16, 21, 37, 38]. Likewise, many studies suggest that CSCs can retain stem or progenitor cell phenotypes, including cell surface marker expression [15, 16, 21, 31, 37]. However, recent evidence also shows that in certain contexts, cell populations with limited self-renewal capacity may become more ‘stem-like’ through autocrine (e.g. epigenetic) mechanisms as well as via paracrine interactions with their environment [34, 39, 40].

In humans, the markers $CD44^{+}/CD24^{-/lo}/EpCAM^{lo}$ are frequently used to enrich for tumor initiation among several subtypes of breast tumors [3, 32, 35, 41]. The $CD24^{-/lo}/EpCAM^{lo}$ cancer stem cell phenotype is similar to that of $CD49f^{+}/ESA^{-/lo}$ normal human mammary stem cell, as well as a $CD44^{+}/CD24^{lo}$ CSC population observed in cultured human mammary epithelial cells (HMECs) [14, 39]. Also shared among normal and tumor cell hierarchies, $CD24^{hi}/EpCAM^{hi}$ denotes normal cells committed to the luminal lineage as well as cancer cells that have been molecularly profiled as luminal-like [1, 3, 41]. ALDH1 and CD133 have been proposed as additional CSC markers in both breast and lung, and ALDH1 also appears to mark normal mammary stem cells [41–43]. However, conflicting reports on the utility of CD133 in lung cancer, and indeed other cancers, suggest that CSC markers may be subtype and context specific [44].

In the mouse, it appears that the luminal progenitor marker, CD61, enriches for CSCs in the MMTV-Wnt1 model of mammary carcinogenesis [15]. However, CD61 did not enrich for CSC activity in the MMTV-Neu/erbB2 breast cancer model, and only enriched for CSC activity in some p53-deficient tumors [15]. Similarly, in the Kras/p53 model of lung adenocarcinoma, sorting for the bronchioalveolar stem cell (BASC) marker, Sca1, enriched for tumor propagating potential. However, in lung tumor models that rely only on Kras or EGFR activation, this same sorting strategy did not show consistent CSC enrichment [45]. This research exemplifies the importance of onco-genotype in determining a cancer’s CSC population. It appears that genotype directly influences whether the CSCs retain the cell of origin phenotype as the cancer evolves. It also exemplifies the importance of functionally

validating the tumor initiation capacity of sorted populations in each new tumor type under study.

Despite the fact that human breast CSCs are phenotypically reminiscent of normal breast stem cells, several groups have found that the luminal progenitor population, rather than a multipotent mammary stem cell (i.e., one capable of giving rise to all mammary epithelial lineages), is the likely cell of origin of both luminal and basal-like breast carcinomas [16, 37, 38]. This idea was clearly demonstrated in studies of tumors from patients and mice carrying the BRCA1 mutation. Although BRCA1^{-/-} tumors are classified as a basal-type breast cancer, these studies demonstrated that luminal progenitors gave rise to the tumors. Similarly in lung cancer, it has been hypothesized that a distinct cell of origin population can give rise to either adenocarcinoma or squamous cell carcinoma, the two major subtypes of non-small cell lung cancer [46]. However, if a lesson is to be learned from the BRCA1 story, it is that the resultant tumor may have little in common with its cell of origin. The reality is likely very complicated; the cell of origin along with the genetic, epigenetic, and microenvironmental aspects of the tumor all likely contribute to unique tumor and CSC phenotypes [47].

As with normal stem cells, the panoply of mesenchymal cells, cytokines, and extracellular matrix components available to the tumor is likely to have important influence on CSC phenotypes and activities. Indeed, it is impossible to describe stem cell behavior without bringing the stem cell environment into the conversation.

4. The bed: Components of Tumor Microenvironment

Stem cell niches are discrete and dynamic functional domains that influence stem cell behavior to govern tissue homeostasis under diverse physiological (development and aging) and pathological (injury and disease) conditions. The contemporary idea that specialized environments, or “niches”, within tissues can preserve proliferative potential and block maturation of adult stem cells has existed since the 1970s [48]. As described above for lung and breast tissue, some clues about normal stem cell niches have been garnered from understanding where within a tissue the stem cells reside. For example, highly specialized and precisely localized niches have been identified for intestinal stem cells in the colon crypt, hematopoietic stem cells in the bone marrow, and stem cells in the skin [49]. The niche must be flexible in order to coordinate stem cell behavior with homeostasis and repair; however, the plasticity of a niche may be co-opted by cancers and chronic diseases.

While much has been learned in recent years about mesenchymal cell types and niche-derived factors that modulate CSC properties, it is not clear whether CSCs direct the formation of the tumor microenvironment (TME), create their own “micro-niches” within the TME, or take advantage of the pre-existing tissue environment. Indeed, although involvement of the local microenvironment in tumor initiation or metastasis has been postulated for over 100 years [50], the TME is quite different in composition and function than the normal parenchyma in which a tumor arises. Precisely how various TMEs are formed is still not clear and it is thought that there are many mechanisms by which stromal activation occurs (for review, see [51]). Our understanding about how CSCs interact with their environment starts with a general understanding about the tissue and tumor microenvironments.

The solid tumor microenvironment, in most cases, is composed of a multitude of mesenchymal cell types and extracellular components, including endothelial cells of the blood and lymphatic circulation, pericytes, fibroblasts and myofibroblasts, adipocytes, and a variety of bone marrow-derived cells, including various immune cells and mesenchymal stem cells [52]. Fibroblasts constitute the majority of stromal cells within most tumor

microenvironments [53]. Activated fibroblasts (known as cancer-associated fibroblasts, or CAFs), regulate a variety of pro-tumorigenic processes through their production of growth factors, chemokines, and extracellular matrix proteins [52, 54–57]. CAFs can also secrete plasminogen activators as well as several types of matrix metalloproteinases, which induce direct degradation of the extracellular matrix (ECM). Collectively, these processes are thought to modulate tumor expansion, invasion and angiogenesis, and activation of latent forms of ECM-associated cytokines and growth factors, many of which are commonly associated with self-renewal [58], as we describe later in this review.

Various bone marrow derived cells, such as macrophages, neutrophils, mast cells, myeloid cell-derived suppressor cells, dendritic cells, B-cells, T-cells, and T-regulatory cells, as well as mesenchymal stem cells and adipocytes also have been identified as important components of the TME. Inflammatory cells of the humoral immune system play a prominent role in cancer progression [59]. One of the most studied types of tumor-infiltrating inflammatory cells is the macrophage, or tumor-associated macrophage (TAM) [60]. TAMs have been well documented since the late 1970s and, for the most part, their presence is associated with poor prognosis [61]. TAMs produce several growth factors capable of directly promoting tumor progression [60]. Also among the stromal cell types that have been implicated in tumor promotion are endothelial cells, which comprise the blood and lymphatic circulatory systems, and pericytes, which surround blood vessels to provide contractility. Together, these two cell types form the neovasculature, which supplies tumor cells with nutrients and oxygen [62].

In addition to the local microenvironment that influences tumor progression in a paracrine fashion, mouse models using tumor cell lines and primary tumor specimens from patients have revealed that factors secreted into the bloodstream also influence the construction and composition of the TME. Soluble factors secreted from certain proliferating tumors can render cells in the bone marrow pro-tumorigenic, such that when they are mobilized and recruited to sites where disseminated and indolent tumor cells reside, they induce a desmoplastic stroma and promote malignant outgrowth in a process called systemic instigation [54, 63].

Therefore, both the systemic and local environments have become a focus of much attention. It is clear that the TME provides a source of cytokines and growth factors, some of which are discussed below, that are necessary for tumor growth and progression. Nevertheless, it is currently not clear if all carcinoma cells within a given tumor respond similarly to their TME or if particular subsets of tumor cells are endowed with the ability to respond to the TME. The advent and popularity of the stem cell theory has lead to more recent efforts to identify TME factors that particularly or exclusively modulate CSC behavior, or even generate, *de novo*, CSCs. Likewise, recent attention has been drawn to TME factors that are particularly mandated by the CSC population.

5. Cross-talk between the bed and the bugs

The concepts of tumor heterogeneity and the interdependence of tumor cells and their surrounding microenvironment lend themselves well to the idea that cancer stem cells are “bugs” that cannot live without the “bed”. Whereas normal stroma can delay or prevent tumorigenesis, pathogenic stromal components can promote tumor growth [64]. Demonstration that stromal genetic alterations actually facilitate tumor progression has been difficult in human studies; however, a number of animal models address this point and show that genetic lesions in the stroma can make a positive and even causative contribution to epithelial tumorigenesis, particularly with respect to tumor-initiating phenotypes.

A number of processes are thought to establish a niche that supports tumorigenesis. Such conditions include chronic inflammation, wound healing, and systemic instigation. In the lung, chronic carcinogen exposure, primarily in the form of cigarette smoke, is by far the largest contributing factor to the development of lung cancer [65]. Pulmonary inflammation caused by continuous exposure to cigarette smoke or asbestos can contribute to lung cancer and mesothelioma progression, respectively [66]. In the breast, mammographic density and altered endocrine homeostasis (e.g. changes in hormone levels) are associated with increased risk of developing breast cancer [67]. Chronic inflammation after wounding or exposure to a chemical irritant enhances cell proliferation and activates the microenvironment through mobilization of inflammatory cells [67]. In turn, the signals that epithelial cells receive from the reactive microenvironment can lead to altered gene expression and epigenetic changes that are conducive to tumorigenesis.

However, whether tissue stem cells, differentiated epithelial cells, incipient transformed cells, or all of the above are the targets of microenvironmental cues leading to carcinogenesis is not well understood. Moreover, it is not clear if the target cells of these early events represent the CSCs, as defined in detail above. Understanding how the bed and the bugs first arise thus remains incompletely understood and is the subject of intense research efforts. No matter how they arise, it is clear that within developed tumors, crosstalk between the bed and the bugs occurs. For the purposes of this review, we focus exclusively on heterotypic interactions between CSCs and the TME. However, just as in normal tissue, one aspect of the CSC niche that cannot be overlooked is the differentiated progeny that a bug produces; these progeny could very well provide factors that support their progenitor [68].

Here, we describe tumor biology using two different models, based on the crosstalk between epithelial cancer stem cells and their surrounding mesenchymal tissue (Figure 1). The following two models are not mutually exclusive; indeed it is possible that both cover important aspects of tumor progression.

“The bed determines the bug fate” (Figure 1A)

A number of pathogenic processes and TME-derived soluble factors have been implicated as important regulators of the tumor-initiating phenotype and CSC-like behavior, which includes, by definition, processes of self-renewal and asymmetric division (Table 1). We focus attention on some of these factors in detail below.

Transforming growth factor-beta (TGF- β)

Members of the TGF- β family of cytokines are involved in the maintenance of stem cell phenotypes and are the best characterized inducers of the process known as the epithelial-mesenchymal transition (EMT), which occurs during the course of development, wound healing, fibrosis, and cancer pathogenesis [69, 70]. The EMT has also recently been associated with the CSC phenotype [71] and in maintaining human embryonic stem cell pluripotency [72]. Irradiation of the mammary-gland stromal component has been shown to promote ECM remodeling and activation of latent TGF- β in breast tissue [73]. In contrast, inhibition of stromal-derived TGF- β results in increased colony-forming efficiency of lung progenitor stem cells *in vitro* [24]. Thus, whether TGF- β signaling exerts stem cell suppression or promotion seems to be dependent upon tissue type.

Platelet-derived growth factor (PDGF)

PDGF family proteins signal through transmembrane tyrosine kinase receptors to stimulate various cellular functions, including growth, proliferation, and differentiation. Fibroblasts, endothelial cells, and epithelial cells express PDGFs. TGF- β -mediated induction of PDGF

expression in tumor cells enhances cell migration *in vitro* as well as proliferative capacity during tumor formation *in vivo* [74]. PDGF, secreted from adipose tissue-derived stem cells, has been shown to contribute to EMT and expansion of breast CSCs [75, 76].

Fibroblast Growth Factor (FGF)

FGF is a growth factor that is expressed by reactive stroma and is implicated in tumor promotion through its pro-angiogenic effects [77] as well as effects on stem cell phenotypes. FGF has been shown to induce EMT in tumor cells [78] and to support pluripotency in human embryonic stem cells [79]. In an organotypic epithelial colony-forming assay *in vitro*, together with hepatocyte growth factor, FGF induced self-renewal of adult lung epithelial stem/progenitor cells [25]. In a study of breast cancer, the FGF/FGFR/Tbx3 signaling pathway, regulated by estrogen signaling, expanded a pool of functional breast CSCs [33].

Insulin-like growth factor-1 (IGF-1)

During mammary gland development, expression of IGF-1 and its receptor, IGF1R, are apparent in both the stromal and epithelial compartments [80]. During cancer progression, the TME is considered to be the source of IGF1 [81–83] and high levels of IGF-1 and its activated receptor are associated with poor patient prognosis [84]. IGF induces the EMT program in several epithelial cell lines *in vitro* [85] and has been demonstrated to play an essential role in maintaining human embryonic stem cell pluripotency [79, 86].

Epidermal growth factor (EGF)

EGF is another soluble factor involved in the crosstalk between epithelial cells and the microenvironment. During cancer progression, tumor-associated macrophages are thought to be the main source of EGF [87, 88]. EGF induces the EMT program in several epithelial cell lines *in vitro* [85] and, like FGF, enriches for stem/progenitor cell self-renewal [29]. Its importance in maintaining populations of stem cells is emphasized by the fact that it is a necessary component of most *in vitro* stem cell assays [17].

Wingless/integration-1 (Wnt) ligands

Wnt proteins comprise a family of secreted factors that regulate cell growth, self-renewal, motility, and differentiation during embryonic development [89]. Canonical (β -catenin-dependent) and non-canonical (Wnt5a and Wnt16 mediated) Wnt signaling, in combination with TGF- β 1, induce and maintain mesenchymal-like and stem cell traits via induction of an EMT program in mammary epithelial cells [40]. In lung cancer, Wnt-5a is associated with poor prognosis, especially for patients with squamous cell carcinoma, and overexpression of Wnt-5a in tumor cells is associated with expression of β -catenin in stromal cells, suggesting the importance of a tumor-stromal interactions [90]. Indeed, stabilization of β -catenin leads to expansion of lung stem cells [91]. As Wnt ligands are necessary for maintenance of some stem cells, relieving the repression of Wnt inhibitors, specifically Dickkopf-1 and sFRP1, might also be necessary in order to regulate stem cell behavior [40, 89].

Hypoxia

Hypoxic conditions and oxygen levels have been demonstrated to be instrumental in the maintenance of undifferentiated cell states in various organs. Stem cell niches are often located in anatomical regions characterized by hypoxia, and it is thought that stem cells require low levels of oxygen to minimize damage caused by DNA oxidation [92]. Most solid tumors, in the clinic and in experimental models, are characterized by poorly vascularized regions and many tumor cells flourish under hypoxic conditions. The ability of malignant cells to survive hypoxic conditions is under the control of hypoxia-inducible factors (HIFs),

which regulate expression of a variety of tumor-modulatory factors –Notch, VEGF, cMyc, Wnt, p53 and Oct4 – that are known to be crucial regulators of angiogenesis and stem cell maintenance [93].

“The bugs modify the bed” (Figure 1B)

While a growing body of literature addresses mechanisms by which tumor cells modify their microenvironment, much less is known about how CSC bugs might directly influence the TME bed. CSCs have been shown to trigger a reactive stroma by releasing soluble factors, such as TGF- β [94]. These factors, in addition to their autocrine effects on the CSCs, can also activate CAFs in the surrounding stroma, increasing inflammation and bone marrow derived cell infiltration [70]. Results from a recent study demonstrated that secretion of TGF β 2/TGF β 3 from breast cancer cells that disseminated to the lung served to induce stromal fibroblast expression of periostatin (POSTN), a component of the extracellular matrix. In turn, TME-derived POSTN induced recruitment of Wnt ligands, thereby increasing Wnt signaling in cancer stem cells [95]. Likewise, use of a xenograft lung metastasis model revealed that metastatic breast cancer cells secreted tenascin C to recruit lung stromal sources of Wnt and notch ligands, thereby enriching tumor-initiating phenotypes [96]. However, it is not clear from these studies if the tumor cells that modulated the metastatic environment were CSCs. It was recently demonstrated that skin CSCs secreted VEGF, which operated in an autocrine fashion to expand the CSC pool, and in a paracrine manner to promote angiogenesis within the TME [97]. In this situation, the “bugs” modified the “bed” in order to serve their own purposes (Fig. 1B). Whether other CSC-derived factors modulate the niche and the extent to which this might occur in other tumor types remains to be answered.

6. Burning the bed to kill the bugs: The CSCs and the niche as targets in cancer therapy

It is evident that the heterogeneity of most adenocarcinomas holds important implications for successful cancer therapies. In fact, the interdependence of the bed and the bug, especially incipient bugs that can create a new bed [95], or beds that can support the expansion of new bug colonies [39], suggest that new therapies must be designed to attack both. Such therapies, both theoretical and currently in use, might include the following considerations:

CSC therapy

Increasing evidence from studies of various tumor types suggests that cells with properties of CSCs are resistant to some commonly used chemotherapeutic treatments [98, 99]. The persistence of CSCs might help to explain the almost-inevitable disease recurrence following apparently successful debulking of human solid tumors by radiation and various forms of chemotherapy. Hence, CSCs may represent a double threat in that they are resistant to therapeutic killing and, at the same time, are endowed with the ability to regenerate a tumor once therapy has been halted. CSC resistance to radiotherapy is primarily due to infrequent cycling, lowered proliferation rates, and increased expression of DNA repair and anti-apoptosis genes, as has been observed in CD133⁺ glioblastoma cells [100] and CD44⁺/CD24^{lo} breast cancer cells [101]. CSCs are also thought to contribute directly to chemotherapy resistance. Low proliferation rates and innate mechanisms, such as amplified checkpoint activation and DNA damage repair, can modulate this response [102]. In addition, membrane expression of specific protein pumps, which cause efflux of chemotherapeutic drugs, have been associated with CSC drug resistance [103]. Identification of CSC-associated antigens has led to the development of antibodies designed to target CSCs [104]. However, usefulness of these antibodies as therapeutics is likely

limited, as CSCs are typically present at very low levels and CSC markers overlap with other normal stem cell markers [105]. Hence, identification and validation of CSC antigens for the generation of antibody therapeutics is still in its infancy.

Tumor microenvironment therapy

Another dimension of the CSC concept is beginning to take shape, whereby the niche provides a “druggable target”. Even if therapy were to eliminate a significant number of CSC bugs, a permissive bed that is allowed to remain intact would aid the residual bugs in their quest to re-colonize. For example, some studies indicate that continuous inflammation, as a result of persistent infection or other irritants, has a causal role in tumor progression [106]. If cancer is conceived as a disease of chronic inflammation, then therapies targeting epithelial tumor cells could reduce tumor proliferation and mass; however, the TME and its inflammatory cytokines, such as those we list above, would persist and would putatively allow future proliferation of any residual tumor CSCs. Consistent with this notion, anti-inflammatory drugs or inhibitors that target the crosstalk between the activated microenvironment and tumor cells hold some promise [107, 108].

Something important to consider is the fact that necrotic cell death (after radiotherapy or chemotherapy, for example) releases proinflammatory signals into the surrounding tissue microenvironment, in contrast to apoptosis and autophagy, which do not. As a consequence, necrotic cells can recruit inflammatory cells of the immune system [109]. As we have reported in this review, immune inflammatory cells can directly and indirectly induce proliferation of the remaining viable tumor cells. Consequently, necrotic cell death, while seemingly beneficial in counterbalancing cancer-associated hyper proliferation, may ultimately do more damage than good. In a similar vein, host systemic responses (i.e., release of cytokines and mobilization of pro-tumorigenic bone marrow cells) to residual disease or even chemotherapy itself are likely to threaten therapeutic success if left intact [54, 63, 110].

Surely the current mandate is to discover therapies that target both the CSC and its microenvironmental niche, with the expectation that such combinatorial approaches will improve tolerability of therapy and outcome for cancer patients. Future studies will likely elucidate whether stochastic or hierarchical models of tumor development predominate during various stages of tumor progression. Understanding the relationships of tumor cells to one another and their environment may aid design of treatment therapies that target pathways upon which the most aggressive, tumor promoting cells rely. Other important unanswered questions are to what extent CSCs are the same cells that fuel local recurrence or seed distant metastases, or if there are organ-specific subpopulations of stem-like cells within a primary tumor that are responsible for successful metastatic colonization of a given organ. In order to answer these questions, future studies will need to dissect the microenvironment both at the primary tumor site as well as within the organs to which the tumors are likely to metastasize. Much work remains to clearly define the bed and the bugs, yet continued discoveries of their symbiotic relationship promise new avenues to understanding cancer biology.

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Abbreviations

TME	Tumor microenvironment
CSC	Cancer stem cell
BASC	Bronchioalveolar stem cell
EMT	Epithelial-mesenchymal transition
CAF	Cancer-associated fibroblast
ECM	Extracellular matrix
TAM	Tumor-associated macrophage

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The Bed Modulates the Bugs

The Bugs Modify the Bed

FIGURE 1. Proposed models of cancer progression based on the crosstalk between cancer stem cells “the Bugs” and tumor microenvironment “the Bed”

(A) The “Bed modulates the Bugs” model describes how tumor-initiating cells (CSCs) are supported by the reactive tumor microenvironment (TME), which, in most solid tumors, is marked by hypoxia, inflammation, and stromal desmoplasia. Cells within the reactive TME release growth factors and cytokines that can serve to expand the CSC pool either by promoting self/renewal (looped arrow) or causing a reversion process whereby differentiated, non-stem tumor cells give rise to CSCs (blue arrow). These processes subsequently lead to tumor tissue formation via asymmetric division and proliferation of CSC progeny to form the bulk tumor tissue. (B) The “Bugs modify the Bed” model describes how CSCs communicate with their surrounding environment through the secretion of soluble factors that act to promote a tumor-supportive TME. In particular, CSC-derived TGF- β and VEGF are known to activate cancer-associated fibroblasts and induce vascularization. These models are predominantly derived from studies of breast cancer. As highlighted in this review, some of the cytokines depicted here have been demonstrated to

operate in tissue- or cell-specific manners such that different models may be designed for other tumor types.

Table 1

Tumor microenvironment-derived factors relevant for stem cell fate.

Soluble Factor	Cell Source	Described Role	References
TGF-β	CAF, TAM, Adipocytes	CSCe, EMT, SCp, lung self-renewal inhibition	[24, 40, 60, 69–72, 111, 112]
FGF	CAF, TAM, PSMC	CSCe, EMT, SCp, self-renewal	[17, 29, 33, 60, 77–79, 111, 113]
PDGF	CAF, TAM	CSCe, EMT	[60, 75, 76, 111]
IGF-1	CAF, Alveolar Macrophages, BMDs, Adipocytes	EMT, SCp	[79, 81, 83, 85, 86, 111, 112]
EGF	CAF, TAM	EMT, self-renewal	[17, 29, 60, 85, 87, 111]
WNT	CAF	EMT, self-renewal	[40, 111, 113, 114]
LIF	CAF	Self-renewal, SCp	[111, 115]
IL6	CAF, Adipocytes	EMT, CSCe	[111, 112, 114, 116]
Hh	Bone Marrow Cells	Self-renewal, SCp, CSCe, EMT	[114, 117]

Transforming growth factor-beta (TGF- β); Fibroblast growth factor (FGF); Platelet-derived growth factor (PDGF); Insulin-like growth factor-1 (IGF-1); Epidermal growth factor (EGF); Leukemia inhibitory factor (LIF); Interleukin 6 (IL6); Hedgehog (Hh); Cancer-associated fibroblast (CAF); Tumor-associated macrophage (TAM); Parabronchial smooth muscle cells (PSMC); Bone marrow derived cells (BMDs); Epithelial-mesenchymal transition (EMT); Expansion of cancer stem cells (CSCe); Maintenance of stem cell pluripotency (SCp); Stem cell self-renewal (Self-renewal)