Cancer Stem Cells

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Abstract

The cancer stem cell model in solid tumors has evolved significantly from the early paradigm shifting work highlighting parallels between the stem cell hierarchy in hematologic malignancies and solid tumors. Putative stem cells can dedifferentiated, be induced by context, and be the result of accumulated genetic mutations. The simple hypothesis that stem cell therapies will overcome the minority of cells that lead to recurrence has evolved with it. Nevertheless, the body of evidence that this field is clinically relevant in patients and patient care has grown with the complexity of the hypotheses, and numerous clinical strategies to target these cells have been identified. Herein we review this progress and highlight the work still outstanding.

Keywords

Cancer stem cells · Stem cell markers · Stem cell models · Stem cell resistance · Microenvironment

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M. Baumann et al. (eds.), *Molecular Radio-Oncology*, Recent Results in Cancer Research 198, DOI 10.1007/978-3-662-49651-0_2

1 Overview of the Evolution of the Cancer Stem Cell Model

Work over the last decade has highlighted the potential importance of stem cell populations in tumors—cancer stem cells (Clarke et al. 2006; O'Brien et al. 2009). Such cells (CSCs) have been argued to represent the critical population for predicting progression and treatment outcome presuming that their number and treatment sensitivity are important for tumor control by radiation and chemotherapy. The prospective demonstration that only small specific populations of cells, derived from a bulk solid tumor population based on expression of specific surface markers, recreated human tumors in outgrowth experiments propelled the subsequent 10 years of cancer stem cell research. The initial concept was that tumors are organized as steep hierarchies from which only a small percentage of cells are capable of self-renewing and recapitulating the tumor heterogeneity. This concept built on normal stem cell data attributing characteristics of normal stem cells—multipotency, unlimited replication potential, and self-renewal—to proposed cancer stem cells. The cancer stem cell hierarchy was initially viewed as a largely rigid top-down progression from the most primitive cancer stem cells at the top to the most differentiated bulk cells at the bottom. It was presumed that individual cancer stem cells reproduced themselves to maintain self-renewal and as needed produced differentiated daughter cells to maintain homeostasis with a small population of cancer stem cells (Fig. 1a). This model presumed the functional and phenotypic differences of stem cells versus differentiated cells were independent of genetic mutation, mediated instead by epigenetics and differentiation commitment. This stood somewhat at odds with the clonal dynamic, driver mutation model of tumor

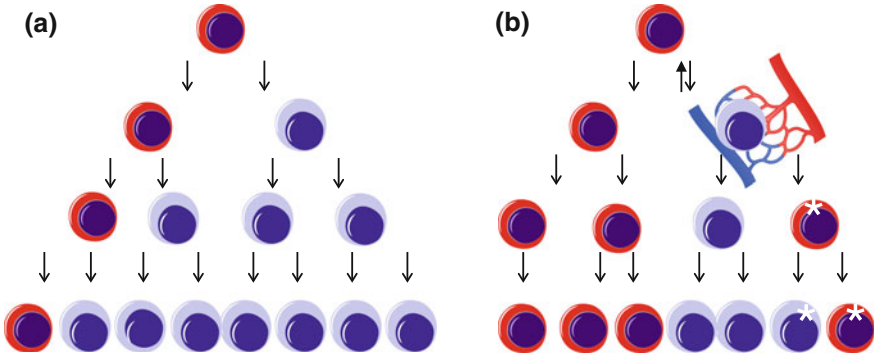

Fig. 1 Increasing complexity in the cancer stem cell model. Drawing from parallels in hematopoietic development, prospective isolation of tumor-initiating cells from solid tumors led to early models of cancer hierarchy similar to the normal state (1A). Primitive, self-renewing cells were presumed to maintain the tumor bulk and the minority population of cancer stem cells. In the last decade has been demonstrated that context and microenvironment can promote tumor initiation, that mutations (*) can confer self-renewing capacity, that some tumors become predominantly composed of self-renewing cells, that markers of self-renewing cells are context dependent, and that functional initiating cells can be both genetically similar or dissimilar (Fig. 1b)

progression and recurrence; the evidence for both was hotly debated. Early on, there were no studies merging genetic analyses with *stemness* studies, and very little consideration was given to the role that context might have in influencing the stem cell population. Over time, however, many new data emerged, challenged the initial paradigm, and were incorporated into this initially simplistic model (Fig. 1b).

After the reports of the first solid tumors to apparently be organized in a cancer stem cell hierarchy (Al-Hajj et al. 2003; Singh et al. 2004), it was demonstrated that the cancer stem cell compartment size and depth of hierarchy depend on tumor type and that self-renewal assays of tumor regrowth in transplants predict for the biology associated with engraftment in animals, which might not faithfully capture the biology of recurrence in situ (Feuring-Buske et al. 2003; Quintana et al. 2008; Notta et al. 2010; Rehe et al. 2013). New markers and strategies to prospectively identify stem cells emerged (Collins et al. 2005; Bao et al. 2006; Dalerba et al. 2007; Ginestier et al. 2007; Hermann et al. 2007; Li et al. 2007; O'Brien et al. 2007; Patrawala et al. 2007; Prince et al. 2007; Ricci-Vitiani et al. 2007; Eramo et al. 2008; Curley et al. 2009; Li et al. 2009a; Piccirillo et al. 2009; Stewart et al. 2011; Wang et al. 2011; Chen et al. 2012; Charafe-Jauffret et al. 2013; Wu et al. 2013; Zhang et al. 2015), and from these, a series of prognostic signatures were derived across tumor types [Table 1 (Gentles et al. 2010; Eppert et al. 2011; Merlos-Suarez et al. 2011; Bartholdy et al. 2014)]. This connection to clinical outcome was reassuring that the cancer stem cell model was relevant. However, the clinical complexity and challenges to incorporation into clinical management were clearly illuminated by findings from patients that the prospectively identified populations that maintain outgrowth potential in tumors may be different in different tumors and patients (Eppert et al. 2011). This further highlighted the need to move to the use of functional demonstrations of stemness rather than the use of markers that are promiscuous, often not linked to function, are potentially transient and depend on context. To this end, the inducible lineage-tracing and re-tracing experiments were developed in genetically engineered mouse models to overcome these issues [reviewed in Roy et al. (2014)], and in some cases, these validated the stem cell model, but they still have some limitations as discussed in more detail below.

Clonal dynamic studies using lineage-tracing approaches in normal tissues demonstrated some common themes across tissues in some cases. In gut and skin, maintaining the frequency of stem cells during homeostasis appeared not as a function of asymmetric division of the primitive stem cell, to create one stem cell and one daughter cell, but rather through maintenance at the population level [reviewed in Blanpain and Simons (2013)]. In other studies, the stem cells identified in lineage-tracing experiments did not align with the prior findings from transplantation experiments, suggesting that transplantation assays may provide circumstances that permit or promote tumor initiation that would not occur in situ. For example, the first lineage-tracing experiments to define cell fate in the developing mammary gland demonstrated that the bipotent differentiation potential of single cells described after transplantation is not identified in situ (Van Keymeulen et al. 2011; van Amerongen 2014). Instead two unipotent basal and luminal stem cells were identified. The same was described in prostate development (Liu et al. 2011;

Table 1 Tumor-initiating cell-related gene signature studies reporting prognostic signatures in independent patient data derived from bulk cells (Glinsky et al. 2005; Phillips et al. 2006; Liu et al. 2007; Shipitsin et al. 2007; Stevenson et al. 2009; Gentles et al. 2010; Eppert et al. 2011; Merlos-Suarez et al. 2011; Becker et al. 2012; Liu et al. 2012; Atkinson et al. 2013; Metzeler et al. 2013; Schwede et al. 2013; Van den Broeck et al. 2013; Peng et al. 2014; Yin et al. 2014; Pfefferle et al. 2015; Yang et al. 2015)

Cancer Type	Signature Source
	Genes identifies in minority stem-like population prognostic in independent tumor samples
Breast, brain, lung prostate	Liu et al. (2007). The prognostic role of a gene signature from tumorigenic breast cancer cells.
Breast	Shipitsin et al. (2007). Molecular definition of breast tumor heterogeneity.
Breast (H2N+)	Liu et al. (2012). Seventeen-gene signature from enriched Her2/Neu mammary tumor-initiating cells predicts clinical outcome for human HER2+: ERalpha- breast cancer.
Breast	Yin et al. (2014). A 41-gene signature derived from breast cancer stem cells as a predictor of survival.
Colon	Merlos-Suarez et al. (2011). The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse.
Pancreas	Van den Broeck et al. (2013). Human pancreatic cancer contains a side population expressing cancer stem cell-associated and prognostic genes.
Leukemia	Gentles et al. (2010). Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia.
Leukemia	Eppert et al. (2011). Stem cell gene expression programs influence clinical outcome in human leukemia.
Leukemia	Metzeler et al. (2013). A stem cell-like gene expression signature associates with inferior outcomes and a distinct microRNA expression profile in adults with primary cytogenetically normal AML.
Leukemia	Yang et al. (2015). Systematic computation with functional gene-sets among leukemic and hematopoietic stem cells reveals a favorable prognostic signature for acute myeloid leukemia.
	Genes extracted based on embyonic or developmental correlation prognostic in independent tumor samples
Breast	Pfefferle et al. (2015). Luminal progenitor and fetal mammary stem cell expression features predict breast tumor response to neoadjuvant chemotherapy.

(continued)

Table 1 (continued)

Cancer Type	Signature Source
Prostate, breast, lung, ovarian, bladder, lymphoma, mesothelioma, brain, and leukemia	Glinsky et al. (2005). Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer.
Lung	Stevenson et al. (2009). Characterizing the clinical relevance of an embryonic stem cell phenotype in lung adenocarcinoma.
Liver	Becker et al. (2012). Genetic signatures shared in embryonic liver development and liver cancer define prognostically relevant subgroups in HCC.
Brain	Phillips et al. (2006). Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis.
Ovary	Schwede et al. (2013). Stem cell-like gene expression in ovarian cancer predicts type II subtype and prognosis.
Prostate	Peng et al. (2014). An expression signature at diagnosis to estimate prostate cancer patients' overall survival.
	Genes identified in minority stem-like population prognostic in independent normal breast samples from patients with tumor
Breast	Atkinson et al. (2013). Cancer stem cell markers are enriched in normal tissue adjacent to triple negative breast cancer and inversely correlated with DNA repair deficiency.

Wang et al. 2014). Later, Rios et al. highlighting the potential caveats of these approaches identified a single bipotent stem cell in the mammary gland (Rios et al. 2014). Speculation regarding contributors to the dramatic differences in the results of these studies includes differences in promoter specificity and/or transcriptional activity due to approaches used, labeling efficiency in different lineages, and confocal imaging variation across the studies [reviewed in Oakes et al. (2014)]. Alternatively or in addition, it may simply reflect the heterogeneous results of a legitimately complex system revealed through differing studies.

Fate mapping was carried out in tumors including benign papilloma and squamous cell carcinoma, glioma, and intestinal adenomas (Chen et al. 2010; Driessens et al. 2012; Schepers et al. 2012). Progression in skin cancer appeared to track with a decrease in the steepness of the hierarchy (Driessens et al. 2012). Through the fate mapping/lineage-tracing experiments as well as with the use of animal models, it was additionally demonstrated that the stem cell frequency could be altered by genetic mutations in the stem cells or background (Vaillant et al. 2008; Curtis et al. 2010; Vermeulen et al. 2013) and that for some normal tissues and many tumors, the pool of stem cells could be replenished, if significantly depleted, via dedifferentiation of a previously non-self-renewing cell (Debeb et al. 2012; van Es et al. 2012;

Buczacki et al. 2013; Schwitalla et al. 2013). The latter challenged the idea that targeting cancer stem cells within bulk tumors would be curative since the remaining more differentiated cells could potentially replace the targeted pool and might be driven to do so by a shift in stem-differentiated cell equilibrium caused by therapy targeting one side of the equation. This demonstration of plasticity led to the concept that stem cells in fact represent a heterogeneous compartment into which cells readily transit and exit becoming temporarily primed for specific stem cell activity [reviewed in Blanpain and Simons (2013)]. It was clear that pressure on the tumor cells such as therapy could shift this equilibrium and that specific signaling pathways could be identified that mediated these transitions.

The most plastic of tumor cells were also presumed to transition between epithelial and mesenchymal states to escape from the primary soil into the circulation and beyond to reseed distant soil (Liu et al. 2014). It further became clear that the microenvironment, including a niche of cells that supported the stem cell state, contributed to maintaining this proposed transient stemness compartment [reviewed in Inman et al. (2015)]. As numerous normal cells were identified as niche conspirators, including macrophages and mesenchymal stem cells, distinct niches for active versus quiescent or dormant stem cells were proposed (Ehninger and Trumpp 2011), and subsequently, the possibility of end organ-specific niches, bone marrow versus lung versus brain, was added to the emerging complex picture.

Alongside the progress made through lineage-tracing experiments, progress in genetic analysis began to converge on the cancer stem cell field. Studies merging these fields led to a direct demonstration that the pressure of therapy to select surviving clones indeed did not in all cases select genetically hardy clones, but rather phenotypically hardy clones, supporting the cancer stem cell hypothesis (Kreso et al. 2013). It was further shown in this work transplanting 150 single cells from 10 colorectal cancer patients that there can be genetic variability within a clone derived from a single stem cell (Kreso et al. 2013). Kreso and Dick proposed a unified model drawing on the genetic and cancer stem cell data and hypothesized that the accumulation of cancer-promoting mutations in the most primitive normal stem cells at the top of the hierarchy led to the most undifferentiated and aggressive cancers, while mutations in more differentiated cells might confer self-renewal and therefore lead to less aggressive cancers (Kreso and Dick 2014). Consistent with this proposal, Tomasetti and Vogelstein report a strong correlation extending over five orders of magnitude between lifetime incidence of multiple cancers and the estimated number of normal stem cell divisions in the corresponding tissues over a lifetime. This suggests that random errors occurring during DNA replication in normal stem cells are a major contributing factor in cancer development (Tomasetti and Vogelstein 2015). Without question, the cancer stem cell model has matured making room for greater complexity.

2 Markers and Models

As above, the solid tumor cancer stem cell field was propelled forward by landmark papers in which tumorigenic breast and brain cancer cells were prospectively identified and distinguished from non-tumorigenic cells in the same cancer using

membrane markers (Al-Hajj et al. 2003; Singh et al. 2004). The readout in these studies was tumor outgrowth in an orthotopic xenograft. This work led to a rapid increase in papers across many tumor types identifying marker sets that prospectively identified the tumorigenic population in human tumors and cell lines using outgrowth in a xenograft as the proof of stemness (Collins et al. 2005; Bao et al. 2006; Dalerba et al. 2007; Ginestier et al. 2007; Hermann et al. 2007; Li et al. 2007; O'Brien et al. 2007; Patrawala et al. 2007; Prince et al. 2007; Ricci-Vitiani et al. 2007; Eramoi et al. 2008; Curley et al. 2009; Li et al. 2009a; Piccirillo et al. 2009; Stewart et al. 2011; Wang et al. 2011; Chen et al. 2012; Charafe-Jauffret et al. 2013; Wu et al. 2013; Zhang et al. 2015) (Table 2). In sum, these studies demonstrated minority tumorigenic populations in multiple tumor types including breast, colon, pancreas, head and neck, sarcoma, lung, ovary, AML, and CML. These studies relied on immunocompromised mice to grow human tumors, and it was quickly recognized that mice with greater immune suppression yielded higher frequencies of tumorigenic cells in AML, ALL, melanoma, and lung cancer (Quintana et al. 2008; Taussig et al. 2008; Chiu et al. 2010; Ishizawa et al. 2010; Notta et al. 2011) raising the question of whether the apparent tumor hierarchy was an artifact of the assay or a clinical reality, although studies supporting the reality were also compelling(O'Brien et al. 2009; Ishizawa et al. 2010). It was further noted that not all murine growth factors cross-react with human receptors and that numerous tissue processing issues may impact the outgrowth in a transplantation assay (Bossen et al. 2006; Rongvaux et al. 2013). One approach to address the variability related to altered immunity was to examine tumors in syngeneic mice with intact immune systems. Consistent with the data from the human tumors, several tumor types examined in these studies supported the cancer stem cell model (Neering et al. 2007; Vaillant et al. 2008; Zhang et al. 2008; Read et al. 2009; Ward et al. 2009). These studies did not necessarily yield markers that are relevant in human cancers however, a difference may relate to the fact that many surface markers do not relate directly to stem cell function.

Following the identification of markers in various solid tumor types, there were numerous studies using these markers in vitro and in translational work to identify genetic signatures from these populations, to identify targets to eradicate them, and

Table 2 Markers reported to prospectively identify tumor initiation from human tumors

Prostate	**CD44+**		
Head and Neck	**CD44+**	**SP**	
Breast	**CD44+ ESA+ CD24-**	**ALDH**	**GD2**
Colon	**CD44+ ESA+**	**CD133+**	**CD166**
Pancreas	**CD44+ ESA+ CD24+**	**CD133+ CXCR4+**	
Glioma	**CD133+***		
Lung	**CD133+**		
Ovary	**CD133+**	**CA125**	

[a]Controversial. Abbreviations: SP, side population; ALDH, aldehyde dehydrogenase activity; ESA, epithelial-specific antigen

Blue and *Green* colors denote their relevance across tumor types. *Bold* represent single markers

to demonstrate their relationship to prognosis. While these were supportive of the model in many cases, it was quickly demonstrated that the markers can depend on context [reviewed in Meacham and Morrison (2013)], that they are promiscuous, and that they are not necessarily related to function. In larger studies of patient samples, it was apparent that in some tumors, the tumorigenic potential may reside in varying minority populations, suggesting that functionally determining which cells were CSCs would need to be a component of individual patient sample analysis (Chiu et al. 2010; Eppert et al. 2011; Sarry et al. 2011). Certainly, it is clear stem cell markers identified and validated in one xenograft model cannot be assumed to identify CSCs in new systems or models where this has not been explicitly demonstrated. Further, it remains to be seen how widely results from cancer stem cell models will apply to the clinic, although various clinical studies have reported that the proportion of cells expressing CSC markers, such as ALDH1 or low proteasome activity, correlates with treatment outcome (Lagadec et al. 2014; Atkinson et al. 2013; Ginestier et al. 2007).

While marker studies furthered the field by identifying cells with tumorigenic potential under permissive circumstances, lineage-tracing studies including proliferation kinetics and clonal dynamics [reviewed in Blanpain and Simons (2013)] have allowed more direct examination of the clonal dynamics of the stem cells under more relevant contextual circumstances. Three techniques have been used to study proliferation kinetics in population-based assays. These are pulse-chase, continuous labeling, and label dilution experiments. These can be applied in vivo by targeting inducible reporter constructs with lineage-restricted promoters to a small number of cells and examining the distribution of labeled cells after elapsed time for the organ of interest to develop. Quantitative analysis is performed to assess the clonal dynamics based on the fixed tissue analysis. These approaches cannot definitively distinguish between population balance that is perfectly maintained through either asymmetric division of a single stem cell that results in a stem cell and a differentiated cell versus division of a stem cell into two stem cells. Importantly, although they have been used to demonstrate differences in multipotency among stem cells in their native context, further work to resolve the fate of individual cells is needed to determine whether lineage is specified early (bestowed on only a few cells early on) or instead involves a competition between equipotent precursors.

In the gut, lineage tracing identified two stem cell pools, one LGR5-expressing pool and a second BMI-1-expressing pool. It was further shown that on ablation of the LGR5 pool, the BMI-1 expressing stem cells can repopulate the crypt (Barker et al. 2007; Sangiorgi and Capecchi 2008; Barker et al. 2012). What is not clear, however, is whether these populations are mutually exclusive. Indeed recent studies have raised the possibility that this work may have targeted the same pool using different promoters (Itzkovitz et al. 2012; Munoz et al. 2012; Buczacki et al. 2013), and Blanpain et al. speculate that the stem cell pool may express all of the identified markers at different times specified by different contexts (Blanpain and Simons 2013). Quantitative studies using these models demonstrated that the number of label-retaining cells was maintained over time by increase in the size of remaining clones as the total number of surviving clones diminished and largely ruled out the

likelihood that ingrained hierarchy accounts for self-renewal, demonstrating instead that neutral competition for limited access to the niche dominates this process (Lopez-Garcia et al. 2010; Snippert et al. 2010b). Similar to the findings in the gut, lineage tracing in the skin also revealed that clones are lost over time and that the constant label-retaining pool is accounted for at the population level by proliferation of the remaining pool (Clayton et al. 2007; Doupe et al. 2010). Quantitative studies here suggested that the tissue was maintained by a single progenitor population, which divided asymmetrically most of the time, but may also divide symmetrically or terminally differentiate to maintain balance. Studies of response after injury mentioned below in aggregate support the model in which the pool is maintained by progenitors and a slower cycling stem cell pool sit ready in response to injury (Ito et al. 2005; Levy et al. 2007; Jaks et al. 2008; Snippert et al. 2010a). The possibility that these progenitors revert into the slow-cycling stem cell pool as described in esophagus (Doupe et al. 2012) cannot easily be ascertained or ruled out. It was reported that location within the niche predetermines the likelihood of a given cell to remain uncommitted or to differentiate, but that committed cells can replenish the stem cell pool after depletion (Rompolas et al. 2013). Using genetic lineage-tracing strategies, similar dedifferentiation behavior as that described in the skin has been reported for the Delta-like 1-expressing cells in the mouse intestine where lineage tracing demonstrates these normally committed, differentiated cells can be recruited into the stem cell compartment if needed upon injury (van Es et al. 2012). Similarly committed Paneth cells can apparently repopulate the stem cell compartment when needed(Buczacki et al. 2013). This important role of position and context has also been demonstrated to regulate the proliferation or quiescence of cancer stem cells (Bissell and Inman 2008).

Fate mapping in tumors was similarly informative. Expression of a conditional reporter in a small population of benign papilloma cells confirmed a hierarchical organization, which became more shallow on progression to squamous cell carcinoma (Driessens et al. 2012). In intestinal adenomas, the previously identified stem cell marker Lgr5+ was tracked through the development of benign lesions using a multicolor lineage reporter. The marked normal stem cells gave rise to the adenomas, and these cells in the adenoma contributed extensively to the tumor growth. The preponderance of Lgr− progeny led to the speculation that the Lgr+ cells gave rise to largely non-proliferative Lgr− cells. Reflecting what is likely a clinical reality, similar studies of intestinal adenomas in different context yield a dissimilar story. Upon Wnt pathway activation, Vermeulen et al. found the Lgr− cells could contribute to the adenoma formation and Lgr− cells gave rise to Lgr+ cells (Vermeulen et al. 2013). It has not yet been established what fraction of adenomas have a hierarchical organization, and how it relates to progression to invasive cancer has not been studied. In glioma studies, the presumptive Nestin+ stem cell population was selectively depleted extending the animals' lives (Chen et al. 2012). Regrowth after therapy with temozolomide was attributed to the Nestin+ population correlating this population to cancer stem cell status although it was not conclusively demonstrated that Nestin− cells did not contribute (Chen et al. 2012).

3 Role of the Tumor Microenvironment

The stromal components and cell–cell interactions in a tumor play an important role in its growth and response to treatment. Stroma within a tumor includes the vasculature, various populations of cells derived from the bone marrow (BMDC) such as monocytes/macrophages and a variety of immune cell populations, cancer-associated fibroblasts, and non-cellular tissue components such as collagens, fibronectin, and laminin. Further, the poorly organized structure of the vasculature in most tumors (Vaupel et al. 1989) creates an environment in which there is substantial heterogeneity in the supply of nutrients such as oxygen or glucose and in the removal of catabolic products. This leads to regions of low oxygen tension (hypoxia), high levels of acidity due to lactic acid production, increased interstitial fluid pressure due to increased leakiness of the blood vessels, and poor removal of tissue fluid partly caused by lack of functional lymphatics. Specific microenvironmental factors, but also cell–cell interactions and genetically regulated cellular signals, are important determinants for stem cell maintenance and survival. As discussed above, different kinds of 'niches' have been described in which certain stromal cell populations may provide a supportive environment for CSCs and/or help to maintain the stem-like phenotype of tumor cells (Pajonk and Vlashi 2013). For example, in two mouse models of metastatic breast cancer, distinct endothelial sub-niches were shown to regulate disseminated tumor cell dormancy with vascular homeostasis maintaining quiescence but stimulation of vasculature causing outgrowth of the tumor cells (Bissell). It has also been reported that glioblastoma cells may sit in a perivascular niche involving endothelial cell contact (Heddleston et al. 2010) but it has also been reported that both glioblastoma and breast cancer CSCs may sit at a distance from functional vasculature and can be at low oxygen levels (i.e., in an hypoxic niche) (Heddleston et al. 2010; Liu et al. 2014; Peitzsch et al. 2014). Interestingly, hypoxia can suppress miRNA levels by repression of both the DICER and DROSHA enzymes, which are required for miRNA processing (van den Beucken et al. 2014). This leads to a significant decrease in overall levels of certain miRNA in hypoxic cells, which in turn can lead to the acquisition of stem and metastatic phenotypes. In a genetically engineered mouse model of soft tissue sarcoma, deletion of one allele of DICER can decrease miRNA expression and increase the rate of metastasis to the lung (Mito et al. 2013). In breast cancer, reduction in DICER results in a selective loss of the miR200 family of proteins, which stimulates an epithelial to mesenchymal transition (EMT) (van den Beucken et al. 2014). This transition has been associated with a CSC phenotype in breast cancer cells (Mani et al. 2008; Liu et al. 2014). Exposure to hypoxia has also been reported to result in changes in the methylation levels of certain genes, due to a requirement for oxygen by some of the enzymes that cause demethylation. This results in a more primitive phenotype similar to that of stem cells. Thus, exposure to hypoxia may cause various epigenetic changes that promote a stem cell phenotype.

4 Response to Therapy

There are many datasets which support a higher treatment resistance of CSC to both radiation and chemotherapeutic drugs compared to non-CSC (Krause et al. 2011; Alison et al. 2012; Sebens and Schafer 2012; Alisi et al. 2013; Holohan et al. 2013; Crowder et al. 2014; Rycaj and Tang 2014; Cui et al. 2015). The increased resistance to chemotherapy has been variably associated with the proliferative quiescence of CSCs and their resistance to DNA damage and reduced susceptibility to induction of apoptosis. A high expression of ABC transporters that can pump drugs out of cells has also been observed in CSCs. Early studies demonstrated an increase in the ex vivo fraction of CD133 positive cells, confirmed as CSC by transplantation assays, following in vivo irradiation of glioma xenografts. Interestingly, DNA damage checkpoints were preferentially activated in marker-positive versus marker-negative cells (Bao et al. 2006). Higher levels of antioxidant molecules have also been observed in CSCs suggesting increased ability to inactivate reactive oxygen species (ROS), a mediator of radiation damage in cells (Diehn et al. 2009). Compared to progenitor cells, breast CSCs have been shown in vitro to contain a lower level of ROS with higher expression of genes involved in ROS scavenging. Moreover, the initially higher post-irradiation clonogenic cell survival of breast CSC can be altered by pharmacological modulation of the ROS levels. Recent studies by Pajonk and colleagues have also reported that low proteasome activity is associated with a stem cell phenotype and that cells from tumors with low proteasome activity are more resistant to chemotherapy and radiation treatment (Lagadec et al. 2012, 2014; Vlashi and Pajonk 2015). However, a higher intrinsic resistance of CSC cannot be regarded as a general phenomenon, since heterogeneity seems to exist between individual tumors of the same histology (Zielske et al. 2011). Further, the early work of West et al. (West et al. 1993) reported a wide range of radiosensitivities for cells derived from different tumors of the cervix and head and neck that were capable of growing in in vitro clonogenic assays in agarose. This was a spheroid-like environment, although not the same as currently accepted stem cell assays.

A link between hypoxia and putative stem cells has also been shown by an increase in the fraction of CD133-positive cells in brain tumor cells exposed to hypoxia in vitro (Blazek et al. 2007; Platet et al. 2007) and the preferential expression of HIF2α- and HIF-regulated genes in glioma stem cells (Li et al. 2009b). This might be expected to affect their relative radiosensitivity, although it should be noted that the level of hypoxia in 'hypoxic' niches is not well defined and may represent a level of hypoxia more consistent with increased levels of HIF-1α and HIF-2α ($<\sim$10–20 mm Hg) rather than the levels required for full hypoxia-induced radioresistance (<1–5 mm Hg) (Wilson and Hay 2011). Important in this context is that EMT (which as noted above can be induced by hypoxic exposure) has also been previously associated with increased radiation resistance (Theys et al. 2011; Bhat et al. 2013; Al-Assar et al. 2014; Zhang et al. 2014), as well as increased metastatic potential. The hypoxic niche has also been reported to

protect colon cancer CSCs from chemotherapy (Mao et al. 2013). However, a recent study has reported that hypoxia does not affect the radiation survival of breast cancer stem cells cultured as mammospheres putatively because of their high levels of antioxidant molecules capable of scavenging reactive oxygen species (Lagadec et al. 2012). Thus, the potential role of hypoxia in modifying the treatment sensitivity of CSCs in vivo remains uncertain and may vary from tumor to tumor.

The number of CSCs in tumor is highly heterogeneous but will also play an important role in overall response to treatment. In animal models, it was demonstrated that the number of (putative) stem cells assessed by transplantation assays correlated with the single radiation dose required for tumor control (Hill and Milas 1989; Baumann et al. 2008). Similar results have been reported for experimental studies in animal models using fractionated radiation treatment (Yaromina et al. 2007) and the expression of the stem cell-related marker CD44 has been reported to correlate with local control in early laryngeal cancers treated with radiation (Baumann and Krause 2010). Two important considerations in the context of the analysis of the treatment sensitivity of CSC are the increasing evidence for the plasticity of the cancer stem cell phenotype and the method of assessment. The concept that early progenitor cells may regain stem cell properties induced by treatment would result in an effective increase in the number of CSCs in the tumor and hence the level of cell killing required to achieve tumor control (Pajonk and Vlashi 2013; Vlashi and Pajonk 2015). The results of Bao et al. (2006) mentioned above, in which radiation-treated gliomas demonstrated increased CSC content, may be explained by the concept that the radiation treatment induced progenitor cell populations in the tumor to reacquire stem cell properties, consistent with findings in normal tissues described above concerning rebalancing of stem and progenitor cell populations after traumatic injury. Assessing the sensitivity of CSCs in vivo using an experimental tumor response assay to determine treatment outcome rather than tumor control assay is also a concern. The essence of the CSC model is that it is the killing of these cells that is ultimately responsible for tumor control, whereas tumor response can reflect the sensitivity of both stem cells and progenitor cells (Baumann et al. 2008). This concern makes it particularly difficult to use experimental studies to assess the response of CSC to drug treatment in vivo, since such treatments are rarely capable of achieving tumor control on their own, and thus, they reflect the response of both stem and progenitor cells. Combination treatments with radiation can potentially address this concern, and such studies have indicated the failure of certain drugs to target stem cell populations (Baumann/Krause). A further complication is that these two considerations are independent of one another but may, of course, both occur during tumor treatments in vivo; thus in some cases, the interpretation of studies assessing the treatment sensitivity of CSC may be impacted by factors that do not directly relate to the sensitivity of the individual tumor cells. In vitro studies of the drug or radiation sensitivity of cells expressing putative stem cell markers can partially overcome this concern but the different environments found within tumors require that such observations are confirmed by in vivo studies.

5 Conclusion

Without question, the cancer stem cell model has been refined numerous times in the last decade and is far more complex than initially proposed. Heterogeneity within tumors and even in clonal populations within tumors, between tumors in the same patient, and between patients as well as across tumor types are the common themes that emerge across fields. The prognostic value of signatures from small populations of cells may imply that there are ways to clinically identify patients whose tumors are driven by a stem cell phenotype that may be amenable to directed therapy and get around the challenges of variation in prospective markers between patients as well as the limited feasibility in profiling each tumor for stemness markers in order to make treatment decisions. Nevertheless, real-time tumor profiling will likely be needed to select patients for therapy, and to date, there are still no trials selecting patients for treatment using such approaches and strategies to merge stem cell targeting with genetics-based targets are in their infancy. Certainly, advances in clinical imaging-based identification of stem cell-driven tumors would greatly enhance the translatability of these models as would liquid biopsy advances still very much unexplored in this area. Still, in spite of the work yet to do, the progress in the last decade has been rapid and continues on.

Acknowledgements Dr. Woodward is supported by the National Institutes of Health R01CA138239-01 and 1R01CA180061-01 and The State of Texas Grant for Rare and Aggressive Breast Cancer Research Program.

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Molecular Radio-Oncology
Baumann, M.; Krause, M.; Cordes, N. (Eds.)
2016, IX, 201 p. 30 illus., 24 illus. in color., Hardcover
ISBN: 978-3-662-49649-7