Rb Pathway

Retinoblastoma (Rb) is another essential tumor suppressor protein that regulates the G1 checkpoint (Classon and Harlow 2002). Hypophosphorylated form of Rb sequesters E2F transcription factor and arrest cells at the G1 checkpoint. Once Rb is hyperphosphorylated by cyclin D and cdk4/6 complex, phosphorylated Rb releases E2F, E2F induces the expression of cell cycle regulators, and then the cells enter S phase. In contrast, p16/Ink4a cdk inhibitor binds to cdk4/6, prevents the complex formation of cdk4/6 and cyclin D, and maintains Rb hypophosphorylation. Mutations in Rb pathway have been frequently identified in many types of malignant tumors. For example, mutations in Rb signaling pathway, including cdk4 amplification and p16/Ink4a deletion, was found in about 80% of GBM (Cancer Genome Atlas Research Network 2008; Parsons et al. 2008; Schmidt et al. 1994).

Activation of Receptor Tyrosine Kinase Pathway

Signaling pathways (Ras/Raf/MAPK and PTEN/AKT pathways) of Receptor Tyrosine Kinases (RTKs) including PDGFR, EGFR, FGFR, and IGFR, many of which play a role for the maintenance of TSCs and amplifying precursors, are frequently mutated in tumors (Schubbert et al. 2007). For instance, activation of RTK pathway was found in about 90% of GBM (Cancer Genome Atlas Research Network 2008; Parsons et al. 2008). In particularly, it has been shown that small GTP protein Ras, one of essential oncogenes, and its negative regulator, type1 Neurofibromas gene (NF1), are mutated in many kinds of human cancers and that phosphatase tensin homolog (PTEN), which inhibits function of phosphoinositol tri-phosphate kinase (PI3K) that activates Akt, is frequently inactivated in malignant tumors (Duerr et al. 1998).

Notch Signaling Pathway

Notch receptors are involved in a number of biological functions, including cell proliferation, differentiation, survival, and tumorigenesis (Radtke and Raj 2003). There are four known mammalian Notch receptors, Notch 1–4, and five ligands, Delta-like-ligand (Dll) 1, 3, and 4, and Jagged 1 and 2 in mammals. Following the activation, Notch is cleaved in its extracellular region by metalloproteases and in its intracellular region by presenilins (PS), releasing the Notch intracellular domain (NICD) from the plasma membrane. The NICD then translocates into the nucleus, associates with the CSL transcription factor CBF1/RBP-Jk, and activates a number of target genes, including the hairy and enhancer-of-split (Hes) genes

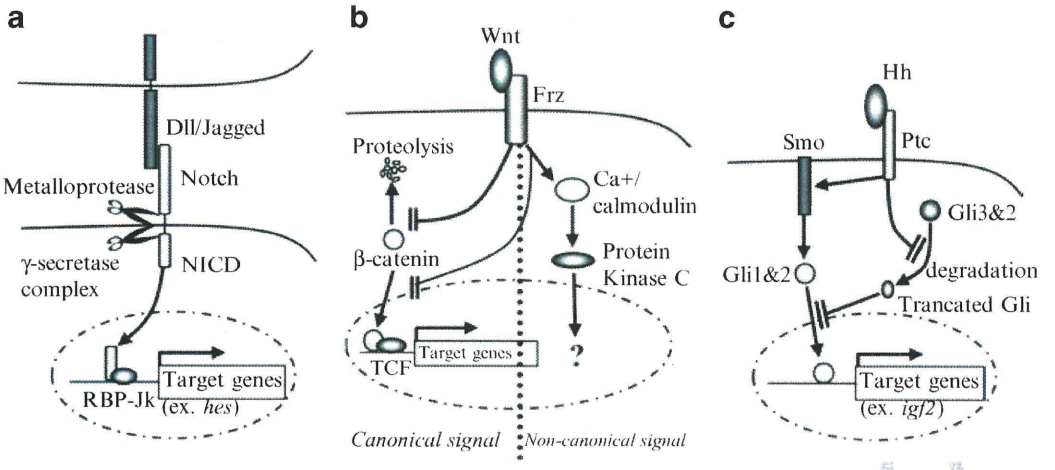

Fig. 2.2 Notch, Wnt, Hh signaling pathways are involved in CSC maintenance. Notch (**a**), Wnt/Frz (**b**), or Hh/Ptc/Smo (**c**) signaling pathway activates a number of genes, which regulate cell proliferation and cell fates. The constitutive activation of any of these pathways leads to abnormal development and tumorigenesis

(Fig. 2.2a). It has been shown that the inactivation of Notch signaling leads to serious developmental defects: Jagged1, Notch1, Notch2, and PS1and 2 knockout mice are all embryonically or perinatally lethal (Krebs et al. 2000; Swiatek et al. 1994; Xue et al. 1999). There is accumulating evidence that Notch activation not only maintains the multipotentiality of NSCs but is also involved in tumorigenesis. Depletion of Notch1, Dll1, or Jagged1 by RNAi was shown to block proliferation of glioma cells in vivo and in vitro (Purow et al. 2005). Together, these findings suggest that Notch signaling is involved in tumorigenesis, as well as in normal development.

Wnt Signaling Pathway

The Wnt family of secreted proteins coordinates diverse developmental processes, including cell proliferation and fate decisions (Logan and Nusse 2004; Moon et al. 2004; Reya and Clevers 2005). In mammals, there are 20 Wnt members, 10 Wnt receptors (called Frizzled, Frz), and 5 soluble forms of Frz, which are natural inhibitors of Wnt signaling. Once Frz is activated, β-catenin, which is a central player in canonical Wnt signaling, accumulates in the nucleus and induces the expression of Wnt target genes, including *c-myc* and *cyclin D1*, by associating with LEF/TCF transcription factors (Fig. 2.2b). The noncanonical Wnt signaling pathway activates calcium/calmodulin-dependent protein kinase and protein kinase C, although the molecular details are still uncertain (Logan and Nusse 2004; Moon et al. 2004; Reya and Clevers 2005). [AU4]

Wnt signaling is also crucial for CNS development. Wnt1 and 3a, Frz5 and 8, and β-catenin, for example, are expressed in the ventricular and subventricular [AU5] zones (VZ/SVZ) in the developing brain (Chenn et al. 2000; Ikeya et al. 1999; Lee et al. 2000). Inactivation of Wnt1, Wnt3a, or β-catenin causes developmental brain defects (McMahon and Bradley 1990; Reya and Clevers 2005). Moreover, overexpression of a stabilized form of β-catenin in neural precursor cells caused a hyperplasia of lateral ventricles (Chenn et al. 2000). Some factors in the Wnt signaling pathway, including β-catenin and axin1 (an inhibitor in the pathway), are mutated in medulloblastomas (Dahmen et al. 2001; Zurawel et al. 1998). Thus these findings suggest that hyper-activation of Wnt signaling may promote brain tumorigenesis.

Hedgehog Signaling Pathway

Hh signaling is also involved in proliferation, development, and tumorigenesis [AU6] (Pasca di Magliano and Hebrok 2003; Ruiz i Altaba et al. 2002a, b). In mammals, there are three Hh members, Sonic, Desert, and Indian, all of which are secreted proteins. When Sonic Hh (Shh), for example, binds to the Patched1 (Ptc1) transmembrane receptor, another transmembrane protein, Smoothened (Smo), which is normally restrained by Ptc, is relieved and activates the zinc-finger transcription factor Gli. Activated Gli accumulates in the nucleus and induces the expression of target genes, including *wnt*, *insulin-growth factor 2 (igf2)*, and *pdgf receptor α* [AU7] (Fig. 2.2c). There are three Gli transcription factors in mammals. Gli1 and 2 function as activators of Shh signaling, whereas the cleaved form of either Gli2 or Gli3 antagonizes the Shh-Gli1/2 signaling pathway. The Shh signaling pathway is essential for CNS development: Shh, Ptc, Gli2, or Gli3 knockout mice die before birth with severe defects in the brain, although Gli1 knockout mice develop normally (Ding et al. 1998; Matise et al. 1998; Palma and Ruiz i Altaba 2004; Park et al. 2000). Conditional inactivation of Smo blocks NSC proliferation in vivo and in vitro (Machold et al. 2003). Together with the finding that Glis, Ptc1, and Smo are all expressed in the VZ/SVZ, these observations suggest that Shh signaling may be essential for the maintenance of NSCs.

Ectopic activation of Hh signaling in CNS is likely to lead to brain tumor formation (Pasca di Magliano and Hebrok 2003; Ruiz i Altaba et al. 2002a, b). For example, Gli1 is highly activated in many brain cancers, including medulloblastoma, glioblastoma, and primitive neuroectodermal tumors, some of which also have mutations in Ptc1 (Goodrich et al. 1997). It was shown that overexpression of Gli1 in the developing tadpole CNS gives rise to brain tumors (Dahmane et al. 2001). Moreover, cyclopamine, which is a specific inhibitor of Smo, blocks the growth of several primary gliomas, medulloblastomas, and glioma cell lines (Berman et al. 2002; Dahmane et al. 2001). Taken together, these findings suggest that Hh signaling plays an important role in brain tumorigenesis.

CSC Models

In Vivo Models

Using a combination of transgenic mice and a retrovirus system, some groups have demonstrated that TSCs and differentiating cells form tumors in vivo. For instance, Holland and his colleagues infected transgenic mice that expressed the avian leukosis virus (ALV) receptor under the regulation of either a *nestin* enhancer or a *gfap* promoter, with recombinant ALVs encoding oncogenic genes, such as platelet-derived growth factor (PDGF) receptor beta, or activated Akt, or activated Ras, and found GBM had developed in the brain (Dai et al. 2001; Uhrbom et al. 2002). De Pinho and colleagues overexpressed a constitutively active form of epidermal growth factor (EGF) receptor in either NSCs or astrocytes from Ink4a/Arf$^{-/-}$ mice, transplanted them into the brain, and found that the cells formed high-grade gliomas (Bachoo et al. 2002). Thus, these findings suggest that NSCs and astrocytes are cells of origin for brain tumors. However, since tumors would be, in theory, generated from one transformed cell, these tumor models, in which many transformed cells are generated or injected at the same time, may not provide an answer to whether NSCs and astrocytes are *bona fide* cells of origin for malignant glioma.

In Vitro Models

It still remains controversial whether CSCs arise from TSCs, committed precursor cells, or differentiated cells. In addition, the relationship between cell of origin for CSCs and genetic alterations have not yet been elucidated, although a number of oncogenes and tumor suppressor genes have been well characterized in tumorigenesis. Using cell lineage markers and new methods including FACS, it is possible to purify the cells. We can then overexpress oncogenes or knock down tumor-suppressor genes in the cells, examine the relationship between cell of origin for tumors and genetic alterations and find therapeutic targets (Fig. 2.3). Indeed, it has been demonstrated that overexpression of exogenous oncogenes can induce hematopoietic stem/progenitor cells to transform into leukemic stem cells (Cozzio et al. 2003; Huntly et al. 2004; Krivtsov et al. 2006). We and others also succeeded in generating glioma stem cells by overexpressing glioma-related oncogenes in neural lineage cells and in finding therapeutic targets by comparing gene expression profile of induced CSC models with that of human tumor spheres (Hide et al. 2009; Ligon et al. 2007). Thus these data suggest that, using similar methods, we might generate any CSCs from TSCs, amplifying precursor cells and/or differentiated cells, characterize them, and identify targets for curable therapy.

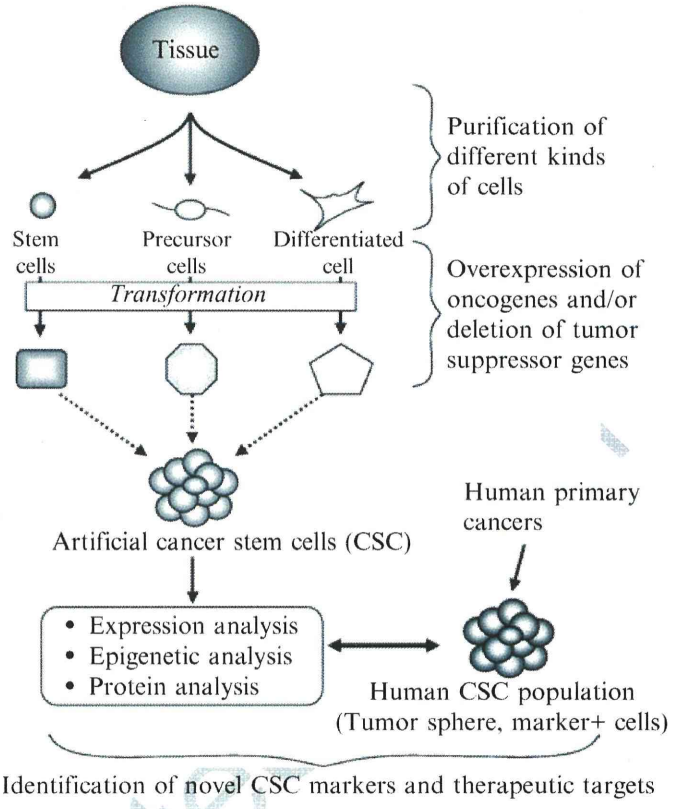

Fig. 2.3 Strategy for identifying factors specific to CSCs. Purified TSCs, committed precursor cells, and differentiated cells that are transfected with various types of oncogenes and/or siRNA/shRNA for tumor suppressor genes, transform into CSCs that are capable of self-renewal, positive for TSC markers and show malignancy. By comparing gene expression profiles of such induced CSCs with that of human CSC-enriched population (tumor spheres and TSC marker-positive cells), novel CSC markers and therapeutic targets would be identified

Conclusion

A number of new stem cell markers and techniques have been utilized to identify and purify CSCs during last several years. However, it is not yet known whether or not such CSCs consist of homogenous population, as $CD133^-$ and non-SP cells as well as $CD133^+$ and SP cells contain tumorigenic cells. Therefore it is still essential to establish experimental strategies, including the single cell analysis, to identify bona fide CSCs and to characterize them, leading to the discovery of novel therapeutic targets and methods.

Conflict of Interest

None declared.

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Queries	Details Required	Author's Response
AU1	Please check clarity of the sentence beginning "Finally, stem cells and precursor cells…"	
AU2	Please provide publication details for "Reed et al. 2009" (or) delete this citation from the text.	
AU3	Please check whether the cross-reference "Fig. 2.3c" could be changed to "Fig. 2.2c".	
AU4	Please note that cross-reference "Fig. 2.3B" has been changed to "Fig. 2.2b" given in the sentence "Once Frz is activated…"	
AU5	Please provide publication details for "Chenn et al. 2000" (or) delete these citations from the text.	
AU6	"a, b" have been introduced in the reference Ruiz i Altaba et al. (2002) since two such references exist in the list. Please retain the appropriate one and check for the same throughout the text.	
AU7	Please note that cross-reference "Fig. 2.3C" has been changed to "Fig. 2.2c" given in the sentence "Activated Gli…"	
AU8	References Blazek et al. (2007), Chenn and Walsh (2002), Bredel and Zentner (2002), Hayflick (1965), Matsui et al. (2004), Read et al. (2009), Suetsugu et al. (2006), and Yuan et al. (2004) are not cited in the text. Please check.	