Chapter 4
TGF-β in Cancer Stem Cells

Hiroaki Ikushima and Kohei Miyazono

Abstract Cancer stem cells are tumor cells characterized by their ability to induce tumorigenesis and to self-renew. Recent research advance in cancer research field has demonstrated heterogeneity in cancer cell population and established the cancer stem cell model. Simultaneously, this model has shed new light on the mechanism of cancer recurrence, tumor metastasis, and several other cancer-related phenomena. The most important issue of cancer stem cells in the clinical situation is their resistibility against conventional therapies. The mechanisms in which cancer stem cells maintain their stem-like abilities have thus been energetically studied in the last decade. TGF-β is a well-recognized factor responsible for maintenance of normal tissue stem cells. In addition to that, recent studies have unveiled the roles of TGF-β signaling in cancer stem cells. In this chapter, we first consider the basic concept of the cancer stem cell model and describe their special characters in cancer cell population. We then discuss the roles of TGF-β signaling in cancer stem cells, focusing on some types of cancers. We also address perspectives on TGF-β signaling as a potential target against cancer stem cells.

Keywords Cancer stem cells • Heterogeneity • Niche • SP cells • Tumorigenicity

Abbreviations

ABC	ATP-binding cassette
ALDH	Aldehyde dehydrogenase
AML	Acute myeloid leukemia
CML	Chronic myelogenous leukemia

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EMT	Epithelial–mesenchymal transition
FOXO	Forkhead O transcription factor
LIF	Leukemia inhibitory factor
MP	Main population
ROS	Reactive oxygen species
SP	Side population

4.1 Cancer Stem Cell Model

The basic principle of the cancer stem cell model is that not all cells in tumors are equal. In the cancer stem cell model, like the growth of normal proliferative tissues such as skin, intestinal epithelium, and bone marrow, the growth of tumors is accelerated by limited numbers of cells that have capacity to self-renew and have the exclusive ability to drive tumor progression (Reya et al. 2001; Jordan et al. 2006; Lobo et al. 2007; Visvader and Lindeman 2008). The bulk of a tumor consists of rapidly proliferating cells and differentiated non-dividing cells. A defined subset of cancer cells, or cancer stem cells, can give rise to more differentiated cancer cells (Fig. 4.1).

Two important observations led to the hypothesis that cancer stem cells may be responsible for tumor initiation and progression. One is that not all cells within a tumor are identical. Although most tumors arise from a single transformed cell, there are many different types of cells in a tumor. This observation is known as tumor heterogeneity (Park et al. 1971). The other observation is that a certain number of cancer cells were required to grow a tumor in a recipient mouse (Hamburger and Salmon 1977). In contrast, in the model in which cancer cells are usually clonal in origin and every cancer cell has the potential to form a new tumor (clonal evolution model), even a few cancer cells would be able to form new tumors.

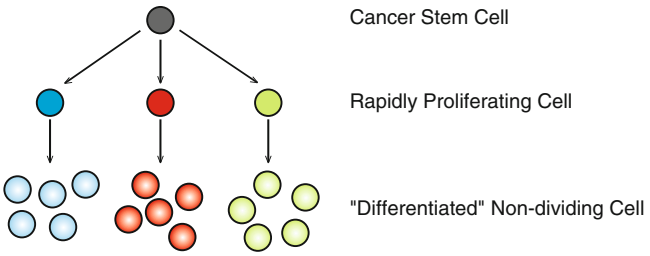

Fig. 4.1 In the cancer stem cell model, only a rare subpopulation of cells within the tumor, the cancer stem cells, has the capacity to generate and maintain a tumor. They can give rise to more differentiated cancer cells, which in turn form a hierarchy

4.2 History of Cancer Stem Cell Model

The concept of a cancer stem cell is not new and the connection between tumors and stem cells has a long history (Clevers 2011; Nguyen et al. 2012). In 1937, Furth and Kahn reported that a single cell from mouse leukemia could initiate a new tumor in a recipient mouse and that the tumor in a recipient mouse shares pathological characters similar to those of the original tumor (Furth and Kahn 1937). In 1941, Jackson and Brues showed that malignant teratocarcinomas contain many types of differentiated cells (Jackson and Brues 1941), suggesting their origin from tumorigenic cells with multilineage potential. Furthermore, in 1963, Bruce and Van Der Gaag reported the first in vivo colony assay for a mouse lymphoma-initiating cell (Bruce and Van Der Gaag 1963). In 1960s, studies by Pierce and his colleagues demonstrated that the morphologically undifferentiated cells in teratocarcinomas showed high tumorigenic activities and could differentiate into multiple differentiated non-tumorigenic cell types (Kleinsmith and Pierce 1964). These studies implicated these undifferentiated cells as candidate teratocarcinoma stem cells. Pierce's group also showed that, in mouse squamous cell carcinoma, well-differentiated cancer cells were derived from undifferentiated cells, using radiolabeling techniques (Pierce and Wallace 1971). From these and other experiments, Pierce defined the cancer stem cell concept as "a concept of neoplasms, based upon developmental and oncological principles, states that carcinomas are caricatures of tissue renewal, in that they are composed of a mixture of malignant stem cells, which have a marked capacity for proliferation and a limited capacity for differentiation under normal homeostatic conditions, and of the differentiated, possibly benign, progeny of these malignant cells" (Pierce and Speers 1988).

In 1970s, epoch-making discoveries were reported in the cancer research field: mutations in proto-oncogenes and tumor suppressor genes were found to cause most human cancers. After the existence of proto-oncogenes and tumor suppressor genes was established, the focus of cancer research shifted to the clonal evolution model as symbolized by the Knudson's two-hit theory and the Vogelstein's multistep carcinogenesis model. In the clonal evolution model, "tumor progression results from acquired genetic variability within the original clone allowing sequential selection of more aggressive sublines" (Nowell 1976; Merlo et al. 2006).

Until 1990s, it had been virtually impossible to directly prove the existence of cancer stem cells in human malignant tumors. However, development of immunocompromised mice, progression of cell sorting techniques, accumulation of knowledge on normal stem cells, and a variety of other scientific progression led Dick and his colleagues to revive the study of functional heterogeneity in leukemias (Lapidot et al. 1994; Bonnet and Dick 1997). They reported that only a subset of acute myeloid leukemia (AML) cells were able to transplant AML into immunodeficient recipient mice. These tumorigenic cells were defined as $CD34^{+}CD38^{-}$, suggesting similarities between leukemia stem cells and normal hematopoietic stem cells. In addition, the xenograft assay allowed measurement of the frequency of the leukemia stem cells; the order of one per million tumor cells. The remaining AML cells that

grew from the transplanted cells were in various stages of differentiation. These data suggest that heterogeneity in leukemias reflects the similar organizational hierarchy to that in normal blood cells.

Clarke and his colleagues applied these concepts and approaches to breast cancer cells and reported that only a minor fraction of breast cancer cells can form a tumor (Al-Hajj et al. 2003). This report was the first reported isolation and characterization of cancer stem cells from solid tumors. They isolated breast cancer stem cells on the basis of surface marker expression ($CD44^+CD24^-$) and tumor regeneration potential in the mammary fat pads of NOD/SCID mice. The tumorigenic subpopulation could be serially passaged and, even after secondary and tertiary transplants, the new tumors showed histopathological characteristics similar to the original tumor. There were no significant differences in cell morphology between the tumorigenic and non-tumorigenic breast cancer cells. This study was followed by similar studies on other solid tumors such as brain tumors (Singh et al. 2003; Singh et al. 2004; Galli et al. 2004), melanomas (Fang et al. 2005; Schatton et al. 2008), prostate cancers (Patrawala et al. 2006; Patrawala et al. 2007; Li et al. 2008), ovarian cancers (Zhang et al. 2008; Curley et al. 2009; Stewart et al. 2011), and colon cancers (O'Brien et al. 2007; Ricci-Vitiani et al. 2007; Dalerba et al. 2007a). In each type of cancer stem cells, small numbers of cells defined by specific markers have ability to form tumors in immunodeficient mice and the transplanted tumors recapitulate the heterogeneity of the original tumors. However, the quantification of cancer stem cells in a population of tumor cells is never absolute, but instead depends on the tumor-initiating assay used to test for their presence (Quintana et al. 2008). Furthermore, recent studies have suggested that reversible regulatory mechanisms allow cancer cells to dynamically cycle between a tumor-initiating "cancer stem cell" state and a non-tumor-initiating cell state (Sharma et al. 2010; Roesch et al. 2010; Quintana et al. 2010; Scheel and Weinberg 2011).

Thus, the clonal evolution model and the cancer stem cell model constitute two major frameworks for interpreting clinical and experimental phenomena of malignant tumors. Of course, these two models are not mutually exclusive, as evidenced by substantial genetic heterogeneity in populations of putative cancer stem cells (Anderson et al. 2011; Notta et al. 2011).

4.3 Characteristics of Cancer Stem Cells

4.3.1 Tumorigenic Activity and Self-Renewal Capacity

The most characteristic feature of cancer stem cells is their high tumorigenic activity. This character is usually proven by the ability to form tumors from a small number of cells in immunodeficient mice, and the newly formed tumor usually shows histopathological characteristics similar to the original tumor. The frequency of cancer stem cells in a tumor can be estimated by limiting dilution analysis. Serial transplantation assay is also used to assess tumorigenic activity and self-renewal capacity of cancer stem cells.

4.3.2 Multilineage Potential

The well-known characters of normal tissue stem cells include multipotency as well as self-renewal capacity. Likewise, cancer stem cells undergo a process similar to differentiation under certain circumstances. In vitro incubation of breast cancer stem cells gives rise to cells positive for cytokeratin 8 (CK8) or mucin 1 (MUC1), both of which are breast epithelial cell markers. Glioma stem cells acquire similar cell morphology and marker expression pattern to astrocytes or oligodendrocytes when cultured in respective differentiation media. These results indicate that cancer stem cells have multilineage potential like normal tissue stem cells, although it is still controversial whether these processes can be defined as "differentiation." Thus, some researchers use the term "cancer-initiating cells" instead of "cancer stem cells" to literally and purely describe their ability to initiate tumor formation.

4.3.3 Resistance to Conventional Cancer Therapy

Another intriguing character of cancer stem cells is their resistance to conventional chemotherapy and radiation therapy (Dean et al. 2005; Baumann et al. 2008). In addition to their ability to self-renew, they are in the dormant phase and are quiescent and divide infrequently. Although our current therapeutic strategies against cancer succeed at eliminating rapidly proliferating bulk cells, they often miss slow-dividing cancer stem cells, which are the source of recurrence and metastasis.

Furthermore, cancer stem cells express high levels of specific ATP-binding cassette (ABC) drug transporters (Dean et al. 2005). The two ABC transporters that have been studied most extensively in cancer stem cells are ABCG2 (also known as breast cancer resistance protein 1, BCRP1) and ABCC2. By using the energy of ATP hydrolysis, these transporters actively efflux drugs from cancer stem cells, serving to protect them from cytotoxic agents (Gottesman et al. 2002).

Cancer stem cells contain lower levels of reactive oxygen species (ROS) than their matched bulk cancer cells (Diehn et al. 2009). Low ROS levels help to protect genomes in cancer stem cells from endogenous and exogenous ROS-mediated damage, including conventional radiation therapy. The mechanism for low ROS levels is at least partially due to the increased production of free radical scavengers. In addition, it has been reported that cancer stem cells show preferential checkpoint response and undergo DNA repair to counteract radiation damage (Bao et al. 2006a).

4.3.4 Invasion and Metastasis

The cancer stem cell model has shed new light on the mechanism of invasion and metastasis, and explain why, despite extensive intratumor heterogeneity, comparison of paired samples of primary tumors and distant metastases usually reveals

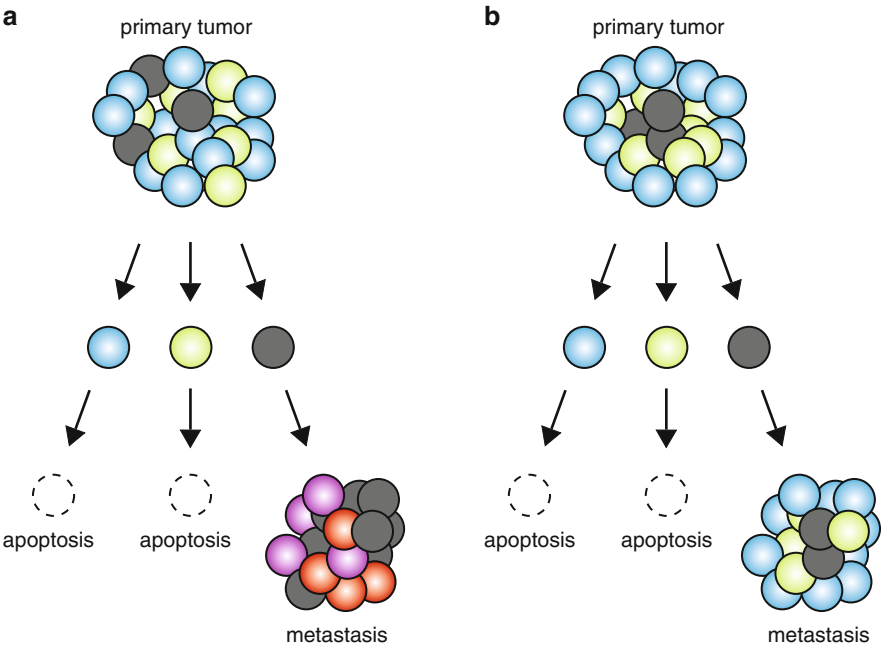

Fig. 4.2 (**a**) Based on the conventional stochastic cancer model, tumors are composed of heterogeneous mixtures of independent subclones with divergent genetic mutations. Different subclones are endowed with different functional properties and only selected clones (*dark gray*) can acquire ability to migrate and form metastases. The metastasis originates from a homogeneous monoclonal expansion of an individual subclone, which in turn can accumulate further mutations and diverge even further from the primary tumor. Therefore, the conventional stochastic cancer model predicts that primary tumors and corresponding metastases are substantially different. (**b**) In the cancer stem cell model, intratumor heterogeneity is mainly caused by cell differentiation and only cancer stem cells (*dark gray*) can migrate and form metastases. The cancer stem cells in metastatic sites undergo differentiation programs that closely resemble those observed in the corresponding primary tumors

striking similarities over a wide range of parameters, including tissue morphology (Brabletz et al. 2001), repertoire of genetic mutations (Losi et al. 1992; Khan et al. 2000; Zauber et al. 2003), and overall transcriptional profile as defined by gene expression arrays (Weigelt et al. 2003; Weigelt et al. 2005; D'Arrigo et al. 2005). These observations are not explained by the clonal evolution model, in which metastases are considered to originate from monoclonal expansions of specific individual tumor subclones endowed with specific genotypic and phenotypic features and therefore are postulated to be substantially different from primary tumors (Fig. 4.2a). However, under the cancer stem cell model, we can predict that, if two lesions share identical genetic abnormalities, they will also undergo similar differentiation programs and display similar patterns of intratumor heterogeneity (Fig. 4.2b).

In addition, C-X-C chemokine receptor type 4 (CXCR4) was reported to be highly expressed in prostate cancer stem cells (Dubrovska et al. 2012), suggesting the role of cancer stem cells as "pacemakers" of tumor metastasis.

4.4 Cancer Stem Cell Markers

Cancer stem cells have been prospectively isolated on the basis of their expression of particular markers such as CD44 and CD133 (Alison et al. 2010), although "non-cancer stem cells" defined as subpopulations negative for certain reported "cancer stem cell markers" also have tumorigenic activities in some contexts (Shmelkov et al. 2008; Quintana et al. 2010). Interestingly, cancer stem cells often share their markers with their normal counterparts, suggesting characteristic similarities between cancer stem cells and normal tissue stem cells.

Stem cells are known to efficiently extrude dyes such as Hoechst 33342. Cells with the capacity to efflux the dye were referred to as side population (SP) cells (Challen and Little 2006). SP cells have been identified in a variety of tissues, and they express high levels of stem-like genes and possess multi-potent differentiation potential (Goodell et al. 1996; Goodell et al. 1997; Pearce et al. 2004). The mechanism regulating the efflux of Hoechst dye can be explained, at least in part, by the expression of ABC transporters, including ABCG2 (Zhou et al. 2001).

SP cells have been also identified in a large variety of cancer cells (Kondo et al. 2004; Chiba et al. 2006; Haraguchi et al. 2006; Ho et al. 2007; Wang et al. 2007). When compared to non-SP population, SP cells isolated from cancer cells are highly enriched for the capacity to initiate tumor formation in immunodeficient mice. They also have the capacity to initiate tumors upon serial transplantation. Furthermore, SP cells in cancer cell population have increased expression of genes which are believed to be involved in the regulation of stem cell function, such as ABCG2 transporter. These data suggest that SP cells in tumors act as cancer stem cells.

4.5 Cancer Stem Cell Niche

Normal stem cells reside in a special microenvironment called "niche." They interact with the niche through adhesion molecules and exchange signals to maintain the specific features of stem cells (Wilson and Trumpp 2006; Martino and Pluchino 2006; Kiel and Morrison 2008; Wang and Wagers 2011). It has been suggested that there is a functional microenvironment to support cancer stem cells as well (Iwasaki and Suda 2009). This should also be considered a niche and is thus called "cancer stem cell niche."

Dick and colleagues demonstrated that anti-CD44 antibody-treated mice transplanted with AML cells exhibited a significantly lower rate of disease onset (Jin et al. 2006). In addition, Van Etten and colleagues showed that there was impaired

induction of chronic myelogenous leukemia (CML)-like myeloproliferative disease among recipient mice transplanted with BCR-ABL-transduced CML progenitors from CD44-mutant donors (Krause et al. 2006). These results indicate that CD44 is required for the homing and/or engraftment of leukemia stem cells to the niche, and that the binding of CD44-expressing leukemic stem cells to the niche is crucial for the maintenance of their tumorigenic activities. Interestingly, this role of CD44 in leukemia stem cells resembles that in normal hematopoietic stem cells, suggesting that cancer stem cells and normal stem cells share the maintenance system within their niches.

In brain tumor stem cell research, Gilbertson and colleagues revealed that brain tumor cells co-expressing Nestin and CD133 exist near the capillary vessels in the brain tumor (Calabrese et al. 2007). In addition, the CD133-positive subpopulation in human medulloblastoma developed brain tumors in a recipient nude mouse only when xenografted with endothelial cells. These results suggest that brain tumor stem cells rely on endothelial cells, which form a vascular niche to maintain the stem-like characters of brain tumor stem cells, such as self-renewal capacity and tumorigenic activity. Conversely, brain tumor stem cells themselves can elicit angiogenic effects by secreting factors such as vascular endothelial growth factor (VEGF) and stromal-derived factor 1 (SDF-1) (Bao et al. 2006b; Folkins et al. 2009). Furthermore, under certain conditions, brain tumor stem cells can even directly transdifferentiate into the endothelial lineage (Ricci-Vitiani et al. 2010; Wang et al. 2010), indicating a close connection between cancer stem cells and vascular niches.

4.6 Roles of TGF-β Signaling in Cancer Stem Cell Model

Cancer stem cells have been suggested to make use of a microenvironment similar to that found in normal stem cell niches for the maintenance of their stem cell-like properties. TGF-β signaling has been identified as a niche signal in the control of hematopoietic stem cells and hair follicle stem cells (Yamazaki et al. 2009; Oshimori and Fuchs 2012), and so a broader role for TGF-β signaling in the maintenance of cancer stem cells has been proposed. Recent studies have revealed crucial roles of TGF-β signaling in interaction between cancer stem cell and niche, as well as cancer stem cell-autonomous signaling pathways (Ikushima and Miyazono 2010a).

4.6.1 TGF-β Signaling in Breast Cancer Stem Cells

Breast cancer stem cells are, as described above, the first identified solid tumor stem cells (Dalerba et al. 2007b). Since the prospective identification using $CD44^{+}CD24^{-}$ subpopulation (Al-Hajj et al. 2003), the involvement of TGF-β signaling in breast cancer stem cells has been energetically studied. Recent reports have uncovered bilateral characters of TGF-β signaling in breast cancer stem cells: deprivation of

tumorigenic activities in some contexts and maintenance of stem cell-like characters in other contexts.

Wakefield and her colleagues showed that the suppression of the TGF-β pathway increased the size of the putative breast cancer stem cell compartment and promoted tumorigenesis by a mechanism that was independent of direct effects on proliferation (Tang et al. 2007). They demonstrated that the introduction of a dominant negative TGFBR2 enhanced the proliferation of immortalized and transformed human breast epithelial cell line, Ca1h, although the expression level of the cyclin-dependent kinase inhibitor p21 was unchanged. They also showed that TGF-β stimulation resulted in the loss of stem cell-like properties and the ability to form mammospheres. The ability of TGF-β to deprive breast cancer stem cells of tumorigenic activity was dependent on the down-regulation of Id1, which is highly expressed during embryogenesis and has been implicated in the regulation of self-renewal and differentiation. Id family proteins (Id1 through 4) act as inhibitors of differentiation and stimulators of cell growth by antagonizing the function of basic helix-loop-helix (bHLH) transcription factors (Miyazawa et al. 2002). Id1 has been reported to inhibit lineage commitment and differentiation in many cell types (Norton 2000) and to be a direct TGF-β target (Kang et al. 2003). These findings suggest that breast cancer stem cells benefit from similar mechanisms that regulate the function of normal stem cells.

Weinberg and his colleagues demonstrated that TGF-β signaling has an important role in the maintenance of stem cell-like properties and tumorigenic activity through the induction of epithelial–mesenchymal transition (EMT). A $CD44^{+}CD24^{-}$ subpopulation that was isolated from breast cancer specimens exhibited mesenchymal properties, and transformed mammary epithelial cells, in which EMT was induced by TGF-β stimulation, acquired stem cell-like properties, including mammosphere-forming ability (Mani et al. 2008; Scheel et al. 2011). Transformed mammary epithelial cells with TGF-β-induced EMT also showed higher tumorigenic activity in vivo and fewer cells were required to initiate tumor formation than cells without TGF-β treatment. These results connect TGF-β-induced EMT and the gain of epithelial stem cell properties of carcinoma.

4.6.2 TGF-β Signaling in Brain Tumor Stem Cells

Glioma stem cells are characterized by the expression of neural stem cell antigens (Singh et al. 2004; Kondo et al. 2004; Hirschmann-Jax et al. 2004) and share several characteristics with normal neural stem cells (Vescovi et al. 2006). The overexpression of TGF-β commonly seen in malignant glioma has been variously implicated in glioma cell proliferation, decreased apoptosis, migration, and tumor-specific immunosuppression (Golestaneh and Mishra 2005). TGF-β signaling also has important roles in the regulation of the stem cell properties of neural stem cells (Watabe and Miyazono 2009). These facts have shed some light on the role of TGF-β signaling in the maintenance of glioma stem cells.

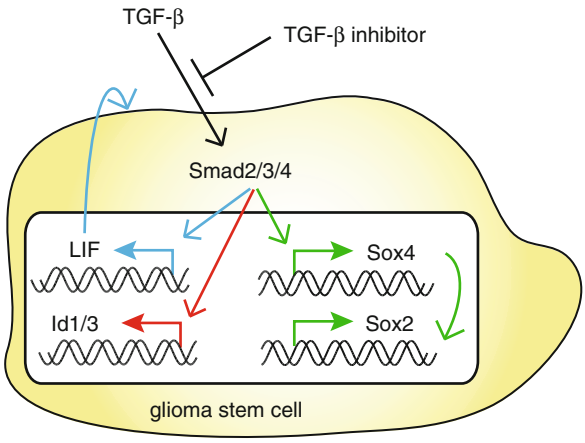

Fig. 4.3 TGF-β-Smad signaling pathway maintains stem cell-like properties and tumorigenic activity of glioma stem cells through many independent pathways, including the induction of the Sox4-Sox2 cascade, the activation of the LIF pathway, and the up-regulation of Id1 and Id3 expressions

TGF-β signaling has been reported to play pivotal roles in the maintenance of tumorigenic activity and stem cell-like properties of glioma stem cells (Peñuelas et al. 2009; Ikushima et al. 2009; Anido et al. 2010; Ikushima et al. 2011). TGF-β inhibitors markedly deprived glioma stem cells of glioma sphere-forming activity and self-renewal capacity in vitro and tumorigenic activity in vivo. Inhibition of TGF-β signaling also suppressed marker expressions that are associated with stem cell-like properties. These results indicate that microenvironmental niche-derived or glioma stem cell-autonomous TGF-β signaling maintains tumor-initiating abilities of glioma stem cells. TGF-β mediates this activity through the direct induction of the leukemia inhibitory factor (LIF) expression (Peñuelas et al. 2009). LIF activates the JAK-STAT pathway in glioma stem cells, leading to their increased tumorigenicity. Independently of this mechanism, TGF-β induces the expression of Sox2, a self-renewal gene that helps to maintain stem cell-like properties in embryonic stem cells and neural stem cells (Kamachi et al. 2000; Graham et al. 2003; Ferri et al. 2004). TGF-β induces the expression of Sox4, and this subsequently induces the expression of Sox2 (Ikushima et al. 2009; Ikushima et al. 2011). The maintenance of tumorigenic activity of glioma stem cells by TGF-β is also mediated by induction of Id1 and Id3 expressions (Anido et al. 2010). TGF-β signaling thus maintains the stem cell-like properties and tumorigenic activities of glioma stem cells through multiple pathways (Fig. 4.3), although interactions among these pathways have been still unclear.

4.6.3 TGF-β Signaling in Leukemia Stem Cells

TGF-β signaling is known to be a candidate niche signal in the control of hematopoietic stem cell hibernation (Yamazaki et al. 2009). Also, it plays important roles in the maintenance of leukemia stem cells.

CML is caused by a t(9;22)(q34;q11) translocation that generates a constitutively active tyrosine kinase, BCR-ABL (Ren 2005). BCR-ABL activates AKT signaling to suppress the forkhead O transcription factors (FOXO) in CML cells (Ghaffari et al. 2003; Essafi et al. 2005). In CML, a rare Lineage$^-$(Lin$^-$)Sca-1$^+$c-Kit$^+$ population was identified as leukemia stem cells (Hu et al. 2006; Neering et al. 2007; Zhao et al. 2009). Although the use of the tyrosine kinase inhibitor imatinib is a breakthrough for CML therapy, it does not deplete the leukemia stem cells, which drive the recurrence of CML (Graham et al. 2002; Michor et al. 2005; Roeder et al. 2006).

TGF-β signaling has crucial roles in the maintenance of leukemia stem cells in CML. TGF-β regulates AKT activation and FOXO3a localization in leukemia stem cells. Furthermore, this TGF-β-FOXO pathway maintains the stem cell-like properties and tumorigenic activities of leukemia stem cells (Naka et al. 2010). It was also demonstrated that a combination of TGF-β inhibition, FOXO3a deficiency, and imatinib treatment led to the efficient depletion of CML cells in vivo. These results indicate the central roles of TGF-β signaling in leukemia stem cells of CML. However, when leukemia stem cells were cultured with TGF-β inhibitors in a stroma-free system, colony formation was not inhibited (Naka et al. 2010), suggesting that the maintenance of leukemia stem cells depends not only on TGF-β produced by leukemia stem cells themselves but also on TGF-β in the surrounding microenvironment.

HOXA9 is involved in human AML caused by the translocation t(7;11)(p15;p15), through which HOXA9 gene is fused with the gene encoding NUP98, a nucleoporin protein (Nakamura et al. 1996; Borrow et al. 1996). Expression of the NUP98-HOXA9 fusion protein enforces expression of HOXA9 target genes and immortalizes hematopoietic myeloid progenitors, resulting in development of AML. In wild-type mouse hematopoietic stem cells transduced with NUP98-HOXA9, Smad4 binds to HOXA9 and inhibits nuclear transportation of HOXA9. In contrast, there is no cytoplasmic accumulation of HOXA9 in Smad4$^{-/-}$ hematopoietic stem cells, and as a consequence increased levels of HOXA9 is observed in the nucleus, leading to increased immortalization in vitro (Quéré et al. 2011). In addition, loss of Smad4 accelerates the development of NUP98-HOXA9-induced AML in vivo, and NUP98-HOXA9-transformed Smad4$^{-/-}$ leukemic cell population contains a higher amount of the leukemia stem cells than wild-type leukemia cells (Quéré et al. 2011). These results indicate that the cytoplasmic binding of Smad4 to HOXA9 is a mechanism to protect NUP98-HOXA9-induced transformation and acquisition of leukemogenic activity.

4.6.4 TGF-β Signaling in Gastric Cancer Stem Cells

Some molecules, including CD44 and aldehyde dehydrogenase 1 (ALDH1), have been reported as markers for gastric cancer stem cells (Takaishi et al. 2009; Katsuno et al. 2012). In addition, flow cytometric analyses using Hoechst 33342 have disclosed that SP cells in gastric carcinomas have higher tumorigenic activities, suggesting their roles as gastric cancer stem cells (Nishii et al. 2009; Ehata et al. 2011).

Mutations of TGFBR2, Smad4, and Smad2 have been reported to be responsible for progression of gastrointestinal tumors, indicating the tumor-suppressive activity of TGF-β signaling in gastrointestinal tumors (Wakefield and Roberts 2002; Bierie and Moses 2006). Besides suppression of cell proliferation and induction of apoptosis, TGF-β decreases the number of SP cells and so attenuates the tumor-forming ability of gastric cancer cells. TGF-β transcriptionally represses ABCG2 expression through direct binding of Smad2/3 to its promoter (Ehata et al. 2011). TGF-β also reduces the expression of ALDH1 and the size of the $ALDH1^+$ cell population in diffuse-type gastric cancer cells. In addition, ALDH1 expression inversely correlates with phosphorylation of Smad3 protein in human diffuse-type gastric cancer tissues (Katsuno et al. 2012). These results suggest that TGF-β reduces the cancer stem cell subpopulation through suppression of ABCG2 and ALDH1 expressions and inhibits tumor-initiating capacity of gastric cancer stem cells.

It has not yet been fully elucidated why TGF-β signaling suppresses tumorigenicity of gastric cancer stem cells while it maintains that of brain tumor stem cells and leukemia stem cells. Possible answers for this question may include the differential roles of TGF-β in the corresponding normal tissue stem cells (See Sect. 8).

4.6.5 TGF-β Signaling in Pancreatic Cancer Stem Cells

When incubated with TGF-β, pancreatic cancer stem cells enriched by sorting for SP cells change their shape into mesenchymal-like spindle-shaped appearance (Kabashima et al. 2009). This change is associated with significant reduction of E-cadherin expression and induction of Snail expression. Furthermore, SP cells show marked invasion activity in response to TGF-β treatment. Interestingly, such invasive activity is not induced by TGF-β stimulation in main population (MP) cells. These results suggest that TGF-β responsiveness is greater in SP cells than in MP cells, resulting in enhanced induction of EMT and invasiveness (Kabashima et al. 2009), although this study did not provide us direct evidences for the effect of TGF-β on tumorigenicity of pancreatic cancer stem cells.

4.7 TGF-β Signaling as Therapeutic Target in Cancer Stem Cell Model

According to the concept of cancer stem cells, therapeutic strategies that do not eradicate the cancer stem cell compartment are likely to achieve little success. They fail to prevent disease relapse and metastatic dissemination even when they might kill the majority of tumor cells and induce temporary regression of gross tumor lesions (Fig. 4.4). As mentioned above, cancer stem cells are considered to be inherently resistant to the toxic effect of conventional chemotherapeutic regimens and

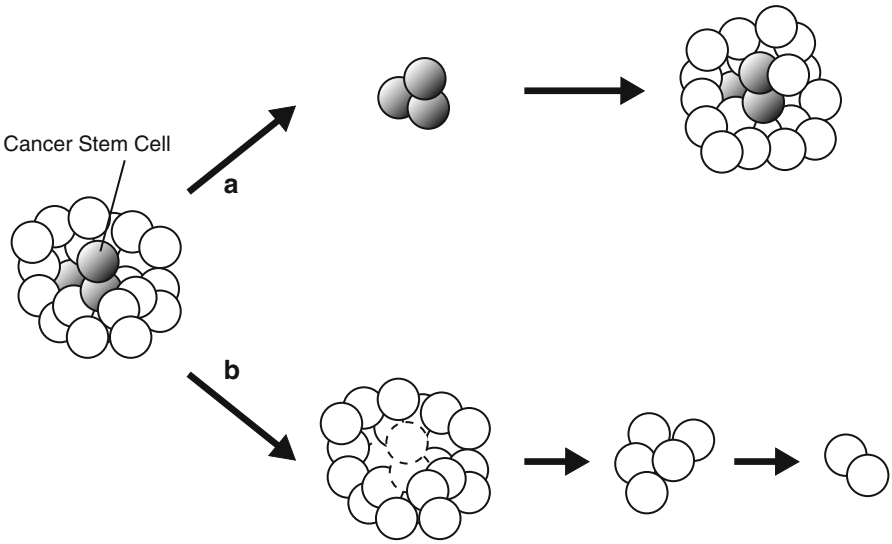

Fig. 4.4 Anti-tumor treatments designed for broad cytotoxic effects (**a**) may kill the majority of cancer cells within a tumor and induce dramatic regression of tumor masses. However, if a certain number of cancer stem cells are spared, tumor tissues can be regenerated, resulting in cancer recurrence. In contrast, anti-tumor treatments specifically designed to target cancer stem cells (**b**) might achieve long-term disease eradication by exhausting growth potential of cancer tissues, even though they are theoretically unable to cause rapid shrinkage of tumor lesions

radiation therapies. Therefore, investigational therapies should be developed, focusing on their ability to target the cancer stem cell subpopulation and its specific signaling pathways. However, there is one important problem in targeting signaling pathways specific for cancer stem cells: these pathways may also be important for normal stem cells. It has not yet fully determined what is the same or what is different between cancer stem cells and normal stem cells. Therefore, we should bear in mind that cancer stem cell-targeting agents could have adverse effects on maintenance of normal tissue stem cells.

The TGF-β pathway has been targeted using multiple strategies, including small-molecule inhibitors of the TGFBR1 kinase domain, TGF-β-specific neutralizing antibodies, and antisense compounds (Yingling et al. 2004). Some of them have been in clinical trials for human cancers (Schlingensiepen et al. 2006; Hau et al. 2007).

4.8 Concluding Remarks

As we have discussed, TGF-β is involved in the maintenance of the tumorigenic activity of cancer stem cells in several types of tumors in a tissue-specific manner. Targeting the pathways that maintain cancer stem cells might ultimately prove to be

an effective therapeutic strategy against malignant tumors. However, such pathways could have divergent roles in cancer stem cell populations from different patients.

Several in vitro and in vivo studies have uncovered cellular context-dependent diversity in TGF-β-induced cell responses (Ikushima and Miyazono 2010b). Because of such diversity, TGF-β can be both pro-tumorigenic and tumor-suppressive in a cellular context-dependent fashion. In addition, TGF-β promotes differentiation in some kinds of tissue stem cells and maintains stemness properties in others. Likewise, as we have discussed, TGF-β signaling shows positive effects on some kinds of cancer stem cells and negative effects on others. Such diversity among cancer stem cells could reflect both the differences between the oncogenic mutations expressed by the cells and their progeny and the differences in their origin. These differences will need to be taken into account when developing treatments based on TGF-β signaling for any individual patient. Also, we need to elucidate the exact mechanism by which TGF-β shows such complex effects against cancer stem cells in cellular context-dependent manner.

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