Stem Cells and Cancer Stem Cells 5
Therapeutic Applications in Disease and Injury

M.A. Hayat
Editor

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Volume 5
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Although touched by technology, surgical pathology always has been, and remains, an art. Surgical pathologists, like all artists, depict in their artwork (surgical pathology reports) their interactions with nature: emotions, observations, and knowledge are all integrated. The resulting artwork is a poor record of complex phenomena.

Richard J. Reed, MD

One Point of View

All small tumors do not always keep growing, especially small breast tumors, testicular tumors, and prostate tumors. Some small tumors may even disappear without a treatment. Indeed, because prostate tumor grows slowly, it is not unusual that a patient may die at an advanced age of some other causes, but prostate tumor is discovered in an autopsy study. In some cases of prostate tumors, the patient should be offered the option of active surveillance followed by PSA test or biopsies. Similarly, every small kidney tumor may not change or may even regress. Another example of cancer or precancer reversal is cervical cancer. Precancerous cervical cells found with Pap test, may revert to normal cells. Tumor shrinkage, regression, reversal, or stabilization is not impossible.

Another known example of cancer regression is found in pediatric neuroblastoma patients. Neuroblastoma shows one of the highest rates of spontaneous regression among malignant tumors. In addition to the well-known spontaneous regression in stage 4S disease, the high incidence of neuroblastoma remnants found during autopsy of newborns suggest that localized lesions may undergo a similar regression (Guin et al. 1969). Later studies also indicate that spontaneous regression is regularly seen in infants with localized neuroblastoma and is not limited to the first year of life (Hero et al. 2008). Another example of spontaneous shrinkage and loss of tumors without any treatment is an intradural lipoma (Endoh et al. 1998). These and other studies justify the "wait and see" strategy, avoiding chemotherapy and radiotherapy in infants with localized neuroblastoma, unless *MYCN* gene is amplified. Infants with nonamplified *MYCN* and hyperdiploidy can be effectively treated with less intensive therapy. Infants with disseminated disease without *MYCN* have excellent survival with minimal or no treatment.

The pertinent question is: Is it always necessary to practice tumor surgery, radiotherapy, or chemotherapy? Although the conventional belief is that cancer represents an "arrow that advances unidirectionally", it is becoming clear that for cancer to progress, they require cooperative microenvironment (niche), including immune system and hormone levels. However, it is emphasized that advanced (malignant) cancers do not show regression, and require therapy. In the light of the inadequacy of standard treatments of malignancy, clinical applications of the stem cell technology need to be expedited.

Eric Hayat

References

Endoh M, Iwasaki Y, Koyanagi I, Hida K, Abe H (1998) Spontaneous shrinkage of lumbosacral lipoma in conjunction with a general decrease in body fat: case report. Neurosurgery 43(1):150–151; discussion 151–152

Hero S, Simon T, Spitz R, Ernestus K, Gnekow A, Scheel-Walter H, Schwabe D, Schilling F, Benz-Bohm G, Berthold F (2008) Localized infant neuroblastomas often show spontaneous regression: results of the prospective trials NB95-S and NB 97. J Clin Oncol 26:1504–1510

Guin P, Gilbert E, Jones B (1969) Incidental neuroblastoma in infants. Am J Clin Pathol 51:126–136

Preface

Stem Cells is nature's indispensable gift to multicellular organisms.

This is volume 5 of the seven-volume series, STEM CELLS AND CANCER STEM CELLS: Therapeutic Applications in Disease and Injury. A stem cell is defined as a cell that can self-renew and differentiate into one or more specialized cell types. A stem cell may be pluripotent, which is able to give rise to the endodermal, ectodermal, and mesodermal lineages; an example is embryonic stem cells. A stem cell may be multipotent, which is able to give rise to all cells in a particular lineage; examples are hematopoietic stem cells and neural stem cells. A stem cell may be unipotent, which is able to give rise to only one cell type; an example is keratinocytes.

A cancer stem cell is a cell type within a tumor that possesses the capacity of self-renewal and can give rise to the heterogeneous lineages of cancer cells that comprise the tumor. In other words, a cancer stem cell is a tumor initiating cell. A unique feature of cancer stem cell is that although conventional chemotherapy will kill most cells in a tumor; cancer stem cells remain intact, resulting in the development of resistance of therapy. All of these types of stem cells are discussed in this volume.

Vast therapeutic applications of the following specific stem cells in disease and tissue injury are discussed: embryonic stem cells, human mesenchymal stem cells, cancer stem cells, arterial stem cells, neural stem cells, cardiac stem cells, dental stem cells, limbal stem cells, and hematopoietic stem cells.

As stated above, given that human embryonic stem cells possess the potential to produce unlimited quantities of any human cell type; considerable focus has been placed on their therapeutic potential. Because of the pluripotency of embryonic stem cells, they have been used in various applications such as tissue engineering, regenerative medicine, pharmacological and toxicological studies, and fundamental studies of cell differentiation. The formation of embryoid bodies, which are three-dimensional aggregates of embryonic stem cells, is the initial step in the differentiation of these cells. Such embryoid body culture has been widely used as a trigger for the in vitro differentiation of embryonic stem cells.

Therapeutic implications of signaling pathways in cancer stem cells are pointed out. Targeting self-renewal pathways in cancer stem cells are also included. Application of mesenchymal stem cells for treating ischemic brain injury is explained. Neural stem cells proliferation surrounding the area of traumatic brain injury is included. Method for differentiation of human

adipose-derived stem cells into cardiomyocytes is presented. Use of different types of stem cells in the regeneration of heart tissue is emphasized; special attention is focused on ischemic stroke. The importance of stem cells in dental implants for repairing tooth injury is explained. The details of bone reconstruction utilizing mesenchymal stem cell sheets for cell delivery are included.

The effect of limbal stem cell deficiency on eye disorders is pointed out. The importance of hematopoietic stem cell transplantation for patients with human immunodeficiency virus is presented. The danger of cytomegalovirus infection in post-hematopoietic stem cell transplantation is pointed out. Neural differentiation from embryonic stem cells is explained, so is neural stem cell differentiation from embryonic stem cells. The subject of tissue engineering is discussed in this volume; examples are urethral tissue engineering and chondrogenesis.

By bringing together a large number of experts (oncologists, neurosurgeons, physicians, research scientists, and pathologists) in various aspects of this medical field, it is my hope that substantial progress will be made against terrible human disease and injury. It is difficult for a single author to discuss effectively the complexity of diagnosis, therapy, including tissue regeneration. Another advantage of involving more than one author is to present different points of view on a specific controversial aspect of cancer cure and tissue regeneration. I hope these goals will be fulfilled in this and other volumes of the series. This volume was written by 79 contributors representing 11 countries. I am grateful to them for their promptness in accepting my suggestions. Their practical experience highlights their writings, which should build and further the endeavors of the readers in these important areas of disease and injury. I respect and appreciate the hard work and exceptional insight into the nature of cancer and other disease provided by these contributors. The contents of the volume are divided into eight subheadings: Cancer Stem Cells, Tissue Injury, Cardiovascular Applications, Bone Diseases, Eye Disorders, Viral Applications, Neural Cells, and Tissue Engineering for the convenience of the readers.

It is my hope that subsequent volumes of the series will join this volume in assisting in the more complete understanding of the causes, diagnosis, and cell-based treatment of major human diseases and debilitating tissue/organ injuries. There exists a tremendous, urgent demand by the public and the scientific community to address to cancer diagnosis, treatment, cure, and hopefully prevention. In the light of existing cancer calamity, government funding must give priority to eradicating deadly malignancies over military superiority.

I am thankful to Dr. Dawood Farahi and Dr. Kristie Reilly for recognizing the importance of medical research and publishing through an institution of higher education.

M.A. Hayat

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Part I

Cancer Stem Cells

Signaling Pathways in Cancer Stem Cells: Therapeutic Implications

1

Anna Pastò, Alberto Amadori,
and Stefano Indraccolo

Abstract

Established therapeutic approaches for cancer do cause tumor shrinking but often fail to eradicate them, hypothetically due to the existence of cancer stem cells (CSC), which are quite resistant to radio- and chemotherapy due to certain intrinsic biological features. The surviving CSC population is suspected to account for tumor relapse that commonly occurs in patients. Aim of this chapter is to critically discuss upcoming therapies targeting CSC with particular emphasis on key signaling pathways involved in normal and cancer stem cells maintenance. Improved knowledge of the role of signaling pathways in CSC will be fundamental to understand the fine balancing between quiescence and proliferation in tumor initiating cells and to elaborate effective anti-cancer therapies.

Introduction

Evidence from the last 10 years strongly supports the concept that cancer could be considered a stem cells disease. It has been established that cancer develops from a small subset of cells – termed cancer stem cells (CSC) – presenting the ability to perpetuate themselves (self-renewal) and to generate tumors recapitulating the heterogeneity of the original tumor mass when injected into immuno- deficient host at low cell number. CSC share some features with normal stem cells, including unlimited replicative potential and long lifespan that enable them to accumulate oncogenic mutations over years.

Historically, two different approaches have led to the identification of CSC: (I) examination of the expression of tissue-specific surface markers such as CD44, CD133, CD24 that are selectively expressed on CSC but not on the bulk of tumor cells, and (II) examination of specific functional features of CSC. In some studies CSC have been recognized by dual-wavelength flow cytometry as the so-called Side Population (SP) on the basis of their ability to efflux the fluorescent DNA-binding dye Hoechst 33342 (Moserle et al. 2010). The SP has been identified both in several tumors and established cancer cell lines. In breast cancer cell lines, the SP population is characterized by a number of features, including

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ALDH$^+$/CD44$^+$/CD24$^-$ phenotype, spheroid formation ability, clonogenicity, multilineage differentiation potential and tumor initiating capacity upon injection into NOD/SCID mice (Charafe-Jauffret et al. 2009). Similar properties were attributed to SP cells isolated from DAOY and PFSK medulloblastoma cell lines, that contained a small population (1.9%) of CD133$^+$/Nestin$^+$ cells able to form spheroids in vitro and generate xenograft tumors (Fan et al. 2006).

A further approach to identify CSC is based on their replicative potential: under standard culture conditions CSC are poorly or non proliferating cells compared to the bulk of tumor cells. By measuring fluorescence intensity following labelling with membrane-binding dyes such as PKH26 it is possible to identify cells that proliferate and eventually loose the dye from cells that remain in quiescent state hence maintain high intensity of the dye. This technique was recently utilized by the Pelicci's group (Cicalese et al. 2009) to identify breast cancer stem cells, recognized as PKH26^{high} cells.

A large fraction of CSC is likely in a quiescent state and does not respond to conventional chemotherapeutics that kill proliferating cells (Fig. 1.1). It has been shown that the CD133$^-$ fraction of human colorectal cancer cells showed a dose-dependent sensitivity to oxaliplatin and/or 5-Fluorouracil (5-FU), whereas the CD133$^+$ fraction, sorted from the same samples, did not undergo drug-induced apoptosis, even by increasing drug concentration (Todaro et al. 2007). A recent study has demonstrated that SP/CSC express multidrug resistance genes – including MDR-1, ABCG2, ABCA3 and BRC1 – that may contribute to the malignant phenotype and can explain the relative inefficiency of chemotherapeutic drugs (Hirschmann-Jax et al. 2004).

A better understanding of key signaling pathways in CSC and their role in the regulation of CSC quiescence could represent the starting point for a new therapeutic approach of cancer. Although knowledge is still limited, it appears that CSC share with their normal counterpart activation of certain signaling pathways involved in stem cell maintenance and proliferation. In particular, Notch-1, Wnt/β-catenin and Sonic Hedgehog pathways will be examined hereunder, to underline how their alterations could contribute to neoplastic transformation of normal stem cells.

The Notch Pathway

The Notch pathway is implicated in intercellular communication and regulates homeostasis of several tissues including intestinal, neuronal, breast and ovarian by controlling cellular self-renewal, apoptosis, differentiation and proliferation.

In mammals, Notch signals through four different receptors (Notch1-4) and 5 different ligands (Jagged 1, Jagged 2, Delta-like-1,-3 and -4). Binding of a ligand to Notch receptor leads to γ-secretase-mediated proteolitic cleavage of the Notch intracellular domain (NICD) that migrates to the nucleus of the cell (Fig. 1.2a). In the nucleus, NICD interacts with a transcriptional factor complex inducing the transcription of several targets, including members of the Hes and Hey families.

Aberrant Notch activation has been demonstrated in CSC from different tumors, including glioma (Wang et al. 2010), breast (Farnie and Clarke 2007), colon (Katoh 2007) and pancreatic cancer (Ji et al. 2009). Pancreatic CSC, identified by the expression of CD44, CD133, CD24, CD34 and ALDH, showed higher levels of Notch-1 and Notch-2 mRNA associated to the loss of microRNA-34 (miR-34), compared to pancreatic non-CSC (Ji et al. 2009). Since Notch-1 and 2 are downstream targets of miR-34, these results suggest that miR-34 could be involved in pancreatic CSC self-renewal via modulation of Notch activity.

Further data support a role of other miRs in the regulation of Notch levels in pancreatic CSC. Li et al. observed low miR-200 levels in gemcitabine-resistant pancreatic cancer cells showing canonical features of CSC; moreover, its forced expression significantly inhibited Notch signals (Li et al. 2009). Thus, modulation of specific miRs could be a successful strategy to target certain CSC features, such as drug resistance.

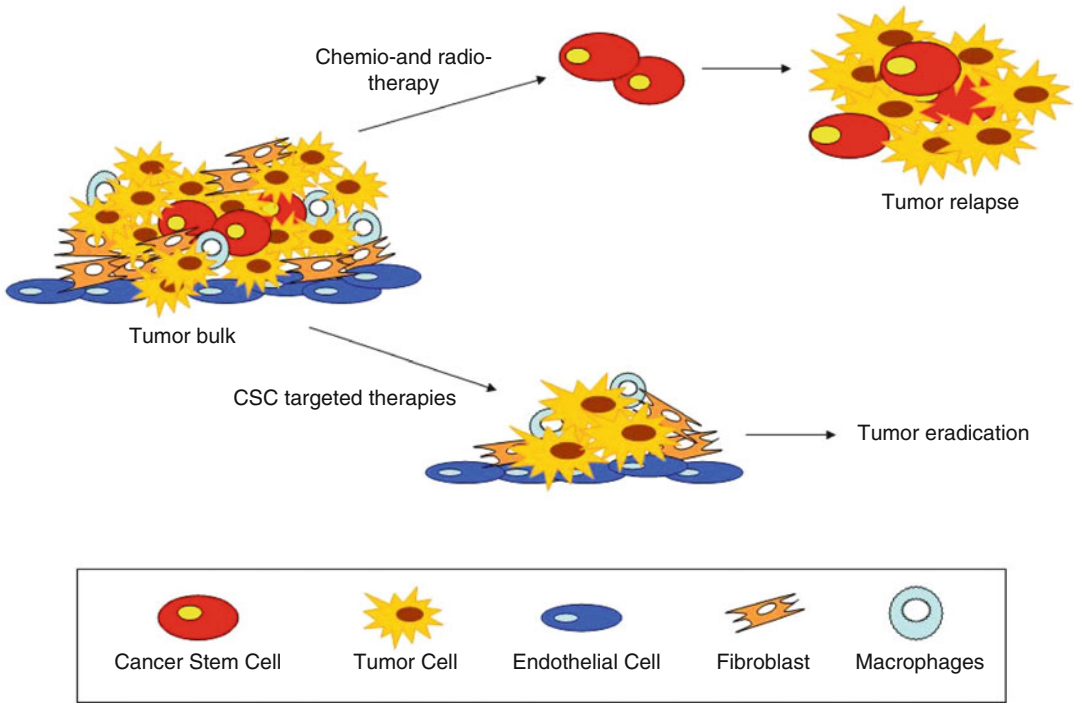

Fig. 1.1 Overview of anti-tumor therapies. Conventional treatments (e.g. chemotherapy and radiation) are able to shrink the tumor mass by killing proliferating cells; since the CSC subset is not damaged, tumors can relapse. CSC-targeting therapies are expected to bring about sustained therapeutic effects

In colon cancer cells with CSC properties, inhibition of Notch-1 induced a reduction in cell proliferation, a cell cycle arrest in Go-G1, and it increased the number of apoptotic cells. Moreover, Notch inhibition reduced both spheroid formation in vitro and tumorigenic capacity in mice, two established CSC features. In contrast, Notch-1 overexpression increased cell proliferation, cell cycle progression and it reduced apoptotic cells (Zhang et al. 2001).

Another approach to inhibit Notch signaling is to use a γ-secretase inhibitor (GSI), such as GSI-18, to enhance the efficacy of temozolomide monotherapy as demonstrated in $CD133^+$ glioma CSC (Ulasov et al. 2011). GSI reduced in culture the CSC population also in some medulloblastoma cell lines (Fan et al. 2006): in fact a threefold reduction of the $CD133^+$ stem-like fraction was observed in DAOY cells treated with GSI as well as in other medulloblastoma cell lines (D283Med, D425Med and PSFK). On the contrary, forced expression of Notch2 intracellular domain (NICD2) in DAOY cells increased the $CD133^+$ fraction to >17%. Similar results were obtained by analyzing the SP in these cell lines: constitutive activation of Notch2 led to a fourfold increase of SP cells, whereas Notch inhibition almost completely ablated SP. In the same study, the CSC fraction expressing the neural stem cells marker Nestin was also analyzed; NICD2 activation increased the $Nestin^+$ cell fraction from 10% to 47%, whereas Notch blockade by GSI reduced this population to about 2.4%. These findings buttress the plasticity of the CSC phenotype, a concept which found experimental support in the last 2 years.

The main problems associated with GSI is gastrointestinal cytotoxicity; for this reason, several "non-toxic natural agents" able to modulate Notch signaling, such as curcumin, quercentin, isoflavone, and sulforaphane are being tested.

More promising approaches are offered by antibodies targeting either Notch receptors or Notch ligands. Hoey et al. showed that a neutralizing

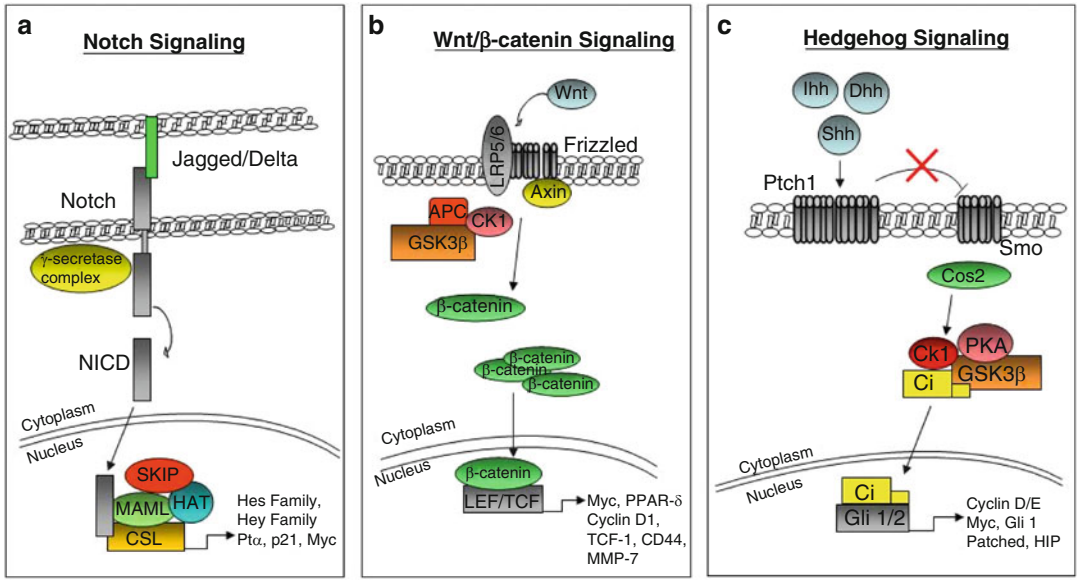

Fig. 1.2 Signaling pathways associated with normal and cancer stem cells. (**a**) The Notch pathway: following cell-to-cell contact, Delta or Jagged ligands bind Notch receptors thus inducing proteolytic cleavage of the receptor. NICD is released into the cytoplasm and translocates to the nucleus where it acts as a transcriptional activator of Notch-associated target genes, including members of HES and HEY family, Myc, Ptα and p21. *Abbreviation*: Notch intracellular domain (*NICD*). (**b**) The Wnt/β-catenin pathway: in the presence of Wnt ligand, the degradation complex composed by Axin, APC, GSK3-β and CK1 is disrupted and β-catenin accumulates in the cytosol with consequent translocation into the nucleus, where it binds Lef/Tcf transcription factors thus activating target genes including Myc, PPAR-δ, Cyclin D1, TCF-1, CD44 and MMP-7. *Abbreviations*: adenomatous polyposis coli (*APC*), glycogen synthase kinase 3β (*GSK3β*) and casein kinase 1 (*CK1*). (**c**) The Hedgehog pathway: in the active state, Shh, Ihh and Dhh ligands bind the Ptch1 receptor on adjacent cells, thus leading to Smo activation. CK1, PKA and GSK3-β complex, released from Cos2 prevent the cleavage of Ci that translocates to the nucleus, leading to activation of the transcription factors Gli1/2 and target genes, including CyclinD/E, Myc, Gli1, Patched and HIP. *Abbreviations*: Sonic (*Shh*), Indian (*Ihh*), Desert (*Dhh*), Patched receptor (*Ptch1*), Smoothened (*Smo*), casein kinase 1 (*CK1*), protein kinase A (*PKA*), Glycogen synthase kinase 3β (*GSK3β*), Costal 2 (*Cos2*), Cubitus interruptus (*Ci*)

antibody against DLL4 decreased by 50% colon CSC population, defined as $ESA^+/CD44^+/CD166^+$ cells. Notably, the combination of anti-DLL4 and irinotecan further decreased the percentage of CSC in treated tumors, whereas irinotecan monotherapy induced an increase from 28% to 43% of the $ESA^+/CD44^+/CD166^+$ population (Hoey et al. 2009). Dontu and colleagues (Dontu et al. 2004) evaluated the effect of Notch signaling agonists and antagonists on breast CSC cultured either as floating mammospheres or on a collagen matrix under conditions that promote differentiation. A synthetic peptide derived from the Delta-Serrate-LAG 2 (DSL) domain conserved in all Notch ligands and a recombinant Delta 1 ligand fused to the immunoglobulin Fc fragment were utilized as agonists of Notch signaling, whereas a Notch4-specific antibody or a GSI were used as antagonists. After treatment with agonists a tenfold increase in mammosphere formation was observed, compared to control cultures; this effect was abrogated by anti-Notch 4 antibody or GSI, while these treatments had no effects on differentiated mammary epithelial cells.

The Wnt/β-Catenin Pathway

Under physiologic conditions, Wnt regulates normal stem cell homeostasis by controlling cell proliferation at an early stage of differentiation. In particular, Wnt signals are necessary to maintain the stem cell compartment in colon crypts: inhibition of Wnt through deletion of TCF4 or overexpression of Dickkopf-1 (a Wnt inhibitor) results

in loss of epithelial cell proliferation and disruption of the intestinal crypt-axis structure (Kuhnert et al. 2004). Furthermore, it has been shown that Wnt/B-catenin acts to maintain pluripotency of Embrionic Stem Cells (ESC) and it is critical for the expansion of neural progenitors and their differentiation (Teo and Kahn 2010).

In the absence of Wnt ligands, this pathway is inactive; the membrane receptor complex formed by Frizzled (Fzd) and low-density lipoprotein receptor-related protein 5/6 (LRP5/6) is not engaged and β-catenin destruction is eventually activated (Fig. 1.2b). The complex, composed by the tumor suppressor protein adenomatous polyposis coli (APC), casein kinase 1 (CK1), glycogen synthase kinase 3β (GSK3β) and axin 2, phosphorylates β-catenin at specific threonine and serine residues inducing its proteosomal degradation. When Wnt ligands bind the receptor, destruction complex is disrupted and β-catenin accumulates in the cytosol with eventual translocation to the nucleus, where in association with the TCF/LEF transcription factor it activates specific target genes. Mutations or alterations in APC or β-catenin translate into defective β-catenin degradation and its accumulation in the nucleus with abnormal persistence of undifferentiated cells (Vermeulen et al. 2010).

The similarities between normal adult stem cells and CSC suggest that this signaling pathway could contribute to the regulation of CSC. Notably, many of the markers used to identify CSC in different tissues (such as LGR5/GPR49, CD44, CD24 and Epcam) are part of the Wnt pathway. LGR5/GPR49, recognized as a putative colon stem cell marker (Barker et al. 2007), has been shown to be overexpressed in the majority of colorectal cancer samples, compared to normal mucosal tissue; in addition, LGR5 expression was correlated to lymphatic and vascular invasion, lymph node metastasis and tumor stage, highlighting the involvement of aberrant Wnt signals in tumor progression driven by CSC (Uchida et al. 2010). Similar results were obtained by Vermeulen's group, reporting that stem-like colon cancer cells with high level of β-catenin have much greater tumorigenic potential than their counterpart with low β-catenin expression (Vermeulen et al. 2010).

Further evidence supports the involvement of this pathway in the pathogenesis of cancer. In transgenic mice, expression of a truncated form of β-catenin resistant to proteolysis led to the development of multiple mammary carcinomas (Sell 2004). Similarly, in colon tissue mutated APC associated with increased levels of β-catenin enhances cell proliferation and crypt expansion, in particular involving the stem cells compartment, with eventual development of colon cancer (Zhang et al. 2001). To date, a large number of Wnt targeting drugs have been tested, but no data on their effects on CSC are available.

The Sonic Hedgehog Pathway

Under physiological conditions, Hedgehog (Hh) signaling regulates development in the embryo and tissue regeneration in adults, but it appears up-regulated in cancer, including leukaemia, basal cell carcinoma, pancreatic and breast cancer (Curtin and Lorenzi 2010). The Hh pathway has a pivotal role in the self-renewal maintenance of both normal and malignant mammary stem cells through regulation of the polycomb gene BMI-1 (Kakarala and Wicha 2008).

Similarly to Notch, the Hh pathway is composed by multiple ligands that interact with the dodecatransmembrane Patched receptor (Ptch1): Sonic (Shh), Indian and Desert. Ptch1 acts as an inhibitor of Smoothened (Smo), a 7-transmembrane protein related to the Frizzled family, one of the components of Hh signaling complex (HSC) composed by the transcription factor Cubitus interruptus (Ci), the serine/threonine kinase Fused (Fu), the kinesin-like molecule Costal 2 (Cos2) and Suppressor of fused (Sufu) (Fig. 1.2c). Cos2 also binds to protein kinase A (PKA), protein kinase CK1 (formerly casein kinase 1) and glycogen synthase kinase 3 (GSK3), which are other kinases implicated in the Hh signaling pathway. In the absence of ligands, Ptch1 represses Smo, thus activating proteolitic cleavage of Ci that converts it into a repressor able to enter the nucleus and inhibit Hedgehog target gene expression. In the presence of ligands the inhibitory effects of Ptch1 on Smo

are relieved. Smo becomes phosphorylated by PKA and CK1 and PKA, CK1 and GSK3 are released from Cos2, preventing the cleavage of Ci. Full length Ci enters the nucleus to induce the transcription of Hh target genes (Cohen 2003).

This pathway has been recognized to play an important role in glioma progenitors or stem cells. Glioma CSC recognized by the expression of Nanog/Oct4/Sox/Bmi1 and the ability to form gliomaspheres in specific culture conditions were treated with cyclopamine, a Smo inhibitor. This treatment induced a decrease in CSC numbers and an increase of their apoptotic rate in a dose-dependent manner; 20 days of treatment were sufficient to irreversibly disrupt gliomaspheres. In contrast, treatment with temozolomide, a drug which affects proliferation and apoptosis as cyclopamine, was unable to prevent reconstitution of gliomaspheres (Clement et al. 2007). In $CD133^{+}$ sorted cells from human glioblastoma specimens, 4 h treatment with cyclopamide reduced expression of Gli1, Ptch1, Nanog, Sox2 and Oct4 by 50%, 40%, 10%, 57% and 20%, respectively, as compared to cyclopamine-treated CD133- cells. These results underline the importance of the Hh pathway in the tumorigenic potential of CSC.

Finally, a recent study has demonstrated that Ptch1 and the transcription factors Gli1 and Gli2 are highly expressed in stem/progenitor mammary cells isolated from human normal breast tissue and cultured as mammospheres (Liu et al. 2006). Activation of Hedgehog signaling through Shh increased by 57% the mammosphere number and size and it induced a sixfold increase in Bmi-1 expression. Also in this study, these effects were blocked by treatment with cyclopamine, which reduced mammospheres formation and inhibited Gli1 and Gli2 expression.

Harnessing Regulators of Cell Proliferation in CSC

Although the majority of stem cells is quiescent, these cells have by definition a great proliferative potential in response to physiological changes in the environment. The exact nature of the signals which regulate the balance between cell quiescence and proliferation in stem cells is still largely uncharted. Some recent studies have highlighted the role of the LKB1 serine/threonine kinase in the regulation of quiescence and metabolic homeostasis of hematopoietic stem cells (HSC) (Gan et al. 2010). LKB1 is an evolutionarily conserved regulator of cellular energy metabolism in eukaryotic cells and functions as the major upstream kinase to phosphorylate AMP-activated protein kinase (AMPK) and other AMPK-related kinases (Shackelford and Shaw 2009). Deletion of the LKB1 gene in mice caused increased HSC division, followed by rapid HSC depletion and pancytopenia. LKB1 deletion had an impact on cell proliferation in HSC, but not in more committed compartments, hinting at context-specific functions for LKB1 in haematopoiesis. Loss of LKB1 was also associated with decreased mitochondrial biogenesis and function, thus affecting cell metabolism. These studies are important because they establish a link between this kinase – previously known mainly for being mutated in certain familiar and sporadic tumors and for its effects on AMPK activation (Shackelford and Shaw 2009) – and regulation of stem cell quiescence. New studies are needed to determine whether LKB1 could effectively contribute to regulate quiescence of CSC.

Published studies have suggested that ubiquitination, proteosomal degradation and protein stability could also control stem cell function in various organisms (Matsuoka et al. 2008). These studies introduced the idea that the fine tuning of the abundance of specific substrates by the ubiquitin-proteasome machinery could control HSC function. The tumor suppressor Fbw7 is a well characterized example of an E3 ubiquitin ligase known to regulate cell cycle progression by specific proteolytic degradation of c-Myc, Cyclin E and Notch (Welcker and Clurman 2008). The E3 ubiquitin ligase Fbw7 has recently been shown to modulate stability of c-Myc in HSC (Reavie et al. 2010). By using c-Myc-GFP mice, Reavie et al. observed that c-Myc protein levels are comparatively low in HSC and increase with HSC differentiation, hinting at the possibility that c-Myc has

a role in the stem cell-progenitor cell transition. The authors found that HSC expressing low c-Myc levels have a greater capacity for *in vitro* replating and *in vivo* reconstitution efficiencies when transplanted into myeloablated recipients, compared to HSC with high c-Myc levels. Consistent with their hypothesis, they also showed that conditional deletion of Fbw7 in HSC results in higher c-Myc levels in HSC, leading to transcriptional activation of downstream genes needed to enable cell proliferation. The unique discovery of a ubiquitin ligase that has a function in stem cell biology expands knowledge of the mechanisms that control stem cell fate. Undoubtedly, it will be important in future studies to investigate whether Fbw7-cMyc or other ubiquitin ligase-substrate pairs might control quiescence of CSC.

Therapeutic Targeting of CSC by Other Approaches

Besides modulation of signaling pathways involved in maintenance of the stem cell state, other approaches were experimented, such as therapies targeting CSC markers, including monoclonal antibodies conjugated to cytotoxic compounds. Herrmann and colleagues treated $CD44^{high}/CD24^{high}/EpCAM^{high}$ cells, recognized as a highly tumorigenic population in the HT-29 colorectal cancer cell line, with MT110, a T cell-engaging antibody with dual specificity for EpCAM and CD3 (Herrmann et al. 2010). This antibody induces the formation of a synaptic conjugate between cytotoxic T cells and CSC, eventually leading to granzyme B-mediated lysis of tumor cells. However, since CSC markers are generally also expressed by normal stem cells, some toxicity is expected. New studies should identify CSC-specific surface markers for immunotherapy approaches, i.e. by using screening assays with ScFv libraries or bioinformatics analysis of transcriptome data from CSC.

Other strategies aim to render CSC sensitive to cytotoxic drugs. In this regard, IL-4 behaves as a chemotherapy-protective cytokine produced by $CD133^+$ colon CSC (Todaro et al. 2007). Treatment with an IL-4 neutralizing antibody in combination with oxaliplatin plus 5-FU induced sensitization to these drugs, as indicated by a decrease in viable cell number in long-term spheroid cultures.

To enhance efficacy of chemotherapy, it is also possible to force CSC to enter the cell cycle. In particular, bone morphogenetic proteins (BMPs) and BMP-4, have been described as non-cytotoxic agents able to reduce proliferation and induce expression of neuronal differentiation markers in $CD133^+$ stem-like tumor-initiating cells. Human glioblastoma tumor-initiating $CD133^+$ cells exposed *in vitro* to BMP-4 lost the capacity to establish glioblastoma when transplanted orthotopically into immunodeficient mice; *in vivo* delivery of BMP-4 induced block of tumor growth and strongly decreased the mortality rate in animals transplanted with $CD133^+$ cells (Piccirillo et al. 2006). Retinoid acid (RA or Vitamin A) is another agent that modulates cells differentiation and proliferation. Treatment of breast CSC with retinoic-acid stealth liposomes induced arrest at G0/G1 phase and differentiation of $CD44^+/CD24^-$ cells. Moreover, xenograft tumors formed by breast CSC into NOD/SCID mice were significantly smaller following treatment with retinoic-acid stealth liposomes (Li et al. 2011). These differentiation-promoting drugs could prospectively be combined with chemotherapy in order to achieve sustained anti-tumor effects.

Conclusions

Cancer stem cells belong to the top areas of biomedical investigation for many reasons, including the fact that canonical CSC features could help to explain some hot questions in oncology, such as long-term latency of certain cancers and resistance to conventional treatments. Quiescence of CSC is a reversible condition and much work needs to be done to uncover the signals that drive CSC into proliferation or, conversely, induce their dormancy. In this regard, we have reviewed here some molecular pathways which are currently known to regulate CSC behavior, but we are aware that this provisional list will expand with

time. With an outlook at therapeutic implications, it is important to keep in mind that these signaling pathways are operative also in normal stem cells and in non-stem cancer cells, which could limit the specificity of intervention at this level. Being conscious of this, the assessment of the therapeutic efficacy of drugs targeting signaling elements in CSC will be mandatory to verify the most important translational implication of the CSC theory, namely the possibility of improving cancer therapy.

References

Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449:1003–1007

Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur MH, Diebel ME, Monville F, Dutcher J, Brown M, Viens P, Xerri L, Bertucci F, Stassi G, Dontu G, Birnbaum D, Wicha S (2009) Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res 69:1302–1313

Cicalese A, Bonizzi G, Pasi CE, Faretta M, Ronzoni S, Giulini B, Brisken C, Minucci S, Di Fiore PP, Pelicci PG (2009) The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell 138:1083–1095

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

Cohen MM Jr (2003) The hedgehog signaling network. Am J Med Genet A 123A:5–28

Curtin JC, Lorenzi MV (2010) Drug discovery approaches to target Wnt signaling in cancer stem cells. Oncotarget 1:563–577

Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS (2004) Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res 6:R605–R615

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

Farnie G, Clarke RB (2007) Mammary stem cells and breast cancer – role of Notch signalling. Stem Cell Rev 3:169–175

Gan B, Hu J, Jiang S, Liu Y, Sahin E, Zhuang L, Fletcher-Sananikone E, Colla S, Wang YA, Chin L, Depinho RA (2010) Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. Nature 468:701–704

Herrmann I, Baeuerle PA, Friedrich M, Murr A, Filusch S, Ruttinger D, Majdoub MW, Sharma S, Kufer P, Raum T, Munz M (2010) Highly efficient elimination of colorectal tumor-initiating cells by an EpCAM/CD3-bispecific antibody engaging human T cells. PLoS One 5:e13474

Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA, Brenner MK (2004) A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci USA 101:14228–14233

Hoey T, Yen WC, Axelrod F, Basi J, Donigian L, Dylla S, Fitch-Bruhns M, Lazetic S, Park IK, Sato A, Satyal XW, Clarke M, Lewicki J, Gurney A (2009) DLL4 blockade inhibits tumor growth and reduces tumor-initiating cell frequency. Cell Stem Cell 5:168–177

Ji Q, Hao X, Zhang M, Tang W, Yang M, Li L, Xiang D, Desano JT, Bommer GT, Fan D, Fearon ER, Lawrence TS, Xu L (2009) MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. PLoS One 4:e6816

Kakarala M, Wicha MS (2008) Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy. J Clin Oncol 26:2813–2820

Katoh M (2007) Notch signaling in gastrointestinal tract (review). Int J Oncol 30:247–251

Kuhnert F, Davis CR, Wang HT, Chu P, Lee M, Yuan J, Nusse R, Kuo CJ (2004) Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. Proc Natl Acad Sci USA 101:266–271

Li Y, VandenBoom TG 2nd, Kong D, Wang Z, Ali S, Philip PA, Sarkar FH (2009) Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. Cancer Res 69:6704–6712

Li RJ, Ying X, Zhang Y, Ju RJ, Wang XX, Yao HJ, Men Y, Tian W, Yu Y, Zhang L, Huang RJ, Lu WL (2011) All-trans retinoic acid stealth liposomes prevent the relapse of breast cancer arising from the cancer stem cells. J Control Release 149:281–291

Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, Suri P, Wicha MS (2006) Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res 66:6063–6071

Matsuoka S, Oike Y, Onoyama I, Iwama A, Arai F, Takubo K, Mashimo Y, Oguro H, Nitta E, Ito K, Miyamoto K, Yoshiwara H, Hosokawa K, Nakamura Y, Gomei Y, Iwasaki H, Hayashi Y, Matsuzaki Y, Nakayama K, Ikeda Y, Hata A, Chiba S, Nakayama KI, Suda T (2008) Fbxw7 acts as a critical fail-safe against premature loss of hematopoietic stem cells and development of T-ALL. Genes Dev 22:986–991

Moserle L, Ghisi M, Amadori A, Indraccolo S (2010) Side population and cancer stem cells: therapeutic implications. Cancer Lett 288:1–9