Immunobiology and signaling pathways of cancer stem cells: implication for cancer therapy

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Abstract Cancer stem cells (CSCs) need to survive cancer treatments with a specific end goal to provide new, more differentiated, metastatic-prone cancerous cells. This happens through diverse signals delivered within the tumor microenvironment where ample evidence indicates that altered developmental signaling pathways play an essential role in maintaining CSCs and accordingly the survival and the progression of the tumor itself. This review summarizes findings on the immunobiological properties of CSCs as compared with cancerous non-stem cells involving the expression of immunological molecules, cytokines and tumor antigens as well as the roles of the Notch, Wnt and Hedgehog pathways in the brain, breast and colon CSCs. We concluded that if CSCs are the main driving force behind tumor support and growth then

understanding the molecular mechanisms and the immunological properties directing these cells for immune tolerance is of great clinical significance. Such knowledge will contribute to designing better targeted therapies that could prevent tumor recurrence and accordingly significantly improve cancer treatments and patient survival.

Keywords Cancer stem cells · Hedgehog · Immunobiology · Notch · Wnt

Introduction

Cancer stem cells (CSCs) can be defined as a rare subpopulation of cancer cells which have the properties of self-renewal, differentiation, multipotency, cancer initiation and resistance to chemotherapy and radiotherapy (Clevers 2011). CSCs have the capability of being resistant to chemotherapy and radiotherapy by many molecular mechanisms. For example, it has been established that increases in the aldehyde dehydrogenase (ALDH) activity in CSC seem to mediate their resistance to particular chemotherapeutics (Hilton 1984; Ginestier et al. 2007). Additionally, CSCs chemo/radiotherapy resistance is depicted to be reliant on interleukin-4 (IL-4) signaling pathway since up-regulation of IL-4 on these cells results in resistance to apoptosis (Francipane et al. 2008). As such, acquisition of these properties by CSCs make them refractory to

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immunotherapy (Ellebaek et al. 2012). In addition, CSCs display an epithelial–mesenchymal cell transition (EMT) which is another mechanism that supports their resistance to chemotherapy and other therapeutic drugs (Kalluri and Weinberg 2009). Another mechanism of chemo resistance that has been overall investigated in CSCs is the role of B-cell lymphoma-2 (BCL-2) protein and its family members (Kelly and Strasser 2011). Taken together, CSCs are equipped with different mechanisms that render them insensitive to chemotherapy, radiotherapy and immunotherapy.

A paramount and fascinating common theme has been the finding that differentially expressed markers on the normal stem cells of the tissue in which a cancer has emerged are often useful for the identification and isolation of the CSCs. However, the exact molecular characterization of CSCs is still unclear because a variety of putative CSC-associated markers have been distinguished up to this point in solid tumors (Table 1) where none of them have been reported to be only expressed by the CSCs (Li and Littera 2012; Magee et al. 2012). Absence of a single CSC-specific marker can explain the inter-patients contrasts of the genetic background and intra- and/or inter-cancer heterogeneity

of the original tumor (Hjelmeland and Rich 2012; Li and Littera 2012). Examples of common CSC markers are listed in Table 1.

Immunological features of CSCs

The expression of both antigen presentation and co-stimulatory molecules by a given cell, including cancer cells, is a prerequisite for them to act as professional antigen presenting cells. As such, the expression pattern of the antigen presentation molecules, including major histocompatibility complex (MHC) class-I (MHC-I) and class-II (MHC-II) molecules as well as the co-stimulatory and co-inhibitory molecules by CSCs determines their immunological signature and if they can present tumor antigens to T cells for immune recognition or eliciting productive immune responses (Comber and Philip 2014).

Indeed, CSCs show down-regulation of MHC-I and a lack of MHC-II expression (Hjelmeland and Rich 2012). Of note, normal hematopoietic stem cells has low MHC-I expression as well (Le Blanc et al. 2003) which suggests that the low expression of MHC-I in both cell types supports the stem cell survival through escaping from the immune attack. As a consequence for the low expression of MHC-I, the antigen processing system is down-regulated in CSCs, especially the low molecular weight protein (LMPs), transporter associated with antigen processing (TAP) and beta macroglobulin have been shown to be downregulated in the CSCs (Di Tomaso et al. 2010).

Considering the expression of the co-stimulatory molecules (CD80, CD86) and the co-inhibitory molecules (CTLA4, B7-H2, B7-H3 and PD-1/-1L) in CSCs, it has been found that these cells are negative for the co-stimulatory molecules where the co-inhibitory molecules were up-regulated in comparison with the conventional cancer cells (Maccalli et al. 2013). Based on this unique phenotype of CSC it could be suggested that the antigen presentation of these cells is dysfunctional.

With respect to the cytokines released by cancerous cells, transforming growth factor (TGF)- β 1, TGF- β 2, tumor necrosis factor (TNF), IL-8, IL-10, IL-6 and IL-13 were found to be released from different cancer cells of different histology. CSCs have been shown to secrete TGF- β , IL-10, IL-13 in vitro (Schatten et al. 2008). Di Tomaso et al. 2010 conducted a

Table 1 List of potential markers of cancer stem cells from different histological origin

Tumor type	Marker	References
AML	CD34 ⁺ CD38 ⁻	Lapidot et al. (1994)
Brain cancer	CD133 ⁺ CD15 ⁺	Singh et al. (2004)
Breast cancer	CD44 ⁺ CD24 ⁻	Al-Hajj et al. (2003)
Colon cancer	CD44 ⁺ CD166 ⁺ LGR5 ⁺	Choi et al. (2009)
Liver cancer	CD133 ⁺ CD90 ⁺ EpCAM ⁺	Yang et al. (2008), Chen et al. (2011)
Prostate cancer	CD44 ⁺ a2b1 ^{high} CD133 ⁺	Collins et al. (2005)
Ovarian cancer	CD44 ⁺ ALDH1 ⁺	Wang et al. (2012)
Pancreas cancer	ESA ⁺ CD44 ⁺ CD24 ⁺	Li et al. (2009a, b)
Osteosarcoma	CBX3 ⁺ ABCA5 ⁺	Saini et al. (2012)
Renal cell carcinoma	HSP DNAJB8	Nishizawa et al. (2012)
Melanoma	ABC5 ⁺	Schatten et al. (2008)

detailed analysis of the soluble factors including cytokines and growth factors expressed by CSCs from glioblastoma *in vitro*. They found that CSCs showed a high release of tumor associated angiogenic factors such as vascular endothelial growth factor (VEGF), macrophage-chemoattractant protein-1 (MCP-1), eotaxin, macrophage inhibitory factor (MIF) and growth related oncogene α (GRO α) (Di Tomaso et al. 2010). Surprisingly, IL-10 or IL-13 were not released by CSCs (Di Tomaso et al. 2010), indicating that CSCs may depend mainly on their angiogenic factors as an intrinsic mechanism to sustain their survival and trafficking.

Tumor associated antigens expressed by CSCs

Expression of tumor associated antigens (TAAs) by cancerous cells is another important factor that determines the immunogenic nature of cancer cells (Lucas and Coulie 2008). Interestingly, CSCs express a variety of molecules that describe their stemness condition and could be conceivably distinguished by the immune system as TAAs (Parmiani et al. 2007). In melanoma for instance, the CSC subpopulation expressing the ABCB5 molecule, which is expressed in human malignant melanoma and supports tumor cell dispersal through mediating chemotherapeutic drug resistance, showed a low level of lineage-related and cancer-testis (CT) antigens (Schatton et al. 2010). The CD133⁺ subpopulation of melanoma cells, however, had high expression of NY-ESO1 CT antigen along with susceptibility to specific T cells (Gedye et al. 2009).

Recently, a new TAA has been characterized in CD133⁺ CSCs in murine melanoma and in many human cancers (called DDX3X), conferring to the immunogenicity of these cells and their capability to induce a T-cell dependent protection against murine cancer growth *in vivo* (Koshio et al. 2013). In contrast, another subpopulation (CD271⁺) of melanoma CSCs are deficient in the expression of both lineage related and CT antigens, making their removal by the immune T cells rather difficult (Boiko et al. 2010) which correlated with the progression and metastasis of these cells. As such, melanoma cells offer an example of multiple CSC subpopulations with different antigen expression patterns. Of note, none of these potential TAAs represents a specific marker of CSCs because

they are often expressed by non-stem cancer cells. Nonetheless, they can be considered as potential useful targets to direct immune response against melanoma and CSC melanoma simultaneously.

The functional *in vitro* and *in vivo* immunogenicity of CSCs has been deliberated in a limited number of cases. Interestingly, a new group of molecules, the heat-shock proteins (HSP), commonly expressed in many murine and human tumors and to be immunogenic *in vivo* and *in vitro* (Parmiani et al. 2007), have been demonstrated to preserve CSCs or at least for a subfamily of them (HSP40). These HSPs demonstrated high expression in most human solid tumors including renal cell carcinoma (RCC), and prompted cytotoxic T lymphocytes (CTL) reactions *in vitro* favored with a therapeutic potential at least in the mouse system (Nishizawa et al. 2012). It has been indicated, however, that other lymphocyte subpopulations (e.g. natural killer (NK) cells or $\gamma\delta$ -T lymphocytes) may distinguish and kill *in vitro* human CSCs isolated from gliomas, CRC, ovarian cancers and melanoma (Avril et al. 2011; Lai et al. 2012).

Indeed, the relative immunogenicity of CSCs makes them a potential target for vaccination based cancer immunotherapy. With this regard, in a recent study of vaccination of mice with dendritic cells pulsed with syngeneic CSC lysate as TAAs, an effective therapeutic and immunological (both antibody and T cell-mediated) reaction has been acquired (Ning et al. 2012). Further, given that characterization of CSCs depends on their expression of ALDH, it is possible to generate anti-ALDH-specific CTLs with a therapeutic activity against human CSCs when xenografted into severe combined immunodeficiency (SCID) mice (Visus et al. 2011). These studies indicate the possible utility of CSCs in anti-cancer vaccination. Since tumors share expression of embryonic gene products (Brewer et al. 2009), it is possible to vaccinate with undifferentiated ESCs to generate anti-tumor immunity. Indeed, vaccination with ESCs was demonstrated to trigger anti-tumor immunity against transplantable colon carcinoma and lung cancer (Li et al. 2009a, b; Dong et al. 2010, 2013) and prevented implantable and carcinogen-induced lung tumors, coinciding with robust anti-tumor-specific effector and memory CD8⁺ T responses, Th1 cytokine response, lower numbers of immunosuppressive cells including regulatory T cells and myeloid derived suppressor cells (Yaddanapudi et al. 2012).

Signaling pathways in cancer stem cells

CSCs express self-renewing genes and show activation of the developmental signaling pathways that are fundamental in maintaining stem cell activity. For example, Notch, Wnt and Hh (Hedgehog) signaling pathways are preserved in controlling normal stem cell activity and their deregulation is linked to oncogenesis (Table 2). Heterogeneity around and between distinctive cancers presents challenges to specifically characterizing CSCs molecularly, genetically, functionally and phenotypically. A few CSCs may be more dependent on the Wnt signaling pathway contrasted with others that may use the Hedgehog pathway. Understanding the basics of what characterizes a CSC signaling pathway inside a particular tumor of interest is essential if CSCs are to be targeted for therapies. If CSCs are the main thrust behind tumor maintenance and development then understanding the molecular mechanisms in establishing CSCs as a stable lineage is fundamentally important. This section will concentrate on the role of

the Notch, Wnt and Hh signaling pathways in CSCs of brain, colon and breast cancers, as these tumors have been the most widely studied.

Notch pathway

The Notch signaling pathway is evolutionarily conserved and plays numerous roles. For example, it impacts stem cell fate, differentiation and cell cycle progression. Notch signaling might be either inhibitory or inductive depending on the context (Purow et al. 2005). Mammals have four Notch receptors (Notch1, Notch2, Notch3, and Notch4) and five ligands including the delta-like proteins (DLL1, DLL3, and DLL4) and Jagged proteins (JAG1 and JAG2). The Notch receptors are transmembrane proteins with an extracellular domain with EGF-like repeats included in the ligand binding and an intracellular domain (ICD) (Blank et al. 2008).

In brain cancers, components of the Notch signaling pathway are up-regulated and several studies have

Table 2 Summary of Notch, Wnt and Hedgehog pathways in different cancer types

Signal	Brain cancer	Breast cancer	Colon cancer
Notch signaling	Notch1 and its ligands DLL1 and JAG1 are over-expressed in primary gliomas (Purow et al. 2005), down-regulation of Notch signaling components expanded apoptosis and diminished cell proliferation (Purow et al. 2005), down-regulation of Notch components sensitized CSCs to radiation or chemotherapeutic therapies and weakened xenograft tumor formation (Wang et al. 2010)	Expression of Notch signaling components is common in human breast cancers, where up to 50 % of the cells showed Notch activation (Pece et al. 2004) and high JAG1 and NOTCH1 expression is connected with poor survival (Reedijk et al. 2005)	Notch signals are high in early colorectal cancers (Reedijk et al. 2008) and appear to be lower in advanced cancers (Fre et al. 2005) and Notch is essential for self-renewal and tumor initiation (Fre et al. 2005)
Wnt signaling	Down-regulation of the secreted Frizzled-related proteins (SFRPs), which are Wnt inhibitors, has been observed in breast cancers (Veeck et al. 2008), Wnt/ β -catenin signaling participates in the radiation therapy resistance of breast CSCs (55)	Mutations in APC, β -catenin, and Axin have been found in medulloblastomas (Baeza et al. 2003), the Wnt antagonist DKK-1 has been ensnared as a medulloblastoma suppressor (59)	Inactivating mutations in APC are found in up to 80 % of sporadic colon cancers and activating mutations of β -catenin are additionally discovered, both of which culminate in actuated Wnt signaling (Pinto et al. 2003)
Hedgehog signaling	Brain cancers have inactivating PTCH1 mutations (Hahn et al. 1996), GLI1 was highly expressed in 26 % of primary GBMs and 57 % of cell lines (Bar et al. 2007) and GLI1 shRNA brought about a critical diminishing in glioma development in vitro (Bao et al. 2006)	Breast cancers showed loss of PTCH1 and expansion in the GLI1 region (Naylor et al. 2005), immunohistological identification of SHH was higher in breast cancers (Cui et al. 2010), Moreover, the Hh signaling pathway is activated in breast CSC (Tanaka et al. 2009)	Hh signaling components are actually activated in advanced human colon cancer cells and their CSCs (Varnat et al. 2010), GLI1 expression was increased in colon CSCs (Varnat et al. 2010). Hence, metastatic colon cancers could conceivably be treated by blocking Hh signaling (Varnat et al. 2010)

ensnared a vital role for Notch signaling in the self-renewal and maintenance of CSCs (Purow et al. 2005). Notch1 and its ligands DLL1 and JAG1 are over-expressed in the primary gliomas and cell lines and are associated with high grade gliomas and medulloblastomas (Purow et al. 2005). In medulloblastomas, up-regulated levels of Notch2 and its downstream targets have been linked with poor survival (Purow et al. 2005). Furthermore, Fan et al. (2006) demonstrated that Notch inhibition in medulloblastomas cells prompted differentiation and indicated a reduction in CD133⁺ cell frequency and HES1 expression. In human glioma cell lines, down-regulation of Notch signaling components expanded apoptosis and diminished cell proliferation (Purow et al. 2005), whereas activation of Notch expanded the formation of neurosphere colonies (Zhang et al. 2008). Notch components have been discovered to be up-regulated in the CD133⁺ fraction (Ulasov et al. 2010) and Notch hindrance in cultured glioma CSCs retarded their proliferation in vitro (Fan et al. 2010). Down-regulation of Notch components additionally sensitized CSCs to radiation or chemotherapeutic therapies and weakened xenograft tumor formation (Wang et al. 2010).

In breast cancer, activation of Notch4 has been shown to be sufficient to impel mammary adenocarcinomas, while Notch1 activation was sufficient to change mouse mammary epithelial cells in vitro and quickened tumor growth in vivo, proposing an oncogenic role for Notch receptors in mammary tumor development (Dievart et al. 1999). Expression of Notch signaling components is common in human breast cancers, with Notch activation reached about 50 % (Pece et al. 2004), whereas high JAG1 and NOTCH1 expression is associated with poor survival (Reedijk et al. 2005). Restraint of Notch1 and Notch4 signaling in both primary breast cancer samples and cell lines resulted in a decreased frequency of CD44⁺/CD24^{low} cells and a diminished capacity to form tumors in vivo (Harrison et al. 2010), whereas inhibition of Notch4 had a more vigorous impact suggesting its involvement in CSC activity.

In colon cancer, it has been suggested that Notch signaling pathway plays a role in the initiation of colon malignancy but not in its progression. Supporting this idea is the finding that Notch signals are high in early colorectal cancers (Reedijk et al. 2008) and appear to be lower in the advanced cancers (Fre et al. 2005).

Notch signaling is active in human colon CSCs (Hoey et al. 2009). In a xenograft model of OMP-C9 colon cancer cells, targeted inhibition of the Notch ligand DLL4 by utilizing an anti-DLL4 antibody hindered tumor development and lessened the expression of HES1, the proliferation of tumor cells and tumorigenicity, coinciding with lower frequency of CSCs (Hoey et al. 2009). Additionally, it was found that colon CSCs highly express Notch signaling factors and that Notch is essential for self-renewal and tumor initiation (Sikandar et al. 2010). Components of the Notch signaling pathway, including Notch1, HES1 and JAG1, were highly expressed in colon CSCs, whereas high Notch activity was detected using a reporter assay. In these studies, blocking of Notch signaling with small molecule inhibitors or shRNAs down regulated HES1 expression and instigated cells to differentiate into MUC2⁺ cells. In summary, Notch signaling is likely to be essential for colon CSC proliferation, survival, and ensuing tumor formation.

Wnt pathway

Two pathways have been recognized for Wnt signaling: the canonical pathway where Wnt signaling happens through β -catenin and is included in cell fate determination; and the non-canonical pathway where Wnt signaling, by means of a β -catenin independent pathway, is involved in cell movement and polarity. While the latter has been indicated to be important in embryogenesis, little is known about its role in cancer stem cell biology (Trowbridge et al. 2006).

In the breast cancer, Wnt signaling was first embroiled in mammary tumors when the mouse mammary tumor virus (MMTV) was found to coordinate into the Int-1 (Wnt1) locus, and overexpression of Wnt1 affected mammary tumorigenesis (Tsukamoto et al. 1988). Various reports have recognized the dysregulation of the Wnt pathway in breast tumors (Zardawi et al. 2009). Down-regulation of the secreted Frizzled-related proteins (SFRPs), which are Wnt inhibitors, has been observed in breast cancers (Veeck et al. 2008). In numerous cancers, mutations in the Wnt pathway are usually found, yet they are less common in breast cancer. Mutations within adenomatous polyposis coli (APC) have been described in 18 % of breast cancers, however mutations have not been recognized in CTNNB1 (the gene encoding β -catenin) (Geyer et al. 2011). Studies in breast cancer

cell lines have demonstrated that stem cell populations are more resistant to radiation therapy and that Wnt/ β -catenin signaling intervenes resistance (Debeb et al. 2009). Recently Zhang et al. (2010) assessed radiation resistance in CSCs isolated from p53-null mouse mammary tumors. These CSCs populations had altered DNA repair because of radiation and increased AKT (a serine–threonine protein kinase) and β -catenin activation. By utilizing the inhibitor perifosine, they were able to block AKT and β -catenin activation and sensitize the cells to radiation.

In brain cancers, aberrant WNT signaling was first implicated in brain tumors through investigation of patients with Turcot's syndrome, which includes an APC mutation connected to a high frequency of colorectal cancers and brain tumors, especially medulloblastoma (Wechsler-Reya and Scott 2001). Mutations in APC, catenin, and Axin were likewise found in sporadic medulloblastomas (Baeza et al. 2003). The Wnt antagonist DKK-1 has been ensnared as a medulloblastoma suppressor that is epigenetically silenced during tumorigenesis, and its re-actuation diminished tumor cell growth by 60 % while expanding apoptosis fourfold (Vibhakar et al. 2007). Altogether, it can be concluded that Wnt regulates self-renewal in normal stem cells and is dysregulated in a group of patients with brain tumors. However, the role of Wnt in brain CSCs has not been widely considered.

In colorectal cancer, the majority of colorectal cancer (CRC) is associated with mutations in key components of the Wnt signaling pathway (Holcombe et al. 2002). APC gene is a well-known tumor suppressor that assumes a focal role in the Wnt signaling pathway by targeting β -catenin for destruction. Dysregulated Wnt activation is likewise prominent around sporadic colon cancers. Inactivating mutations in APC are found in up to 80 % of sporadic colon cancers and activating mutations of β -catenin are additionally discovered, both of which culminate in heightened Wnt signaling (Pinto et al. 2003). Studies concentrating on the Wnt pathway and its role in colon cancer stem cell function are limited. Nonetheless, Wnt activity has been implicated to plan an essential role inside the CD133⁺ population of colorectal malignancy. Evidence for Wnt activity within colon CSCs was additionally reported by Ernst et al. (2011). It was found in this study that upon gene expression profiling of CD133⁺ versus CD133[−] cells from primary patient samples, EGR1 (Early Growth

Response Protein 1) demonstrated the highest expression within the CD133⁺ fraction. EGR1 enhances Wnt signaling through the up-regulation of TCF4, which induces the stem cell marker LGR5 (Ernst et al. 2011) (Fig. 1).

Hedgehog (Hh) pathway

The Hh signaling pathway plays important roles in embryonic development. Distinctive parts of the embryo have diverse concentrations of Hh signaling proteins (Athar et al. 2006). The Hh pathway assumes a fundamental role in the development of skin, sebaceous glands and hair follicles (Palma et al. 2005) as well as postnatal and adult brain development (Lum and Beachy 2004). The pathway gets its name from its polypeptide ligand, an intercellular signaling molecule known as Hedgehog (Hh) found in fruit flies of the genus *Drosophila* (Athar et al. 2006). Components of the mammalian Hh pathway includes the ligands Sonic (SHH), Desert (DHH) and Indian (IHH) Hh, the membrane receptor Patched (PTCH1/2), signal transducer Smoothened (SMO) and the G protein coupled receptor, and the downstream mediators of gene expression, the GLI zinc finger transcription factors (GLI1, GLI2 and GLI3) (Lum and Beachy 2004). GLIs go about as repressors in the absence of Hh signaling. In the absence of the ligand, PTCH1 suppresses SMO and averts GLI modulation (Lum and Beachy 2004).

Sonic Hedgehog (SHH) is one of the best studied pathways in CNS development and it is well known for its role in brain growth and patterning during development (Dessaud et al. 2008). The distinguishing role of signaling pathways in medulloblastoma emerged from the discovery that patients with Gorlin syndrome have inactivating PTCH1 mutations, and that they have a higher inclination for developing medulloblastoma and skin basal cell carcinomas (Hahn et al. 1996). In mouse models, changes in *Ptch1* accelerate the advancement of medulloblastoma (Goodrich et al. 1997). Furthermore, numerous sporadic human medulloblastoma harbors mutations in the Hh pathway (Manoranjan et al. 2012).

Bar et al. (2007) found that the transcription factor GLI1 is highly expressed in 26 % of primary glioblastoma multiforme (GBM), and 57 % of cell lines, and the ligand SHH is expressed in GBM-derived tumorspheres. Restraint of GLI1 with cyclopamine or

Fig. 1 Signaling pathways involved in CSCs. CSC function relies on various signaling pathways like Notch, Wnt and Hh signaling pathways. **A** Wnt pathway is initiated by binding of Wnt to its frizzled receptor. Mutations in APC gene is common in many CSCs leading to over activation of β -catenin promoting

carcinogenesis. **B** Over activity of NICD, a component of the Notch pathway, leads to gene overexpression promoting carcinogenesis. **C** In the Hh pathway, mutation in PTCH1 gene leads to SMO over activity and promotes uncontrolled growth and carcinogenesis

GLI1 shRNA diminished glioma development in vitro as well as primary and secondary sphere formation, indicating the depletion of CSCs. ALDH1 activity and dye exclusion, which are connected with stem-like phenotypes, were also diminished upon restraining GLI1 with cyclopamine treatment. In concurrence with the studies demonstrating that CD133⁺ GBM stem-like cells are impervious to radiation therapy (Bao et al. 2006), other studies indicated that combining cyclopamine with radiation treatment caused a reduction in growth rate in vitro (Clement et al. 2007).

In breast cancers, comparative genomic hybridization analysis of human breast cancer specimens and cell lines uncovered a common loss in the chromosomal PTCH1 region (19 % of samples) and expansion in the GLI1 region (Naylor et al. 2005). PTCH1 was likewise demonstrated to be silenced through promoter methylation (Wolf et al. 2007). SMO is expressed at higher amounts in Ductal carcinoma in situ and invasive ductal carcinoma (IDC), where its constitutive activation in mouse mammary glands brings about ductal dysplasia (Moraes et al. 2009). Immunohistological identification of SHH was higher in breast cancers comparing with normal tissue (Cui et al. 2010). Moreover, the Hh signaling pathway was

discovered to be activated in the CSC-enriched CD44⁺CD24^{-/low} population of breast cancer (Tanaka et al. 2009). The strongest confirmation for Hh involvement in breast carcinogenesis originates from a study in which restrictive over-expression of GLI1 alone in the mouse mammary gland was sufficient to impel tumor formation (Fiaschi et al. 2009). Altogether, these studies suggest that the Hh signaling pathway is fundamental for self-renewal and proliferation of breast CSCs. Further studies may need to explore the role of Hh in stem cell maintenance, epithelial–stromal interactions and EMT, to identify contributions by this pathway to the initiation and progression of breast cancer.

In colon cancers, Hh activation occurs by means of transcriptional up-regulation of the Hh ligands rather than mutation (Theunissen and de Sauvage 2009). Increased transcript levels of SHH and its downstream target GLI2 are revealed in colon cancers (Douard et al. 2006). Expression of IHH, which is included in the differentiation of enterocytes, has been suggested to be lost in early phases of human colorectal carcinogenesis (van den Brink et al. 2004). It is suggested, however, that Hh signaling increases with colon cancer progression (Yoshikawa et al. 2009). It

was found further that Hh signaling components are actually activated in advanced human colon cancer cells and their CSCs, where GLI1 expression was higher in CD133⁺ cells from patients with advance metastatic cancers (Varnat et al. 2010). This study showed also that active Hh signaling is crucial for the survival and development of the CD133⁺ cells in vivo. Hence, metastatic colon cancers could conceivably be treated by blocking Hh signaling (Varnat et al. 2010).

Conclusion and perspectives

While there has been much debate encompassing the CSC hypothesis, it is clear that there is a subpopulation of cells in numerous tumors that share the molecular signatures with stem cells. Intriguingly, despite the possible biological heterogeneity of the distinctive histological origin of CSCs, they appear to be of low immunogenicity with immune suppressive activity comparing with non-CSC cancerous cells. This fits well with the assumed function credited to CSCs i.e. their capacity to survive in order to give rise to new, more differentiated cancerous cells that will permit the development and expansion of the tumor in the body.

While the Notch, Wnt and Hh pathways clearly play paramount roles in normal and CSCs, there are many additional pathways that likewise also participate in the maintenance of tissue homeostasis whose deregulation can prompt cancer growth. Thus if CSCs are to be targeted therapeutically, a combinational strategy has to be adopted to target multiple signaling pathways. On the other hand, the molecular identification of shared CSC antigens and elucidation of the mechanism of CSC to evade immune surveillance may inspire the development of immunotherapeutic strategies that are broadly effective against cancers, regardless of their origin.

In summary, intensive investigations on the part of developmental signaling pathways and immunobiological properties of CSCs introduce an opportunity for productive collaborations of multiple disciplines including developmental biology, immunology and oncology. Future researches are promised to develop novel therapeutic strategies to target CSCs, thus offering new modalities to end tumor development and eliminate recurrence.

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References

- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 100:3983–3988
- Athar M, Tang X, Lee JL, Kopelovich L, Kim AL (2006) Hedgehog signalling in skin development and cancer. *Exp Dermatol* 15:667–677
- Avril T, Vauleon E, Hamlat A, Saikali S, Etcheverry A, Delmas C, Diabira S, Mosser J, Quillien V (2011) Human glioblastoma stem-like cells are more sensitive to allogeneic NK and T cell-mediated killing compared with serum-cultured glioblastoma cells. *Brain Pathol* 22:159–174
- Baeza N, Masuoka J, Kleihues P, Ohgaki H (2003) AXIN1 mutations but not deletions in cerebellar medulloblastomas. *Oncogene* 22:632–636
- Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444:756–760
- Bar EE, Chaudhry A, Lin A, Fan X, Schreck K, Matsui W, Piccirillo S, Vescovi AL, DiMeco F, Olivi A, Eberhart CG (2007) Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. *Stem Cells* 25:2524–2533
- Blank U, Karlsson G, Karlsson S (2008) Signaling pathways governing stem-cell fate. *Blood* 111:492–503
- Boiko AD, Razorenova OV, van de Rijn M, Swetter SM, Johnson DL, Ly DP, Butler PD, Yang GP, Joshua B, Kaplan MJ, Longaker MT, Weissman IL (2010) Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. *Nature* 466:133–137
- Brewer BG, Mitchell RA, Harandi A, Eaton JW (2009) Embryonic vaccines against cancer: an early history. *Exp Mol Pathol* 86:192–197
- Chen Y, Yu D, Zhang H, He H, Zhang C, Zhao W, Shao RG (2011) CD133(+) EpCAM(+) phenotype possesses more characteristics of tumor initiating cells in hepatocellular carcinoma Huh7 cells. *Int J Biol Sci* 8:992–1004
- Choi D, Lee HW, Hur KY, Kim JJ, Park GS, Jang SH, Song YS, Jang KS, Paik SS (2009) Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. *World J Gastroenterol* 15:2258–2264
- Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A (2007) HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol* 17:165–172

- Clevers H (2011) The cancer stem cell: premises, promises and challenges. *Nat Med* 17:313–319
- Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ (2005) Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 65:10946–10951
- Comber JD, Philip R (2014) MHC class I antigen presentation and implications for developing a new generation of therapeutic vaccines. *Ther Adv Vaccines* 2:77–89
- Cui W, Wang LH, Wen YY, Song M, Li BL, Chen XL, Xu M, An SX, Zhao J, Lu YY, Mi XY, Wang EH (2010) Expression and regulation mechanisms of Sonic Hedgehog in breast cancer. *Cancer Sci* 101:927–933
- Debeb BG, Xu W, Woodward WA (2009) Radiation resistance of breast cancer stem cells: understanding the clinical framework. *J Mammary Gland Biol Neoplasia* 14:11–17
- Dessaud E, McMahon AP, Briscoe J (2008) Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. *Development* 135:2489–2503
- Di Tomaso T, Mazzoleni S, Wang E, Sovena G, Clavenna D, Franzin A, Mortini P, Ferrone S, Doglioni C, Marincola FM, Galli R, Parmiani G, Maccalli C (2010) Immunobiological characterization of cancer stem cells isolated from glioblastoma patients. *Clin Cancer Res* 16:800–813
- Dievert A, Beaulieu N, Jolicoeur P (1999) Involvement of Notch1 in the development of mouse mammary tumors. *Oncogene* 18:5973–5981
- Dong W, Du J, Shen H, Gao D, Li Z, Wang G, Mu X, Liu Q (2010) Administration of embryonic stem cells generates effective antitumor immunity in mice with minor and heavy tumor load. *Cancer Immunol Immunother* 59:1697–1705
- Dong W, Qiu C, Shen H, Liu Q, Du J (2013) Antitumor effect of embryonic stem cells in a non-small cell lung cancer model: antitumor factors and immune responses. *Int J Med Sci* 10:1314–1320
- Douard R, Moutereau S, Pernet P, Chimingqi M, Allory Y, Manivet P, Conti M, Vaubourdolle M, Cugnenc PH, Loric S (2006) Sonic Hedgehog-dependent proliferation in a series of patients with colorectal cancer. *Surgery* 139:665–670
- Ellebaek E, Andersen MH, Svane IM, Straten PT (2012) Immunotherapy for metastatic colorectal cancer: present status and new options. *Scand J Gastroenterol* 47:315–324
- Ernst A, Aigner M, Nakata S, Engel F, Schlotter M, Kloor M, Brand K, Schmitt S, Steinert G, Rahbari N, Koch M, Radlwimmer B, Weitz J, Lichter P (2011) A gene signature distinguishing CD133hi from CD133– colorectal cancer cells: essential role for EGR1 and downstream factors. *Pathology* 43:220–227
- Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, Eberhart CG (2006) Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res* 66:7445–7452
- Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, Gul N, Koh C, Zhang J, Li YM, Maciacyk J, Nikkhah G, Dimeco F, Piccirillo S, Vescovi AL, Eberhart CG (2010) NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells* 28:5–16
- Fiaschi M, Rozell B, Bergstrom A, Toftgard R (2009) Development of mammary tumors by conditional expression of GLI1. *Cancer Res* 69:4810–4817
- Francipane MG, Alea MP, Lombardo Y, Todaro M, Medema JP, Stassi G (2008) Crucial role of interleukin-4 in the survival of colon cancer stem cells. *Cancer Res* 68:4022–4025
- Fre S, Huyghe M, Mourikis P, Robine S, Louvard D, Artavanis-Tsakonas S (2005) Notch signals control the fate of immature progenitor cells in the intestine. *Nature* 435:964–968
- Gedye C, Quirk J, Browning J, Svobodova S, John T, Sluka P, Dunbar PR, Corbeil D, Cebon J, Davis ID (2009) Cancer/testis antigens can be immunological targets in clonogenic CD133+ melanoma cells. *Cancer Immunol Immunother* 58:1635–1646
- Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A, Natrajan R, Reis-Filho JS (2011) Beta-catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. *Mod Pathol* 24:209–231
- Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G (2007) ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 1:555–567
- Goodrich LV, Milenkovic L, Higgins KM, Scott MP (1997) Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* 277:1109–1113
- Hahn H, Wicking C, Zaphiropoulos PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toftgard R, Chenevix-Trench G, Wainwright B, Bale AE (1996) Mutations of the human homolog of *Drosophila* patched in the nevus basal cell carcinoma syndrome. *Cell* 85:841–851
- Harrison H, Farnie G, Howell SJ, Rock RE, Stylianou S, Brennan KR, Bundred NJ, Clarke RB (2010) Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. *Cancer Res* 70:709–718
- Hilton J (1984) Role of aldehyde dehydrogenase in cyclophosphamide-resistant L1210 leukemia. *Cancer Res* 44:5156–5160
- Hjelmeland AB, Rich JN (2012) The quest for self-identity: not all cancer stem cells are the same. *Clin Cancer Res* 18:3495–3498
- Hoey T, Yen WC, Axelrod F, Basi J, Donigian L, Dylla S, Fitch-Bruhns M, Lazetic S, Park IK, Sato A, Satyal S, Wang X, Clarke MF, Lewicki J, Gurney A (2009) DLL4 blockade inhibits tumor growth and reduces tumor-initiating cell frequency. *Cell Stem Cell* 5:168–177
- Holcombe RF, Marsh JL, Waterman ML, Lin F, Milovanovic T, Truong T (2002) Expression of Wnt ligands and Frizzled receptors in colonic mucosa and in colon carcinoma. *Mol Pathol* 55:220–226
- Kalluri R, Weinberg RA (2009) The basics of epithelial–mesenchymal transition. *J Clin Invest* 119:1420–1428
- Kelly PN, Strasser A (2011) The role of Bcl-2 and its pro-survival relatives in tumorigenesis and cancer therapy. *Cell Death Differ* 18:1414–1424

- Koshio J, Kagamu H, Nozaki K, Saida Y, Tanaka T, Shoji S, Igarashi N, Miura S, Okajima M, Watanabe S, Yoshizawa H, Narita I (2013) DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked is an immunogenic target of cancer stem cells. *Cancer Immunol Immunother* 62:1619–1628
- Lai D, Wang F, Chen Y, Wang C, Liu S, Lu B, Ge X, Guo L (2012) Human ovarian cancer stem-like cells can be efficiently killed by gammadelta T lymphocytes. *Cancer Immunol Immunother* 61:979–989
- Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE (1994) A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 367:645–648
- Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O (2003) HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 31:890–896
- Li Y, Laterra J (2012) Cancer stem cells: distinct entities or dynamically regulated phenotypes? *Cancer Res* 72:576–580
- Li C, Lee CJ, Simeone DM (2009a) Identification of human pancreatic cancer stem cells. *Methods Mol Biol* 568:161–173
- Li Y, Zeng H, Xu RH, Liu B, Li Z (2009b) Vaccination with human pluripotent stem cells generates a broad spectrum of immunological and clinical responses against colon cancer. *Stem Cells* 27:3103–3111
- Lucas S, Coulie PG (2008) About human tumor antigens to be used in immunotherapy. *Semin Immunol* 20:301–307
- Lum L, Beachy PA (2004) The Hedgehog response network: sensors, switches, and routers. *Science* 304:1755–1759
- Maccalli C, Volonte A, Cimminiello C, Parmiani G (2013) Immunology of cancer stem cells in solid tumours. A review. *Eur J Cancer* 50:649–655
- Magee JA, Piskounova E, Morrison SJ (2012) Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell* 21:283–296
- Manoranjan B, Venugopal C, McFarlane N, Doble BW, Dunn SE, Scheinemann K, Singh SK (2012) Medulloblastoma stem cells: where development and cancer cross pathways. *Pediatr Res* 71:516–522
- Moraes RC, Chang H, Harrington N, Landua JD, Prigge JT, Lane TF, Wainwright BJ, Hamel PA, Lewis MT (2009) Pth1 is required locally for mammary gland morphogenesis and systemically for ductal elongation. *Development* 136:1423–1432
- Naylor TL, Greshock J, Wang Y, Colligon T, Yu QC, Clemmer V, Zaks TZ, Weber BL (2005) High resolution genomic analysis of sporadic breast cancer using array-based comparative genomic hybridization. *Breast Cancer Res* 7:R1186–R1198
- Ning N, Pan Q, Zheng F, Teitz-Tennenbaum S, Egenti M, Yet J, Li M, Ginestier C, Wicha MS, Moyer JS, Prince ME, Xu Y, Zhang XL, Huang S, Chang AE, Li Q (2012) Cancer stem cell vaccination confers significant antitumor immunity. *Cancer Res* 72:1853–1864
- Nishizawa S, Hirohashi Y, Torigoe T, Takahashi A, Tamura Y, Mori T, Kanaseki T, Kamiguchi K, Asanuma H, Morita R, Sokolovskaya A, Matsuzaki J, Yamada R, Fujii R, Kampinga HH, Kondo T, Hasegawa T, Hara I, Sato N (2012) HSP DNAJB8 controls tumor-initiating ability in renal cancer stem-like cells. *Cancer Res* 72:2844–2854
- Palma V, Lim DA, Dahmane N, Sanchez P, Brionne TC, Herzberg CD, Gitton Y, Carleton A, Alvarez-Buylla A, Ruiz i Altaba A (2005) Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. *Development* 132:335–344
- Parmiani G, Russo V, Marrari A, Cutolo G, Casati C, Pilla L, Maccalli C, Rivoltini L, Castelli C (2007) Universal and stemness-related tumor antigens: potential use in cancer immunotherapy. *Clin Cancer Res* 13:5675–5679
- Pece S, Serresi M, Santolini E, Capra M, Hulleman E, Galimberti V, Zurrida S, Maisonneuve P, Viale G, Di Fiore PP (2004) Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. *J Cell Biol* 167:215–221
- Pinto D, Gregorieff A, Begthel H, Clevers H (2003) Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev* 17:1709–1713
- Purov BW, Haque RM, Noel MW, Su Q, Burdick MJ, Lee J, Sundaresan T, Pastorino S, Park JK, Mikolaenko I, Maric D, Eberhart CG, Fine HA (2005) Expression of Notch-1 and its ligands, Delta-like-1 and Jagged-1, is critical for glioma cell survival and proliferation. *Cancer Res* 65:2353–2363
- Reedijk M, Odorcic S, Chang L, Zhang H, Miller N, McCready DR, Lockwood G, Egan SE (2005) High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. *Cancer Res* 65:8530–8537
- Reedijk M, Odorcic S, Zhang H, Chetty R, Tennert C, Dickson BC, Lockwood G, Gallinger S, Egan SE (2008) Activation of Notch signaling in human colon adenocarcinoma. *Int J Oncol* 33:1223–1229
- Saini V, Hose CD, Monks A, Nagashima K, Han B, Newton DL, Millione A, Shah J, Hollingshead MG, Hite KM, Burkett MW, Delosh RM, Silvers TE, Scudiero DA, Shoemaker RH (2012) Identification of CBX3 and ABCA5 as putative biomarkers for tumor stem cells in osteosarcoma. *PLoS One* 7:e41401
- Schatton T, Frank MH (2009) Antitumor immunity and cancer stem cells. *Ann N Y Acad Sci* 1176:154–169
- Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, Fuhlbrigge RC, Kupper TS, Sayegh MH, Frank MH (2008) Identification of cells initiating human melanomas. *Nature* 451:345–349
- Schatton T, Schutte U, Frank NY, Zhan Q, Hoerning A, Robles SC, Zhou J, Hodi FS, Spagnoli GC, Murphy GF, Frank MH (2010) Modulation of T-cell activation by malignant melanoma initiating cells. *Cancer Res* 70:697–708
- Sikandar SS, Pate KT, Anderson S, Dizon D, Edwards RA, Waterman ML, Lipkin SM (2010) NOTCH signaling is required for formation and self-renewal of tumor-initiating cells and for repression of secretory cell differentiation in colon cancer. *Cancer Res* 70:1469–1478
- Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004) Identification of human brain tumour initiating cells. *Nature* 432:396–401

- Tanaka H, Nakamura M, Kameda C, Kubo M, Sato N, Kuroki S, Tanaka M, Katano M (2009) The Hedgehog signaling pathway plays an essential role in maintaining the CD44+ CD24−/low subpopulation and the side population of breast cancer cells. *Anticancer Res* 29:2147–2157
- Theunissen JW, de Sauvage FJ (2009) Paracrine Hedgehog signaling in cancer. *Cancer Res* 69:6007–6010
- Trowbridge JJ, Moon RT, Bhatia M (2006) Hematopoietic stem cell biology: too much of a Wnt thing. *Nat Immunol* 7:1021–1023
- Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE (1988) Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. *Cell* 55:619–625
- Ulasov IV, Nandi S, Dey M, Sonabend AM, Lesniak MS (2010) Inhibition of Sonic hedgehog and Notch pathways enhances sensitivity of CD133(+) glioma stem cells to temozolomide therapy. *Mol Med* 17:103–112
- van den Brink GR, Bleuming SA, Hardwick JC, Schepman BL, Offerhaus GJ, Keller JJ, Nielsen C, Gaffield W, van Deventer SJ, Roberts DJ, Peppelenbosch MP (2004) Indian Hedgehog is an antagonist of Wnt signaling in colonic epithelial cell differentiation. *Nat Genet* 36:277–282
- Varnat F, Zacchetti G, Ruiz i Altaba A (2010) Hedgehog pathway activity is required for the lethality and intestinal phenotypes of mice with hyperactive Wnt signaling. *Mech Dev* 127:73–81
- Veeck J, Geisler C, Noetzel E, Alkaya S, Hartmann A, Knuchel R, Dahl E (2008) Epigenetic inactivation of the secreted frizzled-related protein-5 (SFRP5) gene in human breast cancer is associated with unfavorable prognosis. *Carcinogenesis* 29:991–998
- Vibhakkar R, Foltz G, Yoon JG, Field L, Lee H, Ryu GY, Pierson J, Davidson B, Madan A (2007) Dickkopf-1 is an epigenetically silenced candidate tumor suppressor gene in medulloblastoma. *Neuro Oncol* 9:135–144
- Visus C, Wang Y, Lozano-Leon A, Ferris RL, Silver S, Szczepanski MJ, Brand RE, Ferrone CR, Whiteside TL, Ferrone S, DeLeo AB, Wang X (2011) Targeting ALDH(bright) human carcinoma-initiating cells with ALDH1A1-specific CD8(+) T cells. *Clin Cancer Res* 17:6174–6184
- Wang J, Wakeman TP, Lathia JD, Hjelmeland AB, Wang XF, White RR, Rich JN, Sullenger BA (2010) Notch promotes radioresistance of glioma stem cells. *Stem Cells* 28:17–28
- Wang YC, Yo YT, Lee HY, Liao YP, Chao TK, Su PH, Lai HC (2012) ALDH1-bright epithelial ovarian cancer cells are associated with CD44 expression, drug resistance, and poor clinical outcome. *Am J Pathol* 180:1159–1169
- Wechsler-Reya R, Scott MP (2001) The developmental biology of brain tumors. *Annu Rev Neurosci* 24:385–428
- Wolf I, Bose S, Desmond JC, Lin BT, Williamson EA, Karlan BY, Koeffler HP (2007) Unmasking of epigenetically silenced genes reveals DNA promoter methylation and reduced expression of PTCH in breast cancer. *Breast Cancer Res Treat* 105:139–155
- Yaddanapudi K, Mitchell RA, Putty K, Willer S, Sharma RK, Yan J, Bodduluri H, Eaton JW (2012) Vaccination with embryonic stem cells protects against lung cancer: is a broad-spectrum prophylactic vaccine against cancer possible? *PLoS One* 7:e42289
- Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PW, Lam CT, Poon RT, Fan ST (2008) Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell* 13:153–166
- Yoshikawa K, Shimada M, Miyamoto H, Higashijima J, Miyatani T, Nishioka M, Kurita N, Iwata T, Uehara H (2009) Sonic hedgehog relates to colorectal carcinogenesis. *J Gastroenterol* 44:1113–1117
- Zardawi SJ, O'Toole SA, Sutherland RL, Musgrove EA (2009) Dysregulation of Hedgehog, Wnt and Notch signalling pathways in breast cancer. *Histol Histopathol* 24:385–398
- Zhang XP, Zheng G, Zou L, Liu HL, Hou LH, Zhou P, Yin DD, Zheng QJ, Liang L, Zhang SZ, Feng L, Yao LB, Yang AG, Han H, Chen JY (2008) Notch activation promotes cell proliferation and the formation of neural stem cell-like colonies in human glioma cells. *Mol Cell Biochem* 307:101–108
- Zhang M, Atkinson RL, Rosen JM (2010) Selective targeting of radiation-resistant tumor-initiating cells. *Proc Natl Acad Sci USA* 107:3522–3527