# Chapter 15
# Cancer Stem Cells and Impaired Apoptosis

**Zainab Jagani* and Roya Khosravi-Far**

**Abstract** For more than 100 years scientists have fervently sought the fundamental origins of tumorigenesis, with the ultimate hope of discovering a cure. Indeed, these efforts have led to a significant understanding that multiple genetic and molecular aberrations, such as increased proliferation and the inhibition of apoptosis, contribute to the canonical characteristics of cancer. Despite these advances in our knowledge, a more thorough understanding, such as the precise cells, which are the targets of neoplastic transformation, especially in solid tumors, is currently lacking. An emerging hypothesis in the field is that cancer arises and is sustained from a rare subpopulation of tumor cells with characteristics that are highly similar to stem cells, such as the ability to self-renew and differentiate. In addition, more recent studies indicate that stem cell self-renewal pathways that are active primarily during embryonic development and adult tissue repair may be aberrantly activated in various cancers. This chapter introduces the cancer stem cell hypothesis; explores evidence for the presence of cancer stem cells, particularly in leukemia; and discusses various classical stem cell self-renewal pathways in relation to cancer. Investigating the role of cancer stem cells in the context of the major characteristics of cancer, especially impaired apoptosis, offers great promise for the design of superior tumor-selective and apoptosis-inducing therapies.

**Keywords** cancer stem cell, therapy, leukemia, Notch, Hedgehog, Wnt, Bmi1

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# 1 Introduction

The inability of conventional chemotherapeutic drugs and even various targeted therapies to produce complete remissions demands a more in-depth understanding of the key cellular events underlying tumor formation, maintenance, and progression, and the molecular pathways that dictate such processes. It has become increasingly apparent that the tumor, rather than consisting of a uniform population of rapidly proliferating cells, is actually composed of a heterogeneous population of cells with variable cellular and molecular characteristics (Foulds, 1965; Heppner, 1984). Therefore, one possible explanation for the failure of chemotherapy is that it cannot eliminate this entire mixed composition of tumor cells, thus necessitating multiple treatment approaches. Along these lines, it has been proposed that a rare group of cells with stem cell-like properties lies within the tumor and gives rise to the heterogeneous tumor cell population (Reya et al., 2001). The existence of these cells indicates that while our current anticancer therapeutics may be successful in debulking a tumor, they remain ineffective in targeting the minute, yet crucial, population of tumor cells that ultimately sustains the tumor. While the "cancer stem cell hypothesis" is supported by seminal findings from hematopoietic cancers, especially acute myeloid leukemia (AML) (Warner et al., 2004), its importance and application in other types of cancers are not clearly understood.

## *1.1 The Cancer Stem Cell Hypothesis*

One intriguing and emerging area of cancer research concerns the striking parallels between cancer cells and stem cells. Both of these cell types have the capacity to self-renew and differentiate. Unlike the highly regulated self-renewal and differentiation decisions of normal stem cells, however, it has been proposed that cancer cells undergo uncontrolled self-renewal and abnormal differentiation. Coincidently, the pathways that regulate stem cell self-renewal and differentiation, such as Notch, Hedgehog (Hh), Wnt, and Bmi1 are dysregulated in various cancers (Reya et al., 2001). In addition, key findings revealing the presence of leukemic stem cells and providing evidence for a stem cell origin for AML are in support of the hypothesis that cancers arise from a small population of tumor-initiating cells known as cancer "stem cells" (Bonnet and Dick, 1997; Buick and Pollak, 1984; Jordan and Guzman, 2004; Lapidot et al., 1994; Mackillop et al., 1983; Reya et al., 2001). These cancer stem cells give rise to the clinically observed, phenotypically diverse tumor population consisting of cells displaying varied capacities for abnormal differentiation, uncontrolled proliferation, and a reduced rate of apoptosis. While the precise identity of a cancer stem cell is difficult to pinpoint, it is possible that cancer stem cells can arise either from the malignant transformation of a stem cell, or the abnormal re-activation of self-renewal pathways in a more committed progenitor cell (Al-Hajj et al., 2004; Burkert et al., 2006; Reya et al., 2001).

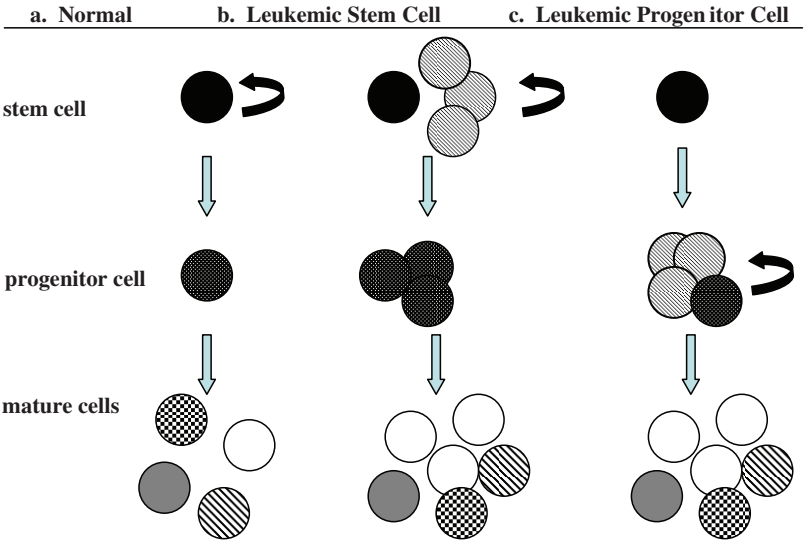


**Fig. 15.1** Cancer stem cells and leukemia. (a) A simplified demonstration of normal hematopoietic development in which the self-renewing stem cell is highly regulated leading to normal progenitor and mature cell production. In leukemia however, and according to the cancer stem cell hypothesis; (b) transformation of a stem cell can lead to uncontrolled self-renewal resulting in an abnormal growth and differentiation program; (c) alternatively, transformation of a progenitor cell can abnormally reactivate self-renewal resulting in the abnormal growth and differentiation of hematopoietic cells

### 1.1.1 Cancer Stem Cells in Leukemia and Other cancers

Since the cellular and developmental biology of the hematopoietic system is well understood, the cancer stem cell hypothesis has been most thoroughly tested in the context of hematopoietic malignancies (Fig. 15.1), such as AML (Dick, 2005). AML is characterized by the uncontrolled growth and accumulation of abnormally differentiated blood cells, or leukemic blasts, which rapidly overwhelm normal blood cell function. Initial studies using various in vitro systems, such as the clonogenic; suspension culture-initiating cells (SC-IC); and long-term culture-initiating cells (LTC-IC) quantitative stem cell assays revealed that only a minor fraction of AML cells are capable of supporting growth in vitro (Warner et al., 2004). These studies were followed by key experiments performed in vivo using the NOD/SCID-leukemia xenotransplantation model. In this model, transplantation of leukemic cells from AML patients into mice can produce leukemic disease resembling human AML (Bonnet and Dick, 1997). It was demonstrated that only a minor percentage (0.1–1%) of AML cells with primitive CD34 + CD38– surface expression was capable of initiating AML in the NOD/SCID mice, thereby providing the first evidence for the presence of cancer stem cells (Bonnet and Dick, 1997; Lapidot et al., 1994). The discovery of leukemic stem cells thus set the groundwork for an

investigation of the existence of cancer stem cells in other types of cancers. While the origin of cancer stem cells has not been conclusively defined, recent studies have also identified a subpopulation of tumor-initiating cells in solid tumors, such as breast (Al-Hajj et al., 2003), melanoma (Grichnik et al., 2006), brain (Singh et al., 2003), prostate (Xin et al., 2005), and ovarian (Bapat et al., 2005) cancers. Together, these studies raise important questions regarding the target cells of our current anticancer therapeutics, and the study of cancer signal transduction pathways in the appropriate cellular context.

### 1.1.2 Targeting Cancer Stem Cells

In the case of the CML-causing oncogene *BCR-ABL*, accumulating evidence suggests that the target cell for transformation is a hematopoietic stem cell (HSC) rather than a committed progenitor cell (Elrick et al., 2005; Huntly and Gilliland, 2005; Huntly et al., 2004). Unfortunately, research has shown that while the Abl kinase inhibitor, Gleevec, can eradicate the majority of proliferating CML progenitors and differentiated granulocytes, it is unable to target the minute population of CML progenitor stem cells that can sustain the disease (Bhatia et al., 2003; Elrick et al., 2005; Graham et al., 2002). In accordance with the cancer stem cell hypothesis, Gleevec treatment can be used continuously to manage chronic phase CML, but not to eliminate leukemic disease, since the remaining cancer stem cells are still able to sustain the disease. Further research must specifically target this cancer stem cell population.

It remains important to determine whether abnormal survival and antiapoptotic signaling, as has been intensively investigated in primary tumor cells, tumor cell lines, and mouse tumor models, actually plays a significant role in the transformation and maintenance of the tumor-initiating cell, or, more specifically, the cancer stem cell population. One goal of such studies is to determine how to selectively induce apoptosis in leukemic stem cells, but not in normal HSCs. Recent studies have shown that the prosurvival pathways, such as NF-κB and PI3-K, are highly activated in the leukemic stem cell population in AML (Guzman et al., 2001; Xu et al., 2003; Zhao et al., 2004). Interestingly, AML leukemic stem cells preferentially undergo apoptosis, unlike normal HSCs, upon combined treatment with the chemotherapeutic agent idarubicin and the proteasome inhibitor MG-132 (Guzman et al., 2002). Such treatments lead to the inhibition of NF-κB activity, along with other currently unidentified mechanisms, and also activate p53, causing the expression of target genes, such as *GADD45, p21*, and the proapoptotic gene *Bax* (Guzman et al., 2002).

## *1.2 The Role of Stem Cell Regulation Pathways in Tumorigenesis*

As early as 1855, the scientist Rudolph Virchow recognized elements of dysregulated embryonic development in tumors, proposing his embryonal-rest hypothesis. In accordance with these earlier findings, there is now evidence for a molecular link between the pathways that regulate stem cell self-renewal during

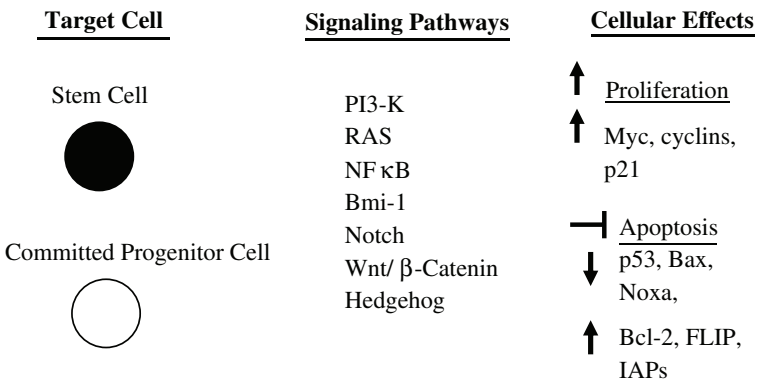


**Fig. 15.2** Cancer stem cells and signaling pathways. A summary of the signaling pathways implicated in the survival of cancer stem cells. In general, these signaling pathways could either be aberrantly activated in a stem cell or a committed progenitor cell. Whereas, the outcomes of activating such pathways are numerous, key cellular effects include increase in cellular proliferation, and the inhibition of apoptosis. This figure outlines only a few of the various downstream genes that play important roles in proliferation and apoptosis

development and tumorigenesis (Fig. 15.2) (Burkert et al., 2006; Reya et al., 2001). The major developmental pathways such as Notch, Hh, and Wnt, which intricately control the self-renewal of stem cells during both embryonic development and adult tissue repair and homeostasis, are found to be upregulated in various cancers. These observations have brought forth important questions as to whether these pathways critically contribute to tumor formation and maintenance and whether their inhibition can be utilized in future anticancer therapeutic strategies. Selective inhibition of these developmental pathways in tumor cells may also have the potential to eliminate the elusive population of tumor-initiating cells that share common characteristics with stem cells. Furthermore, determining the direct impact of inappropriate activation of self-renewal pathways on apoptosis in a tumor cell will lead to a better understanding of how to combine therapies that attack upstream self-renewal pathways, with those that unleash downstream apoptotic cascades.

### 1.2.1 Bmi-1

The *Bmi-1* proto-oncogene was first identified as a target of the Moloney murine leukemia viral insertion in the Eμ-myc lymphoma mouse model (Haupt et al., 1991; van Lohuizen et al., 1991), with further studies suggesting a cooperative role with c-myc in inducing murine lymphogenesis (Haupt et al., 1993). Bmi-1 is a Polycomb-group gene which functions as a transcriptional repressor and plays a role in regulating cellular proliferation and senescence through repression of the INK4A locus (Jacobs et al., 1999). Recently, the *Bmi-1* gene has been shown to play a critical role in the generation of self-renewing adult HSCs, as mice deficient in Bmi-1 show reduced numbers of HSCs (Park et al., 2003). In addition, the *Bmi-1*

gene has not only been implicated in regulating the proliferative activity of normal hematopoietic cells, but also of leukemic stem and progenitor cells, in which lack of Bmi-1 leads to proliferation arrest and characteristics of differentiation and apoptosis (Lessard and Sauvageau, 2003).

### 1.2.2 Notch Signaling

Notch signaling functions in a diverse set of cellular processes during embryonic and postnatal development, including the maintenance of stem cells, cell fate specification, differentiation, and proliferation (Artavanis-Tsakonas et al., 1999; Kadesch, 2004). Interestingly, research points to a role for constitutively active Notch signaling under certain cellular contexts, such as in tumorigenesis (Callahan and Egan, 2004; Hansson et al., 2004; Radtke and Raj, 2003), yet the precise mechanisms underlying this effect remain to be determined. In mammalian systems, the Notch signaling pathway consists of four receptors (NOTCH1–4) and five ligands, Delta-like 1, 3, 4 (DLL1, DLL3, and DLL4), Jagged 1 and Jagged 2 (JAG1, JAG2) (reviewed in Artavanis-Tsakonas et al., 1999; Hansson et al., 2004; Kadesch, 2004). Notch receptors are synthesized as precursors, with Notch receptor activation occurring in a series of proteolytic cleavages upon interaction with its ligand. While the first cleavage is facilitated by TACE (tumor-necrosis factor $\alpha$-converting enzyme/metalloproteinase) (Brou et al., 2000), the second is mediated by the $\gamma$-secretase activity of presenilins, and results in the release of the intracellular cytoplasmic portion of Notch, which then translocates to the nucleus (De Strooper et al., 1999; Mumm et al., 2000; Saxena et al., 2001). The known targets of Notch activation are the HES (hairy/enhancer of split) and HERP (Hes-related repressor protein) families of transcription factors, which regulate the transcription of various genes through development (Bailey and Posakony, 1995; Davis and Turner, 2001). The set of target genes activated by Notch signaling has not been completely defined, and may vary with cellular context. In transformed cells, transcription of the *erbB2* (Chen et al., 1997) and *cyclin D1* (Ronchini and Capobianco, 2001) genes have been reported to be upregulated in response to activated Notch.

The earliest evidence for the involvement of activated Notch in human cancers arose from the identification of a translocation involving the *Notch1* gene in cases of T-cell acute lymphoblastic leukemia (T-ALL) (Ellisen et al., 1991). In particular, the t(7;9) chromosomal translocation fuses a truncated Notch consisting mainly of the intracellular domain (NOTCH1-IC) to the TCR$\beta$ promoter/enhancer locus. The oncogenic property of NOTCH1-IC was confirmed by a murine bone marrow transplant model wherein reconstitution with hematopoietic progenitors expressing NOTCH1-IC led to the development of T-cell leukemias (Pear et al., 1996). The presence of activated Notch is not limited to leukemias, as its overexpression or gain-of-function mutations, resulting in expression of a truncated active Notch, have also been observed in tumors of epithelial origin such as breast, cervical, and colon carcinomas (Callahan and Egan, 2004; Callahan and Raafat, 2001; Gray et al., 1999; Zagouras et al., 1995). A role for constitutive Notch signaling

in the development of mammary tumors was first found with the discovery that the *Notch4* gene is a common integration site for the mouse mammary tumor virus (MMTV) in about 18% of virus-induced mouse mammary tumors (Gallahan and Callahan, 1997; Gallahan et al., 1987). MMTV interruption of *Notch4* results in the expression of a transcript that encodes the transmembrane and intracellular regions for Notch4, but that lacks the extracellular regulatory domain. Transgenic mouse models expressing the Notch4 intracellular domain develop mammary tumors (Jhappan et al., 1992; Smith et al., 1995), and therefore support a causative role for activated Notch signaling in mammary tumorigenesis. The relevance of Notch activation in human breast cancers has recently been investigated using tissue microarrays of breast tumor samples from various clinical stages. In these studies, elevated expression of Notch-1 and the Notch ligand, Jag1, was associated with poor survival (Reedijk et al., 2005).

Among the primary mechanisms for Notch-induced tumorigenesis, in addition to increased proliferation, is the inhibition of apoptosis. Activated Notch-1 renders T cells resistant to Fas receptor-mediated signaling, as well as to drugs including dexamethasone and etoposide, via upregulation of antiapoptotic molecules such as Bcl-2, FLIP, and IAPs (Sade et al., 2004). Additional mechanisms for Notch-induced survival include inhibition of p53 tumor suppressor expression, and activation of the RAS, PI3-K, and NF-κB pathways (Leong and Karsan, 2006).

While the precise value of Notch signaling inhibition in cancer therapy remains to be determined, preliminary studies have shown the potential for gamma secretase inhibitors (GSI) (Lanz et al., 2004; Wong et al., 2004), which can block Notch proteolytic processing, to induce apoptosis in various tumor cell lines (Curry et al., 2005; Nickoloff et al., 2005). Treatment of chemoresistant melanoma cells with a small molecule, GSI, induced the expression of the proapoptotic BH3 family member, NOXA, and caused apoptotic cell death (Nickoloff et al., 2005). Future studies will determine which downstream survival or antiapoptotic pathways play a role in the context of Notch activation in leukemias, as well as in solid tumors. In addition, the precise role of each of the four Notch receptors in tumorigenesis, and the development of specific inhibitors and/or antibodies against these receptors, will be crucial for an understanding of the overall role of Notch signaling in cancer and for investigating the potential of Notch inhibition in anticancer therapy. Finally, it will also be important to perform these studies at the cancer stem cell level in order to determine the cellular context in which dysregulated Notch signaling can potentially exert its oncogenic effects.

### 1.2.3 Hedgehog Signaling

The Hh pathway, first discovered in *Drosophila* (Nusslein-Volhard and Wieschaus, 1980), is highly conserved across vertebrates, with important functions during embryonic development, as well as in adult tissue homeostasis, such as in postembryonic tissue repair and stem cell regulation (Lum and Beachy, 2004; Taipale and Beachy, 2001; Zhang and Kalderon, 2001). The mammalian Hh pathway includes

three secreted Hh ligands (Sonic, Indian, and Desert), their 12-pass transmembrane receptors Patched1 (PTCH1) and Patched2 (PTCH2), and the 7-pass transmembrane signal transducer Smoothened (SMO). Hh ligands activate the Hh pathway by inducing the activation of SMO, followed by a signal transduction cascade that causes the nuclear translocation of the GLI family of transcription factors (GLI1, 2, 3), and the subsequent induction of a distinct transcriptional regulatory program (Cohen, 2003; Hooper and Scott, 2005; Kalderon, 2005). The targets of Hh pathway activation include various cell cycle, proliferation, and survival-regulating genes such as the cyclins (Kenney and Rowitch, 2000), c-myc (Kenney et al., 2003), and Bcl-2 (Bigelow et al., 2004; Regl et al., 2004), and also Hh pathway genes themselves, such as Ptch1, Gli1, and Hip (Hh-interacting protein), which in turn regulate pathway activation (Chuang and McMahon, 1999; Goodrich et al., 1996; Lee et al., 1997).

Notably, gene mutations within the Hh pathway have been linked with several human diseases. Mutations resulting in unrestrained Hh pathway activity have been found in Gorlin's syndrome, which is characterized by developmental defects in the brain, spinal cord, and skeleton, and a predisposition for skin and brain cancers, such as basal cell carcinomas (BCCs) and medulloblastomas, respectively (Hahn et al., 1999). Subsequent investigations have substantiated aberrant Hh signaling in BCCs and medulloblastomas (Gailani et al., 1996; Xie et al., 1998). Recent studies have revealed that the Hh pathway is also active in more common tumors such as those of the lung, breast, pancreas, stomach, and prostate (Berman et al., 2003; Karhadkar et al., 2004; Kubo et al., 2004; Pasca di Magliano and Hebrok, 2003; Sheng et al., 2004; Thayer et al., 2003; Watkins et al., 2003). Cyclopamine is a plant-derived steroidal alkaloid that inhibits the Hh pathway by antagonizing SMO (Taipale et al., 2000). Various studies have shown the ability of cyclopamine to induce apoptosis in a variety of tumor cell lines, and to inhibit tumor progression in medulloblastoma, pancreatic, and lung mouse tumor models (Berman et al., 2002; Thayer et al., 2003; Watkins et al., 2003).

### 1.2.4 Wnt/β-catenin Signaling

Similar to the Notch and Hh pathways, the Wnt signal transduction pathway also plays a critical role during development. Among several functions, Wnt signals regulate the self-renewal of hematopoietic, epidermal, and intestinal stem cells. The canonical Wnt pathway involves signaling through the cytoplasmic protein, β-Catenin. The binding of a Wnt ligand to a complex of a Frizzled receptor and the LRP5/6 receptor leads to a series of signaling events resulting in the inhibition of a destruction complex that promotes the proteasomal degradation of β-Catenin. Therefore, Wnt pathway activity causes the accumulation of β-Catenin and its translocation to the nucleus where it binds to the Lef/Tcf family of transcription factors. This binding elicits the transcriptional activation of various target genes involved in the promotion of cellular proliferation and invasion, and the inhibition of apoptosis (reviewed in Fuchs et al., 2005; Reguart et al., 2005; Reya and Clevers, 2005).

Interestingly, the first *Wnt* gene was identified in mouse mammary tumors induced by the integration of the MMTV (Rijsewijk et al., 1987). Since then, there have been numerous studies on the aberrant activation of Wnt signaling in various cancers, including those of the colon, ovary, prostate, pancreas, breast, and lung, along with melanomas, multiple myeloma, and even leukemias (Fuchs et al., 2005; Janssens et al., 2006; Reguart et al., 2005; Reya and Clevers, 2005). While mutations in the Wnt ligands and receptors have not been identified in cancers thus far, mutations have been identified in downstream effectors of the Wnt pathway, especially in colorectal cancers (CRC). Gain-of-function mutations in oncogenic β-Catenin, and loss-of-function mutations in adenomatous polyposis coli (APC) and Axin, the latter of which are components of the destruction complex, can all lead to uncontrolled β-Catenin-mediated Lef/Tcf target gene expression (Fuchs et al., 2005; Janssens et al., 2006). Wnt pathway target genes involved in the inhibition of apoptosis include *MDR1/PGP, COX-2, PPAR-δ*, and Survivin, each of which has been found to be upregulated in CRCs (Fuchs et al., 2005). Considering the activation of the Wnt pathway in various cancers, inhibition of the Wnt pathway may serve as an attractive and promising therapeutic approach. Recent studies have demonstrated the potential for small-molecule antagonists of the TCF/β-Catenin complex to decrease expression of the Wnt target genes, *Myc* and *Cyclin D*, and to inhibit cellular proliferation in colon carcinoma cell lines (Lepourcelet et al., 2004). In another approach, monoclonal antibodies against Wnt-1 and Wnt-2 ligands have shown promise in inducing apoptosis in a variety of tumor cell lines overexpressing Wnt ligands, both in vitro and in vivo (He et al., 2004; You et al., 2004a–c; ). Interestingly, the Wnt-2 antibody was shown to downregulate the expression of Survivin and induce apoptosis in various human non-small-cell lung cancer (NSCLC) cells, while failing to induce apoptosis in normal human airway cells that do not express Wnt-2. In contrast, primary NSCLC tissues showed elevated expression of Wnt-2 (You et al., 2004c).

## 2 Conclusion and Perspectives

Even though the cellular heterogeneity of tumors has long been recognized, the exact reasons for this feature have not always been clearly understood. The genomic instability that is inherent in cancer cells offers one explanation. Interestingly, recent studies, especially in leukemia, have revealed that the abnormal behavior of a malignant stem cell can give rise to the abnormally differentiated and diverse cellular hierarchy observed in tumors. The cancer stem cell hypothesis proposes that the tumor is actually sustained by a minority of cells, the cancer stem cells. The identification of cancer stem cells in leukemia and some solid cancers has yielded great insight into the cellular underpinnings of cancer, and will greatly affect the consideration of which cells to target critically in future anticancer therapeutics. Together, the study of signal transduction pathways that govern the survival of cancer stem cells, the precise role of cancer stem cells in different cancers, and an

analysis of stem cell regulation pathways in cancer offers great promise for the development of more effective treatments in the future.

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