

Cancer stem cells and niche microenvironments

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1. ABSTRACT

The isolation and characterization of cancer stem cells as the cells that initiate cancer has lead to a paradigm shift in our approaches toward cancer management. According to this new concept, only a small percentage of cells, termed cancer stem cells (CSCs), drive tumor formation and progression and give rise to the heterogeneity of tumor cells. It has been a decade since the cancer cell was proclaimed to be “self-sufficient in growth signals”. However, recent researches suggest that even the CSCs rely heavily on the ancillary cells present in the tumor stroma for their persistence in the quiescent state. In this review we will discuss a complex integrated ongoing process in the tumor microenvironment which enables the CSCs to maintain their undifferentiated yet plastic state.

2. INTRODUCTION

Historically cancer has been perceived to be a disease of cells that undergo uncontrolled proliferation. Cancer has been maligned with the dubious distinction of being malignant because of the inexorable rate of expansion of these malignant cells that invade the surrounding tissues and distant organs. A range of therapies have been designed that target these rapidly dividing cells and hope to achieve a cure from this deadly disease. However the last two decades of research have revealed that it would be naïve to consider cancer as a disease caused by a group of crazy cells. It is becoming increasingly clear that cancer cells are more resistant to cytotoxic agents *in vivo* than they are on a petri dish (1,2). This incriminates extrinsic factors to play a role modulating

the response of cancer cells' response to such agents. This has coaxed cancer biologists the world over to embark onto the field of the tumor micro-environment.

For decades it was accepted that metastasis, the earliest established hallmark of cancer, is a late event in the progression of cancer, wherein the cells break off from the primary tumor only after they attain a critical size and spread to distant organs. However this fails to explain the propensity of prostate cancer cells to selectively invade bones or breast cancer cells to migrate to lymph nodes. Instead this would point to the microenvironment in these two sites being conducive for organ specific malignant cells. It is becoming increasingly clear that the tumor microenvironment provides the necessary endothelial precursor cells required to form and maintain vascularity of a tumor thereby sustaining it. Tumors have been documented to be infiltrated with macrophages, neutrophils and other immune cells that secrete a host of growth factors and anti apoptotic factors on which the tumor cells survive. In fact, such is the inflammatory nature of cancer that some scientists have described it as a wound that fails to heal (3). Such has been the pace at which the role of the microenvironment in tumor propagation has been unearthed in the last ten years or so that even the much accepted axiom of self-sufficiency of growth signals in tumor cells is close to being jeopardized.

The development of tissue culture techniques in the early 70s provided cancer researchers with the opportunity to work with malignant cells on a petri dish and characterize these cells in detail. Much has been unraveled as to how these cells behave in response to gene manipulations and external stimuli. However as we learn more about these cells we have realized that as long as we do not have a working knowledge as to how these cells manipulate the multitude of innocent bystander cells into augmenting the malignant program, cure from cancer will mostly remain elusive. In fact, the radicals among the cancer research community clamor for cancer to be considered as a multi-system disease much like an autoimmune disease wherein the malignant cells cry havoc and let slip the dogs of inflammation.

3. THE CANCER STEM CELL THEORY

The origin of cancer has been much debated. Clonal evolution remained the most widely accepted model explaining the origin of cancer. This model theorizes that cancers arise from a single clone of cells that accumulate mutations over a period of time leading to the disruption of their normal cell cycle, culminating in cancer. Consequently one would expect that clonal selection would lead to the evolution of a pool of cells in which every cell is capable of robust proliferation and therefore eradication of this entire pool of cells should provide permanent remission from cancer. On the contrary it is often noted in clinical practice that achievement of clinical remission by chemoradiotherapy does not necessarily warrant complete recovery from cancer. In fact patients can relapse with metastatic disease even in the absence of relapse of neoplasm at the primary tumor site. Such observations have

led to a paradigm shift in our understanding of cancer and have led to the evolution of what we now know and accept as the Cancer Stem Cell (CSC) theory.

It has long been observed that tumors are populated by a heterogenic mass of cells that vary in their lineage and differentiation. Interestingly, the heterogeneity in tumor tissues is not only limited among cell types, but also extends to intra-tumor populations (4). There is considerable variety in the tumor cells' degree of differentiation, malignancy and their ability to give rise to tumors when implanted in immuno-compromised mice. Therefore, instead of the "stochastic model", which presumed that all cancer cells within a tumor participate in the generation of new tumors, the current "hierarchical" or "cancer stem cell" theory envisions that tumors originate from a handful subpopulation of stem cell-like cancer cells, that undergo proliferation for a limited number of cycles giving rise to transient amplifying cells, which further subdivide to form various colonies of cells which are in varying stages of differentiation.

The two basic criteria that define stem cells are: self-renewal and pluripotency. This means that stem cells are capable of going through numerous cycles of cell divisions while maintaining their primitive undifferentiated state. However when needed these cells are also capable of giving rise to terminally differentiated specialized cells belonging to any of the three germ layers. Accordingly, the definition of cancer stem cells can be likened to a population of cells within the tumor mass that possess infinite self-renewal potential as well as the ability to give rise to the heterogeneous lineages of cancer cells that comprise the tumor (5). Cancer stem cells can alternatively be termed as "tumor initiating cells" or "tumorigenic cells" based on their capability to initiate tumors when transplanted in immuno-compromised mice. The number of such tumor initiating cells may vary from a meager 1-5% as in the case of colon cancer to a high proportion of 70-100% as in the case of malignant melanoma.

4. CANCER STEM CELLS AND THEIR NICHE

There is considerable diversity in the opinions as to the origins of cancer stem cells. There is one school of thought that pronounces that cancer stem cells are derived from normal adult stem cells through acquisition of genetic alterations while a second theory proposes that CSCs arise from a pool of dysplastic cells by chance mutations which cause them to acquire stem cell like properties. Whatever may be the source of CSCs, there is little doubt that the ability of CSCs to maintain their undifferentiated state is central to the maintenance of their stem like properties. Recent advancements in the field of stem cell research have shown that pluripotent cells are also capable of undergoing trans-differentiation into cell types from other lineages. This would suggest the presence of an extracellular switch that is turned on to direct the stem cells which lineage to differentiate into. The key to understanding the processes that contribute to this may lie not only in the CSCs themselves but also in the environment in which the CSCs are safely nestled, the Stem Cell niche, which support and

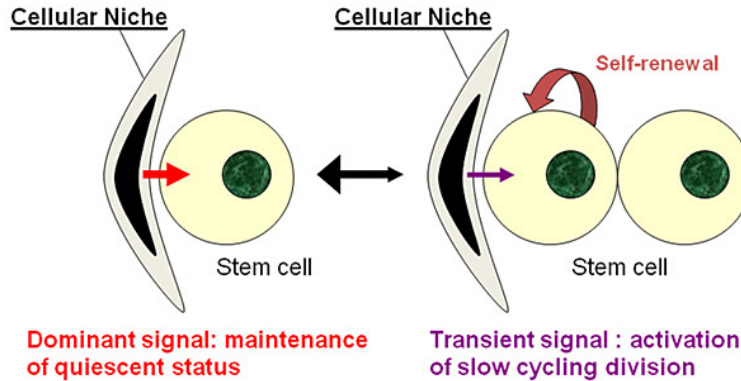


Figure 1. Role of Niche in Cancer Stem Cell regulation. Niche under normal physiological condition is critical to maintain the homeostatic balance of stem cells. The normal microenvironment provides cell-cell contact and secreted factors that keep stem cells in quiescent state dominantly. However, transient signaling for proliferation and subsequent differentiation is also required.

protect the CSCs from stimuli that seek to change their primitive, undifferentiated form. Normally, stem cells remain in a quiescent state through signaling pathways that inhibit cell growth. Regulated cell division, controlled proliferation and proper differentiation of stem cells only occur upon receipt of stimulating signals. According to the cancer stem cell theory carcinogenesis essentially involves all these phenomena but in a dysregulated manner.

There is growing consensus among the research community that cancer can no longer be considered to be a disease of individual neoplastic cells. It is a syndrome complex in which innocent bystander cells play an important role in maintaining the viability of the malignant cells. An accurate understanding of this interplay between the cancer cells and cells of the tumor microenvironment is of paramount importance especially in case of the CSCs. This is because Stem cell function depends on the interaction between intrinsic genetic programs and extrinsic regulatory cues derived from a stem cell's microenvironment or 'niche'. When compared to normal stem cells, cancer stem cells are prodigal in their propensity to undergo deregulated proliferation. This could be attributed to an altered niche in cancerous conditions with abnormally dominant signals that favor proliferation or evasion of apoptosis or a combination of both in tumor cells. Thereby the stem cells with uncontrolled proliferation and improper differentiation are poised to accumulate genetic mutations and promote tumorigenesis. A thorough understanding of the key mediators of this interplay could provide important targets for rational drug therapy.

The concept of the stem cell niche was initially proposed by Schofield in the context of the mammalian blood system (6). Niche cells provide a sheltering environment that protects stem cells from differentiation stimuli, apoptotic stimuli, and other stimuli that would challenge stem cell reserves. A functional niche can maintain the balance of stem cell quiescence and activity. Ever since the existence of the stem cell niche *in vivo* was first confirmed in the *Drosophila* GSC (germ-line stem cells), much progress has been made in identifying stem cell niches in various mammalian tissues, including nerves,

hair follicles (7), intestine (8), teeth, and bone marrow (9,10). Figure 1 summarizes the role of the niche in regulating the actively proliferating and quiescent stages of the cancer stem cells.

5. COMPOSITION OF THE CSC NICHE

The CSC niche essentially consists of cellular elements as well as structural moieties which interplay with each other to maintain the CSCs. In this review we shall consider the important roles played by some of the key players of the CSC niche.

5.1. Cellular elements Of The CSC niche

5.1.1. Macrophages

Macrophages herald the onset of inflammation. These cells serve as Antigen Presenting Cells (APC) that lead to further recruitment of other immune cells. Initially macrophages were considered to act as tumoricidal cells, however recent developments have shown that Tumor Associated Macrophages (TAM) indeed potentiate the perpetuation of inflammation in tumors and thereby help the cancer cells to attain increased malignant potential. Macrophages contribute to a chronic inflammatory setting in the gastric mucosa in response to *H. pylori* infection that leads to epithelial cell activation through NF- κ B (11) as well as Akt and GSK-3 β signaling (12). CD31 and F4/80 positive monocytes directly increase microvascular density thereby sustaining tumor growth (13). They maintain the undifferentiated state of nasopharyngeal cancer cells by increased expression of pluripotency associated genes (14). They have been shown to interact with undifferentiated neural crest-like cells favouring the formation of increased vascular dense neuroblastoma (15). Macrophages have been shown to play pro-oncogenic roles in hematopoietic malignancies as well. Macrophage progenitors have been shown to evolve into acute myelogenous leukemia through activation of the Wnt/ β -catenin pathway (16) and chronic myelogenous leukemia through activation of Hes1 (17) Cancer Stem Cells are masters of immune evasion. The ability of these cells to be regarded as self by APCs is used to some advantage in designing immune vaccines against

CSCs. Vaccines directed against such Neural Crest cells showed considerable promise in reducing CSC population in Glioblastoma Multiforme (18, 19, 20).

5.1.2. Fibroblasts

Fibroblasts are cells that come in to play a major role in the wound healing process. They normally migrate to the site of inflammation and secrete growth factors which help the epithelial cells to proliferate. Fibroblasts also deposit collagen that forms the bulk of the ECM scaffold as well help to remodel the newly formed tissue. They secrete Matrix Metallo-Proteinases (MMP-2 and MMP-14) that help the epithelial cells to degrade the basement membrane (21) and eventually remodel the wound. Fibroblasts comprise the most populous non-malignant cells found inside a tumor (22). These are known as Cancer Associated Fibroblasts (CAF). CAFs are reportedly endowed with increased pro-angiogenic and invasive properties than their normal counterpart (23). There is considerable debate as to whether these specialized fibroblasts arise from mature fibroblasts or from their progenitor cells. Irrespective of their origin, their contribution to the development and progression of cancer is beyond ambiguity. CAFs act as feeder cells that help to maintain the CSC population *in vitro* (24). Fibroblasts secrete growth and differentiating factors that control the stemness of prostate cancer cells (25). There have been reports that basal cell cancers harbour specialized fibroblast cells that secrete Bone Morphogenic proteins (BMPs) that help to maintain the quiescent state of CSCs (26). Fibroblasts can also trans-differentiate into hematopoietic progenitor cells when they are made to express Oct4 ectopically (27). The importance of CAFs in cancer progression can be ascertained from the fact that even other cell lineages differentiate into CAFs when induced by TGF- β signaling originating from malignant cells (28). Prostate CSCs are endowed with increased malignant and invasive potential when they are co-administered with CAFs into immuno-compromised mice (29). Myofibroblasts secrete Hepatocyte Growth Factor which dictates the activity of the Wnt signaling pathway in colon CSCs and determines their clonogenicity (30). Fibroblasts also help CSCs to evade cytotoxic agents. CAFs undergo autophagy when the tumor is under hypoxic stress thereby replenishing the acute shortage of nutrients for the CSCs. This helps the CSCs to acquire chemo and radio resistance (31,32). Fibroblasts also interact with other cells of the tumor microenvironment like macrophages to increase mammary tumor progression (33).

5.1.3. Endothelial cells

Angiogenesis is vital to the cancer cells for their survival and growth. Bone marrow derived endothelial precursor cells play a central role in the formation of the vascular structures at the cancer site. Mesenchymal stem cells have been shown to increase angiogenesis in a mouse model of human lung adenocarcinoma (34). Endothelial cell derived signals promote HNSCC cell survival and self renewal as demonstrated by increased orosphere formation (35). Tie-2 secreted by hematopoietic precursor cells endows breast cancer cells with increased osteolytic and osteoclastogenic potential (36). Disruption of the

perivascular niche by IFN- β decreased the number of CSCs and caused tumor shrinkage in glioma (37). However recent studies have undermined the role of bone marrow derived endothelial precursor cells in cancer progression. Tumor infiltrated vascular cells are capable of giving rise to endothelial cells which then contribute to angiogenesis (38). In the absence of vascular progenitors, glioma cells themselves trans-differentiate into vascular endothelial cells (39,40). Similar vascular mimicry has also been reported in ovarian CSCs (41). It has also been reported by several studies that CSCs themselves secrete several factors which may recruit circulating endothelial cells or other hematopoietic cells to the cancer and these then promote angiogenesis either by themselves or by undergoing trans-differentiation into ECs (42), thus perpetuating a vicious cycle. Such phenomena have been observed in hepatic (43) as well as colonic cancers (44). Figure 2 shows the dominant roles played by the various cellular elements of the CSC niche.

5.2. Structural elements Of The CSC niche

5.2.1. E-cadherin

There is a growing body of evidence that suggests that Cell Adhesion Molecules play a major role in tumorigenesis, cancer progression, tumor cell migration and metastasis (45). Cancer cells have long been known to have anchorage independent growth abilities in their repertoire (46). However it is becoming increasingly clear that the more these cancer cells attain a mesenchymal phenotype wherein they lose their cell-cell adhesion molecules, the more malignant or stem-like they become. Loss of E-cadherin expression is perhaps the most well known marker of the Epithelial to Mesenchymal Transition (EMT) process correlating with increased histological grade of cancer and decreased patient survival (47, 48, 49). Loss of E-cadherin expression is closely correlated to expression of stem cell markers like CD44, CD133 and CD29 and formation of holoclones in HNSCC (50), increased mammosphere formation in breast cancer cell lines (51), expression of Nestin, Bmi-1, Oct3/4 in CD24⁺ ovarian cancer cells (52) and increased expression of CD45 along with increased invasion ability in murine liver cancer cells (53). Suppression of E-cadherin is also correlated with increased potency to vasculogenic mimicry in hepatocellular CSCs (54). In the light of present data it would seem that CSCs have an increasing mesenchymal phenotype than the non-stem cancer cell population. This loss of epithelial character may also in part be aided by other cells present in the tumor microenvironment. It is reported that mesenchymal stem cells help breast cancer cells to lose their E-cadherin expression by activating ADAM10 (55).

6. SIGNALING PATHWAYS IN THE CSC NICHE

Cancer Stem Cells are unique in their ability for self-renewal and maintenance of their primitive undifferentiated state. Several signaling pathways are at work to maintain the CSCs in such an undifferentiated state. Not surprisingly therefore the CSCs are reliant not only on themselves but also on the cross talk between themselves and the ancillary cells. As in the case with the

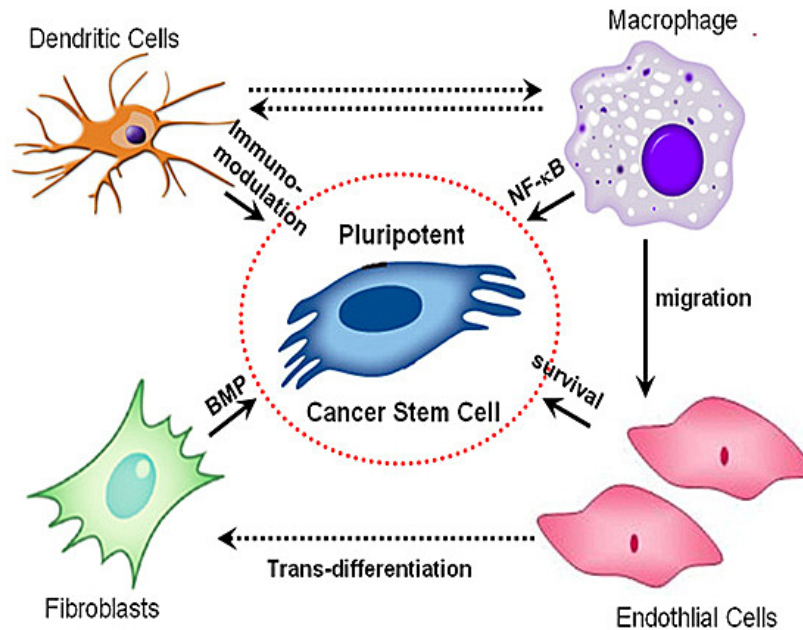


Figure 2. Key cellular elements of the Cancer Stem Cell Niche. Cellular elements maintain CSCs in their pluripotent, undifferentiated state, enhance their survival and evade immune surveillance. There is considerable overlap and cross-talk between these bystander cells.

origin of CSCs, much of these signaling pathways are aimed at explaining either of the two enigmas of CSCs. Signaling pathways that maintain their undifferentiated stem like state and ones that cause their de-differentiation from a pool of transformed cells or their malignant transformation from normal stem cells. Recent advancements tend to show that a culmination of several redundant pathways help the CSCs to maintain their stem cell-like state and this review will try to discuss a few of the major pathways that are implicated.

6.1. Wnt signaling pathway

The Wnt signaling pathways are among the most studied pathways involved in embryogenesis and malignant transformation of cells. In the absence of Wnt protein, β -catenin, which is the downstream effector of the Wnt pathway, is phosphorylated by multiple protein complexes such as Glycogen Synthase Kinase-3 β (GSK3 β), Casein Kinase 1 (CK1), scaffolding proteins APC, Axin1 and Axin2, thus targeting β -catenin for ubiquitination and subsequent proteasomal degradation. Upon ligation with Wnt ligands, the Frizzled and LRP receptors inhibit the inhibitory protein complexes and stabilize β -catenin which then translocates to the nucleus, binds to Tcf/Lef transcription factor family and activates transcription of a broad range of target genes. Alternately, Wnts also signal through tyrosine kinase receptors, particularly the ROR and RYK receptors. The importance of Wnt signaling in stem cell mediated carcinogenesis was first described by studying its role in Embryonal Carcinoma (EC) cells, wherein it was depicted that P19 mouse ECs could be made to differentiate into neuroectodermal cells by the Wnt-1 protein (56), whereas Wnt-6 signaling could differentiate F9 mouse ECs into primitive endoderm (57). Wnt signaling

was found to play a major role in maintenance of stemness in brain tumor derived cell line (58) and in mammary tumors (59) as well as in leukemogenesis (60). However the greatest advances in the field of CSC and Wnt signaling has probably come from studies involving intestinal tumors. Tcf-4 as well as Tcf-3 was reported to maintain the homeostasis in the intestinal crypts wherein they controlled the stemness of the basal crypt cells (61) by controlling the Nanog promoter activity (62). Later it was reported that CD133⁺ IEC are susceptible to undergo malignant transformation due to an aberrant Wnt signaling (63). Similar aberrant Wnt signaling has also been reported in CD133⁺ malignant melanoma cells (64). Recent studies have further described that Bcl9/Bcl9l controls the stemness of the colonic epithelium by modulating the Wnt pathway (65). The myofibroblast and other “normal” cells of the colon are thought to provide the external cues that cause this hyperactivation of aberrant Wnt signaling which seems to define colonic neoplasms (66).

The Wnt signaling has been implicated in the formation of hematological malignancies. Expression of β -catenin in AML cells was clinically correlated with poor prognosis (67), while Lef-1, a transcription mediator of Wnt signaling exhibited CML with lymphoid characteristics with Immunoglobulin G (IgG) DH-JH rearrangements (68). It was also shown that conditional deletion of β -catenin impaired Bcr-Abl induced CML (69). Clinical studies showed that splicing mutations of major β -catenin inhibitor, GSK3 β , were commonplace in stem cells from CML patients in phase of blast crisis (70). Wnt signaling may interact with other signaling pathways culminating in tumorigenesis. MMTV-Wnt signaling and DeltaN89- β -catenin reportedly activate Hedgehog signaling in progenitor cells in mammary tumors (71).

6.2. Notch signaling pathway

The Notch signaling pathway is highly conserved through evolution and it regulates key embryonic development, maintenance of homeostasis in adults, and self-renewal of stem cells in various organs. Hyper active Notch signaling was demonstrated in medulloblastoma and its ablation led to increased apoptosis in these malignant stem cells (72). RTPCR analyses of human tumor samples revealed that human astroglomas expressed increased levels of Notch ligands or receptors or both and this promoted the formation of neural stem-like colonies (73). The role of Notch signaling in cancer has been most studied in the context of breast cancer. Aberrant activation of Notch signaling was found in tissue samples of DCIS (74). This would indicate that this is an early step in breast cancer pathogenesis. Both Wnt and Notch pathways were found to be activated in the stem cell population of MDA-MB-453 breast cancer cells (75). Recent studies have shown that expression of Notch-4 receptor is 8-fold higher and that of Notch-1 is 4-fold lower in breast cancer stem cells compared to differentiated malignant breast cells (76). Notch signaling has also been reported to play a pro-oncogenic role in other forms of cancer such as colon (77), prostate (78) and melanoma (79). Aberrant activation of Notch has been implicated in the development of T-cell leukemia and mammary tumors (80). Notch signaling plays a significant role in chemo-radio resistance. CD24⁺/CD44⁺ breast cancer initiating cells overexpressing Notch-1 were found to be significantly more resistant to radiotherapy (81). Similar conclusions were drawn from radiation studies involving glioma stem cells (82). Inhibition of Notch in these cells resulted in decreased growth of such CD133⁺ glioma stem cell (83).

6.3. Transforming growth factor- β (TGF- β) signaling pathway

The TGF- β is a group of single pass Serine/Threonine Kinase receptors that have long been known to play important roles in embryonic development, tissue regeneration and cell differentiation. As the name suggests, it is responsible for oncogenic transformation of normal epithelial cells into a "transformed" phenotype. TGF- β is unique in that in normal cells it acts to maintain the epithelial nature of cells whereas in transformed cells, it is mostly oncogenic in its activity. This was definitively brought into light by comparing the differential actions of TGF- β signaling in multiple myeloma and normal plasma cells (84). This was further confirmed by comparative studies in normal and CML progenitor cells (85). The earliest reports of TGF- β acting as a cell differentiation pathway was first studied in ECs where multiple groups reported that treatment of NT2/D1 EC with BMP-2 and/or Retinoic Acid induced their differentiation into the neural lineage^(86,87). Several other studies confirmed the role of TGF- β signaling pathway in maintaining the plasticity of embryonal stem cells as well as multipotent tumor cells (88). A xenograft model of breast cancer showed that TGF- β reduces the CSC pool while promoting the differentiation of a more committed, but highly proliferative, progenitor cell population to an intrinsically less proliferative state (89). This ability of

TGF- β signaling to affect the differentiation of pluripotent stem cells into specialized cells has been likened to the EMT process and as such studied in great detail to elucidate the metastatic process. A transgenic mouse model of breast cancer showed that loss of TGF- β increased pulmonary metastasis and enhanced cancer cell survival along with upregulation of a number of inflammatory genes including COX-2, CXCL-1 and CXCL-5 (90). Similar studies in pancreatic cancer revealed that pancreatic CSCs respond to TGF treatment by undergoing the EMT process along with increased metastasis (91). TGF- β also increased the tumorigenic potential of human glioma cells through a TGF- β -Sox4-Sox2 pathway (92). Recent studies indicate that TGF- β also controls the epigenetic silencing of the stem cell marker CD133 through altered methyltransferase activity of DNMT1 and DNMT3 β leading to inhibition of promoter-1 of CD133 (93). Overall the role of TGF- β in cancer stem cell biology is still not fully understood. Though it seems that TGF- β signaling is required for differentiation of multipotent cells, yet the substantial evidence that it can also cause increased tumorigenicity in several tumor types cannot be ignored.

6.4. Nuclear factor-kappa B (NF- κ B) signaling pathway

NF- κ B is a transcription factor which when activated leads to transcription of most notably gene related to inflammation. It plays a major role in immune responses to infection. However deregulated NF- κ B signaling pathway has been implicated in auto-immune diseases as well as cancer. The last decade has seen a renewed interest in treating cancer as an inflammatory disease (94, 95) and this has NF- κ B into the forefront of cancer research (96, 97). Cancer cells have been shown to have constitutive translocation of NF- κ B which thereafter has been shown to increase IL-6 and IL-8 cytokine production leading to immune cell infiltration (98), promote expression of VEGF family of genes causing angiogenesis (99), increase MMP expression causing increased invasiveness of cancer cells (100). It has also been shown to reduce apoptosis by inhibiting Bcl-2 (101) and enhance chemoresistance through the HMGB-1 group of proteins (102). NF- κ B seems to be the single most important signaling factor that orchestrates the tumor microenvironment according to the octets of the cancer cells. Ever since the Cancer Stem Cell theory has caught the imagination of cancer researchers, there has been a growing interest in focusing on the CSCs themselves. However very recently it is being appreciated that inflammation may after all play as big a role in sustenance of CSCs as with the other malignant cells. Canonical NF- κ B signaling was found to play a major role in formation of luminal breast cancer in the progenitor cell population (103) while PDTC, an inhibitor of NF- κ B nuclear translocation was found to preferentially inhibit breast CSC proliferation (104). Similar studies involving prostate CSCs found that inhibition of NF- κ B signaling led to their increased apoptosis (105). Other studies involving ovarian CSCs point that NF- κ B signaling plays a major role in repair following conventional chemotherapies and TNF- α mediated apoptosis in these cells (106). To summarise, it would seem that as of now there are major gaps in our understanding of how the ancillary cells of the tumor

Table 1. Major signaling pathways in the CSC niche

Signaling pathway	Tissue of origin	Effect on CSC biology
Wnt- β -catenin aberrant activity	EC cells	de-differentiation
	breast, brain and colon cancer	maintenance of pluripotency
	colonic crypt	proliferation
Delta-Notch signaling aberrant activity	medulloblastoma	inhibition of apoptosis
	glioma	maintenance of pluripotency
	breast cancer	radio-resistance
TGF- β	breast and colon cancer, leukemia	proliferation
	EC cells	de-differentiation
	breast cancer	differentiation
NF- κ B	pancreatic cancer	metastasis
	ovarian cancer	Inhibition of apoptosis

microenvironment in turn modify the CSCs and lead to their sustenance in the undifferentiated primitive state.

6.5. Other factors controlling CSC niche

The role of Wnt, Notch and TGF- β signaling pathways in maintenance of CSC quiescence and plasticity is well documented. However there is considerable overlap, redundancy and in some cases, antagonism among these pathways. Foremost amongst these is the interplay between the Wnt and Sonic Hedgehog Homolog (Shh) signaling pathway. The Shh pathway maintains the self-renewal potential of Hematopoietic Stem Cells (HSC) and the expansion of myeloid progenitor cells (107). Its abnormal activation is associated with various types of cancers, such as multiple myeloma and prostate cancer (108). However Shh plays a completely distinct role in intestinal epithelial stem cell biology. Wnt pathway is activated in the trans-amplifying region at the crypts of the intestinal epithelium whereas activated Hedgehog signaling can be detected in the well differentiated region of the apex of the villi (109). It is documented that Shh signaling inhibits the canonical Wnt signaling and proliferation in IECs (110). In mesenchymal cells, the hedgehog-dependent Wnt antagonist SFRP1 partially explains the separated activation domains of Shh and Wnt signaling pathways (111). Table 1 lists the signaling pathways thought to play a major role in regulating the stemness properties of cancer stem cells.

Recent studies on transcription factors like Oct4, Sox2, Nanog and Lin28 have shown that these transcription factors (TF) alone can be sufficient to reprogram somatic cells to pluripotent stem cells which can then exhibit the characteristics of Embryonic Stem Cell (ESC), including the expression of normal karyotype, cell surface markers, and telomerase activity as well as the plasticity to differentiate into various cell lineages (112). Oct4 plays a major role in embryonic development without which ESCs cannot undergo self-renewal in vitro (113). Nanog is necessary for maintaining the inner cell mass of the trophoblast as well as self-renewal of ESCs in culture (114). The Sox family genes also play a role in the maintenance of pluripotency in ESCs and embryonic neural stem cells. Overexpression of Sox2 inhibits the differentiation of embryonic neural stem cells and retains them in their primordial state (115). Sox2 is also known to play a role in differentiation in some cell types. It cooperates with Pax6 to activate a battery of genes for early lens development and initiates lens differentiation (112).

7. FUTURE DIRECTIONS

The field of Cancer Stem Cell research in relatively new. However it holds much promise to solving some of the burning issues of cancer treatment like control of metastasis and recurrence. The fact that the origin of this theory stems from a perceived need to explain certain characteristics of cancer cells which cannot be explained otherwise, will continue to keep cancer researchers worldwide keen on this topic. Some forms of cancer like the leukemias, lymphomas and even solid tumors like breast cancer are indeed being considered more as a cancer stem cell disease and a lot of effort is being put in to develop therapies directed at these CSCs. The discovery of transcription factors that can transform somatic cells into a stem like cell is certainly a giant leap in this direction but in the last three decades of cancer research if anything has taught us that to consider cancer to be “stemming” from a group of omnipotent cells would be an oversimplification. As of today it is evident that even the CSCs rely heavily on the other cells present in the tumor stroma for their persistence in the quiescent state. It has been a decade since when the cancer cell was proclaimed to be “self-sufficient in growth signals”. However in the light of recent discoveries, this self-sufficiency is at serious threats. The very fact that CSCs isolated by the presence of surface markers required special medium in order to ensure their undifferentiated state, should lead us to believe that there must be a complex integrated ongoing process at the tumor microenvironment which enables the CSCs to maintain their undifferentiated yet plastic state.

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Key Words: Cancer stem cell, Stem cell niche, Tumor microenvironment, Tumor associated macrophages, Cancer associated fibroblasts, Review

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