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Stem Cell Growth as a Model of Carcinogenesis

Steven Poser[1], Joseph Alisky[2], Kuei-Fang Chung[3], Doreen Ebermann[3], Monika Ehrhart-Bornstein[3], Stefan Bornstein[3] and Andreas Androutsellis-Theotokis[3,4,5]

[1]*Celling Technologies, Austin, Texas,*
[2]*Total Longterm Care, Aurora, Colorado,*
[3]*Carl Gustav Carus University Medical School, Medical Clinic III, Dresden University of Technology, Dresden,*
[4]*Center for Regenerative Therapies, Dresden University of Technology, Dresden,*
[5]*European Brain Research Institute, Rome,*
[1,2]*USA*
[3,4]*Germany*
[5]*Italy*

1. Introduction

Cancers of the central nervous system are typically some of the most challenging of malignancies to treat and often have poor clinical outcomes. Even under the most aggressive of therapies, tumors such as glioblastoma recur at a high frequency. This was long thought to be due in part to the inability of delivering effective therapeutics to the site in combination with the inherent difficulties of surgical intervention in this challenging environment. With the discovery of a specific population of cell that could drive cell expansion in acute mylogenous leukemia (Lapidot et al., 1994) came a fundamental change in the thinking about the therapeutic approach necessary to effectively address certain cancers. Instead of the need to eliminate every cell in a tumor, a distinct group with identifiable properties could be the target. This would deprive the tumor of its proliferative engine, making the remaining more differentiated, and presumably less invasive, tissue dealt with more easily. Evidence has emerged over the past few years suggesting these cancer stem cells (CSCs) might be at the heart of numerous other non-hematological malignancies including breast, prostate, pancreatic, lung, and ovarian tumors (Al-Hajj et al., 2003; Eramo et al., 2008; Collins et al., 2005; Zhang et al., 2008). This concept has great potential implications for treating central nervous system (CNS) neoplasms like glioblastomas and medulloblastomas, which demonstate the kind of hierarchical organization that is considered to be a hallmark of the cancer stem cell hypothesis [Reviewed in (Gupta et al., 2009)]. In this model, tumor heterogeneity arises from distinct cell subpopulations, only some of which are capable of regenerating the tumor as demonstrated through serial transplantations experiments performed in immunocompromised mice. This is opposed to a

clonal or stochastic model where there is a certain probability that any cell will develop mutations that allow for it to show unregulated proliferation and the ability to generate new tumors (Nowell, 1976). There is ongoing debate about how much each of these models contribute to the distribution of cells in any given cancer type. This can partially be attributed to the fact that stem cells can undergo both symmetric and asymmetric divisions, making them clonal and hierarchical in their contribution to the growth of a tumor. For example, clonal evolution may drive the progression of the cancer by producing secondary CSCs with additional mutations that impart further growth advantages (e.g. hypoxia- and chemotherapeutic-resistance), confounding interpretation of the cellular origin of the malignancy. There are a number of recent reviews on the topic of CSCs reflecting the growing interest in understanding in how these cells operate (Frank et al., 2010; LaBarge, 2010; Morrison et al., 2011; O'Brien et al., 2010; Takebe and Ivy, 2010; Shackleton et al., 2009). Still, there remain significant challenges that lie ahead in translating any understanding about this unique cell population into effective therapies. Principal among these are the ability to identify the CSC itself by a set of unique validated biomarkers, developing strategies for addressing their cell biology, whether it be targeting the intracellular signal transduction pathways, or the microenvironment they establish that regulate their self-renewal and survival in a way that minimizes effects on normal stem and non-stem cell populations. Over the course of this chapter, we will look at how our understanding of mechanisms that regulate the proliferation, differentiation and survival of non-transformed neural stem cells has impacted the thinking of how tumors arise and resist current therapeutic approaches in the context of the cancer stem cell hypothesis.

Throughout this Review, the term "stem cell" will refer to non-cancerous stem cells; when we refer to stem cells in or derived from cancerous tissue, we will use the term "cancer stem cell", abbreviated as "CSC".

2. Identification of cancer stem cells

For the cancer stem cell hypothesis to hold, the CSC has to be a distinct and identifiable entity within a progressing cancer. A number of biomarkers have been proposed to label CSCs of many different sources [reviewed in (Frank et al., 2010)] and one that has drawn considerable interest is CD133/prominin1. It is found on different solid tumor types, and in the context of the CNS, is co-expressed with the neural stem cell marker nestin in medulloblastomas, ependydomas, oligodendrogliomas and gliolblastomas (Singh et al., 2004; Calabrese et al., 2007). CD133 expression is linked to the activity of the transcription factor HIF-1 α in glioma cells grown under low oxygen conditions (Platet et al., 2007). The growth of these cells specifically is enhanced during hypoxic exposure (Soeda et al., 2009), a notion consistent with them having a selective advantage, particularly under the stressful conditions present during the rapid expansion phase of a tumor when neovascularization is just beginning, and the tumor is deriving its sustenance from the existing niche environment (Pouyssegur et al., 2006; Keith and Simon, 2007; Louis et al., 2007). However, while selection of CD133 positive cells followed by serial diluted xenotransplantation can recapitulate tumor formation, it was only observed in a subset of glioblastoma tumors. This finding highlights a challenge to the CSC hypothesis, that a CSC population may not only be specific to particular forms of cancer, but that markers may change during the evolution of a tumor from early neoplasm to metastasis. As a consequence, multiple markers will be

needed to provide for an effective screen. Other markers, including CD24, A2B5 and the chemokine receptor CXCR4, are increased in CD133-positive glioma cells grown under hypoxic conditions (Soeda et al., 2009). It is not clear how these relate to the tumorogenic potential of the cells, but do have interesting characteristics such as the role of CXCR4 in controlling the migration of gliomas (Ehtesham et al., 2004).

Hairy and Enhancer of Split 3 (Hes3) may provide an alternative biomarker for CSCs. It is a transcription factor that, like other members of its family (e.g. Hes1 and Hes5), is regulated by activation of the transmembrane Notch receptor. However, Hes3 is an indirect target of Notch signaling (Lobe, 1997; Hirata et al., 2001; Hatakeyama et al., 2004), and has been shown to identify neural stem cells in established cultures from the fetal and adult mammalian brain (Androutsellis-Theotokis et al., 2006; Androutsellis-Theotokis et al., 2009). It also identifies endogenous neural stem cells in the brain and spinal cord of fetal and adult rodents, and the adult human and non-human primate brain (Androutsellis-Theotokis et al., 2010). In addition, Hes3 identifies a subpopulation of cells in biopsies from glioblastoma patients. Hes3 co-localizes with prominin (Androutsellis-Theotokis et al., 2010), suggesting that, like prominin, it marks the CSC population in these brain tumors.

Given the above observations as well as the fact that Hes3 is regulated by signaling pathways that are of critical importance for survival and proliferation of normal stem cells, raises the intriguing possibility that analogous signal transduction mechanisms may control cancer neural stem cells as well. Consequently, the study of normal stem cells can identify core mechanisms that regulate the expansion of cancer stem cells, in an experimental system that is free of the confounding mutations present in cancerous tissue or transformed cell lines. Below, we will discuss the signaling pathway that regulates Hes3 and stem cell survival, and we will review recent literature that shows how this pathway is relevant to both normal and cancer stem cells.

3. Intracellular signaling in stem cells

A number of links have been made between stem cell growth and known oncogenic pathways. This is highlighted by the observation that loss of the tumor suppressor p53 results in greater numbers of neural stem cells in the subventricular zone (Meletis et al., 2006). In fact, many of the genes that are upregulated in putative CSCs are those that define a primitive cell population, such as Oct4, Nanog, Sox2, and Myc (Glinsky, 2008; Stevenson et al., 2009). Furthermore, it is generally recognized that CSCs and normal stem cells share a number of properties, including self-renewal and differentiation. These similarities provide a strong rationale for examining the underlying mechanisms of proliferation in non-transformed population to gain novel insight into the causes of the uncontrolled growth of CSC-driven malignancies.

The developmentally conserved Notch signaling pathway plays many roles in pattern formation, expansion and differentiation processes during embryonic and adult life (Artavanis-Tsakonas et al., 1999), being "context-dependent", to serve different roles in different cells or the same cells at different developmental stages (Louvi and Artavanis-Tsakonas, 2006). For example, in the vertebrate central nervous system, it inhibits the cascade of events required for the formation of neurons and promotes the differentiation of glia (Haddon et al., 1998; Morrison et al., 2000; Tanigaki et al., 2001; Justice and Jan, 2002; Stump et al., 2002; Kamakura et al., 2004; Taylor et al., 2007).

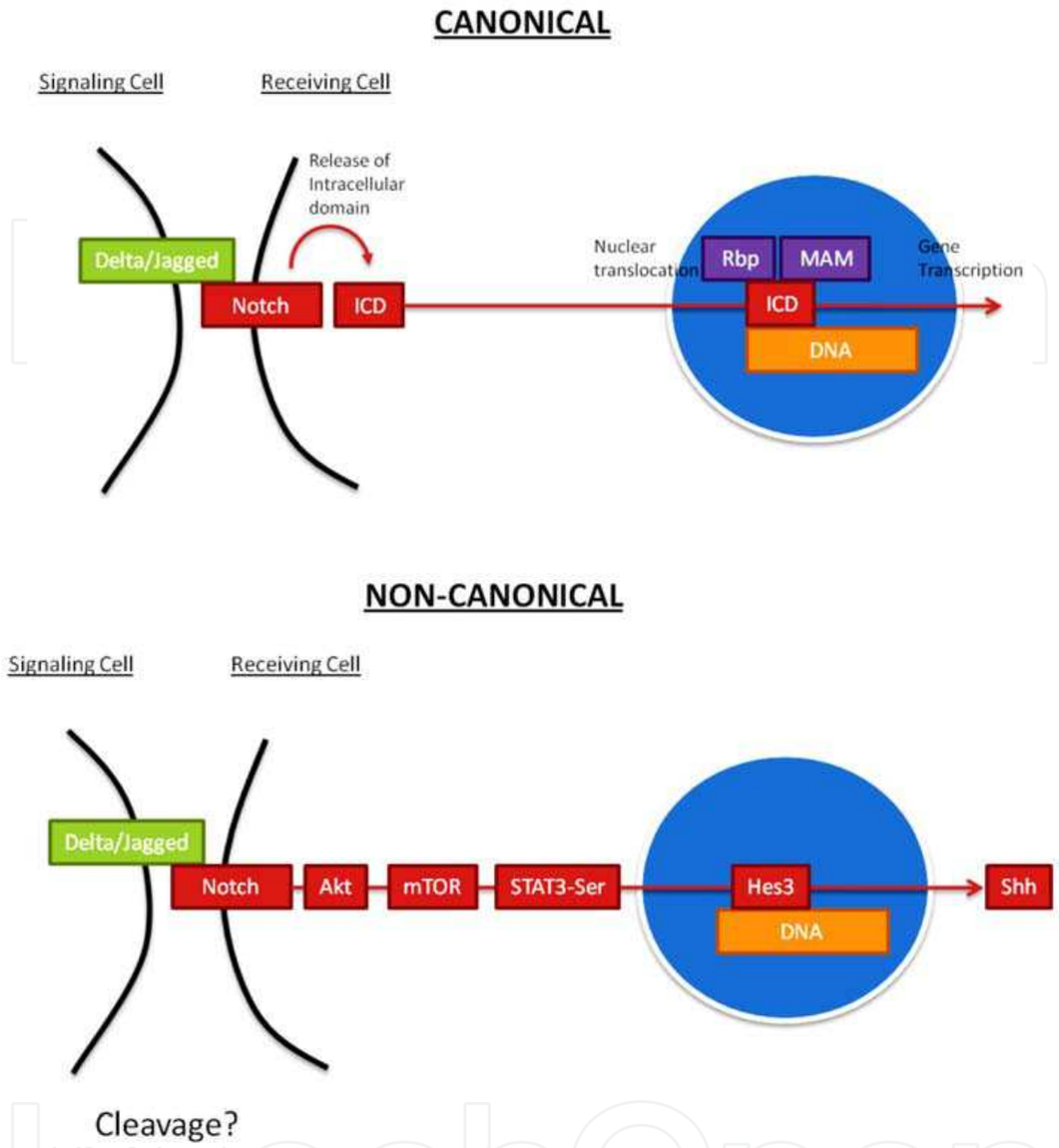

Fig. 1. Canonical vs. non-canonical Notch signaling. Engagement of the Notch receptor by its ligand leads to context dependent activation of Notch signaling. In the canonical pathway, the Notch intracellular domain (ICD) is released by γ-secretase. It then translocate to the nucleus where it complexes with factor such as Rbpsuh (Rbp) and Mastermind (MAM) to directly regulate gene expression. An alternative, non-canonical pathway involves Notch activation of transcription by an indirect mechanism through by sequential phosphorylation of kinase and subsequent increase in the transcription of Hes3. Hes3, in turn, regulates expression of sonic hedgehog (Shh), a mitogen for neural stem cells

Notch ligands and receptors are membrane-bound, so activation relies on cell-to-cell contact (Figure 1). Notch encodes a trans-membrane receptor that is cleaved on activation to release an intracellular domain that is directly involved in transcriptional control and regulates cell fate following association with Recombinant binding protein suppressor of hairless (Rbpsuh) and Mastermind (Heitzler and Simpson, 1991; Ruohola et al., 1991; Greenwald and

Rubin, 1992; Spana and Doe, 1996; Artavanis-Tsakonas et al., 1999). Many of the cellular consequences of Notch activation are mediated by the achate-scute family of bHLH transcription factors (Kidd et al., 1986; Campos-Ortega and Jan, 1991; Chiba, 2006). Together, these components constitutes the canonical Notch signaling pathway.

In Drosophila, Notch function is regulated by the inhibitor Numb (Uemura et al., 1989; Rhyu et al., 1994; Frise et al., 1996). In mice there are two homologues of Drosophila Numb, called Numb and Numb-like, which are required for the maintenance of the sub-ventricular zone and ependymal cells in the post-natal brain (Kuo et al., 2006). This suggests a continuing role for Notch in the maintenance of primitive cells associated with the ventricular surface of the brain. In addition to its function in precursor/stem cell survival, Notch inhibits neurite outgrowth in mature neurons (Sestan et al., 1999).

Soluble forms of Notch ligands increase the survival of cultured neural stem cells within hours (Androutsellis-Theotokis et al., 2006). The survival response in neural stem cells is so rapid that it suggests the involvement of second messengers that had not previously been associated with the Notch receptor. Indeed, Notch receptor activation lead to increased phosphorylation of Akt within minutes, a hallmark of growth and cancer pathways. This was followed by phosphorylation of the mammalian target of rapamycin (mTOR), another staple of cancer signaling (Cantley, 2002). Inhibition of Notch, Akt and mTOR activity resulted in reduced survival of cultured neural stem cells. These findings identified a novel branch of the Notch signaling pathway (refered to from this point as non-canonical Notch signaling) and showed that normal stem cells utilize survival signals which play central roles in cancer biology. This pathway might be a manifestation of the classical role of Notch, regulation of cell fate through lateral inhibition as originally demonstrated in Drosophila (Cabrera, 1990; Simpson, 1990). To better understand how to exploit Notch signaling for potentially therapeutic applications; it is critical to determine the signal transduction steps required for each in the appropriate cellular context.

The family of Signal Transducers and Actvators of Transcription (STAT) proteins are phosphorylated following membrane receptor activation and they mediate multiple cellular responses in the cytoplasm and nucleus of cells (Levy and Darnell, 2002). A member of the family, STAT3, has two phosphorylation sites: a tyrosine phosphorylation site at amino acid position 705 (Tyr), and a serine phosphorylation site at amino acid position 727 (Ser) and plays an important role in promoting the survival of many cell types.

STAT3-Tyr phosphorylation is a critical mediator of survival in many cell types, including transformed cell lines and cancer cells (Kiuchi et al., 1999; Levy and Darnell, 2002). STAT3-Ser phosphorylation, is of minor importance to the survival of many established transformed cell lines. Accordingly, many studies have measured increased STAT3-Tyr phosphorylation levels in many cancers (Kiuchi et al., 1999; Levy and Darnell, 2002), whereas the levels of STAT3-Ser in tumors have been less studied and somewhat unclear in the literature.

As a consequence of stem cells having the ability to both self-renew and differentiate, they interpret signals in ways different from most other cell types. Neural stem cells, for example, respond to STAT3-Tyr phosphorylation not simply by increasing their survival, but by differentiating towards the astrocyte fate (Johe et al., 1996; Bonni et al., 1997; Rajan and McKay, 1998; Song and Ghosh, 2004). In neural stem cells, under conditions that support their "stemness", phosphorylation of STAT3-Tyr is low or absent but phosphorylation of STAT3-Ser plays a major role in survival (Androutsellis-Theotokis et al., 2006). A possible interpretation is that neural stem cells simply cannot use the tyrosine phosphorylation on STAT3 for survival, and are left with only the serine site to drive survival. The serine

phosphorylation is of minor importance to most cell types (which rely mostly on tyrosine phosphorylation for survival), but of critical importance to neural stem cells (which do not have the luxury of using the tyrosine site for survival). Thus, it seems that STAT3-Tyr is required for neural stem cell differentiation, whereas STAT3-Ser phosphorylation is required for self renewal and survival. These distinct signal transduction requirements may be exploited as specific targets for the manipulation of neural stem cells and tumor cells (Figure 2).

The non-canonical Notch pathway described previously involves the fast (within approximately 20 minutes) phosphorylation of mTOR. mTOR phosphorylation has is documented to lead to STAT3 phosphorylation on serine 727 (Levy and Darnell, 2002). Indeed, inhibition of mTOR in neural stem cell cultures also inhibits STAT3-Ser phosphorylation and dramatically compromises survival (Rajan et al., 2003). These results place STAT3-Ser phosphorylation in the non-canonical Notch pathway and provide a signaling point that distinguishes stem cells from most cell types in the body in terms of their survival requirements.

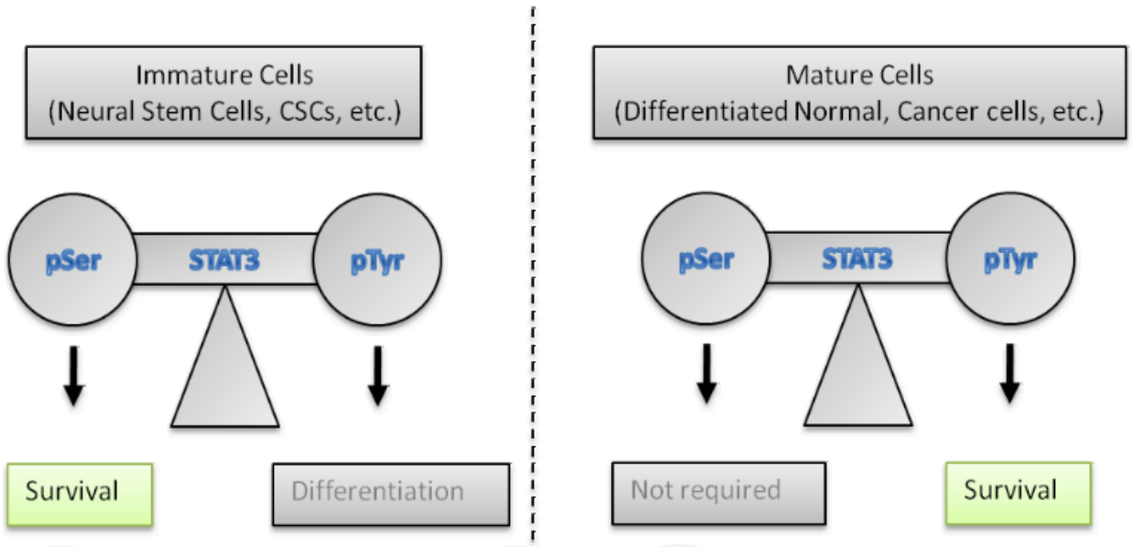

Fig. 2. Stem cell – specific use of STAT3. In neural stem cell cultures, the two phopshorylation sites on STAT3 integrate distinct signaling cascades and mediate different cellular responses. Tyrosine phosphorylation induces their differentiation through the activation of downstream genes, whereas serine phosphorylation (e.g. by the non-canonical Notch pathway) leads to Hes3 transcription, sonic hedgehog expression and increased survival. Distinct signals from multiple plasma membrane receptors and cytoplasmic kinases induce the phosphorylation of STAT3 on the tyrosine 705 or serine 727 residues. JAK2 is the direct kinase for the tyrosine site, whereas mTOR is required for phosphorylation on the serine. In neural stem cells, tyrosine phosphorylation leads to differentiation whereas serine phosphorylation maintains their self-renewal properties and promotes their survival in culture. In contrast, cancer cells rely mostly on the tyrosine site for survival, whereas the serine site is considered auxiliary. Such differences in the signal transduction requirements between cancer cells and stem cells may be exploited to specifically target the stem cell compartment in cancer as well as regenerative medicine applications

Components of the non-canonical Notch pathway are regulated by multiple other pathways in the cytoplasm, providing additional ideas for manipulation in the context of CSC biology. For example, kinases including JAK and p38 negatively regulate this pathway and inhibitors of these two kinases, predictably, promote neural stem cell survival. JAK in particular can be activated by cytokines such as CNTF, LIF or cardiotrophin and directly phosphorylates STAT3. In addition, it phosphorylates p38 (Cohen, 1996), which has a distinct role in mediating the activation of the nuclear localized mitogen- and stress-activated protein kinases that are required for the stress-induced activation of CREB and ATF1 (Arthur and Cohen, 2000; Wiggin et al., 2002). JAK and p38 inhibition, therefore, limit the survival/proliferation functions of the Notch/Akt pathway (Androutsellis-Theotokis et al., 2006).

The phosphorylation events on Akt, STAT3 and mTOR occur and diminish within an hour of Notch activation, but they initiate changes in survival that last for days. The mediators of these survival effects are unknown at present, but likely involve changes in gene expression. The Hes and Hey genes encode a family of bHLH transcription factors that are direct targets of Notch-mediated transcription (Kageyama et al., 2007). Although the function of the Hes/Hey genes remains largely unknown, transgenic animal studies showed their importance for proper brain development (Hatakeyama et al., 2004). The mRNA for one member of this family, Hes3, is elevated within one hour by Notch ligands in neural stem cells (Androutsellis-Theotokis et al., 2006). Pharmacological experiments show that Hes3 mRNA is elevated only in conditions that lead to serine phosphorylation of STAT3 and that JAK -dependent tyrosine phosphorylation is a negative regulator of Hes3 levels. These data suggest that Hes3 is a candidate mediator of the long-term effects of the initial phosphorylation events. Experiments showed that Hes3 elevates expression the protein Shh. Shh is both a morphogen and a mitogen for neural stem cells (Ericson et al., 1995; Rowitch et al., 1999). Transfection of neural stem cells with Hes3 led to long lasting (several days) elevation in Shh levels, providing yet another mechanism to translate short term effects to changes that last for days.

The non-canonical Notch signaling pathway presents a time-ordered mechanism that controls both the survival and growth of stem cells. This pathway is not confined to neural stem cells, but at least parts of it apply to many other stem cell and progenitor systems. In human embryonic stem (ES) cells, STAT3 serine phosphorylation and inhibition of tyrosine phosphorylation correlate with survival (Daheron et al., 2004; Androutsellis-Theotokis et al., 2006).

Recent studies demonstrate that p38 inhibition is essential for the in vitro propagation of adult myocardiocytes (Engel et al., 2005) and we have shown that p38 inhibitors and other treatments described above promote the expansion of fetal pancreatic precursors expressing c-peptide (the pro-insulin protein) and somatostatin. Many of these signals are likely to affect properties of multiple cell types. Activin A, for example, a member of the TGF-β superfamily of proteins (Mason et al., 1985; Shi and Massague, 2003) inhibits the proliferation of neuroblastoma cells and the angiogenic properties of vascular endothelial cells (Panopoulou et al., 2005). Pigment epithelium-derived factor (PEDF) is a factor secreted by several cell types including endothelial cells (Aparicio et al., 2005) and acts as an inhibitor of angiogenesis (Dawson et al., 1999), a trophic factor for various neurons (Steele et al., 1993), and an activator of neural stem cells in the adult brain (Ramirez-Castillejo et al., 2006). Similarly, STAT3 mediates many functions in stem cells and endothelial cells. These results provide a general model for in vitro expansion of embryonic, fetal and adult stem cells.

The core principles of the non-canonical Notch pathway that regulate stem cell number also regulate CSCs. In a prostate cancer cell line, where an equilibrium between CSCs and more differentiated cancer cells is established, manipulation of STAT3-Ser through transfection with mutant constructs selectively affects the CSC component (Qin et al., 2008). These findings provide an example of how studying normal stem cell signaling requirements results in novel therapeutic strategies to target CSCs.

Therapeutics are in various clinical stages of development for modulation of both Notch and Shh signaling (Clinicaltrials.gov). Broad spectrum inhibitors of γ-secreatase, such as MK0752, are in phase I clinical trials in the treatment of refractory or recurring CNS cancers. Also, a number of antibody-based therapies directed against specific isoforms and the Notch receptor and its ligands are in pre-clinical development. In the case of the hedgehog (Hh) of molecules, GDC-0449, LDE225 and BMS-833923 are in clinical trials for treating refractory medulloblastomas in children. Their mechanism of action is not to antagonize the interaction of Hh with their Patched receptors, but prevent the ability of the co-receptor Smoothened to be recruited to a complex necessary for transduction of ligand binding to intracellular signal transduction cascades and gene expression. This highlights an important avenue for achieving greater specificity and potentially limiting the side effect profile of any inhibitor by targeting the interactions between signaling components that are responsible for the aberrant growth driving tumor formation while leaving the interactions with other signaling components intact.

4. The balance between self-renewal and differentiation

We discussed examples of how targeting the specific pro-survival requirements of stem cells can help to kill the CSC population of a tumor. Another possible mechanism for halting the growth of a CSC-driven cancer is to induce the differentiation of the stem cell population, therefore depriving the tumor of its growth engine. This approach has been successfully applied in both hematopoetic and non-hematopoetic cancers. For example, BMP4 treatment impedes the growth of glioblastomas by pushing them to an astroglial cell fate in vitro and in vivo (Piccirillo et al., 2006). The canonical Notch pathway is largely associated with glial differentiation (Furukawa et al., 2000; Hojo et al., 2000; Chambers et al., 2001; Scheer et al., 2001; Tanigaki et al., 2001; Ge et al., 2002; Taylor et al., 2007). In this case, a complex containing Hes1, JAK2 and Tyr705-phosphorylated STAT3 has been identified after Notch1 transfection (Kamakura et al., 2004). As we have discussed above, Notch can also activate a non-canonical pathway triggered by PI3K/Akt activation that requires Ser727 phosphorylation of STAT3. These findings suggest that the site of STAT3 phosphorylation determines whether stem cell renewal or differentiation is triggered. Additionally, treatment of xenografts with the γ-secretase inhibitor GSI-18 resulted in reduced proliferation, increased apoptosis in the nestin positive cell population, and elevated neuronal differentiation (Fan et al., 2006).

The idea that a dynamic tension exists between these pathways is also supported by evidence that Akt activation inhibits canonical Notch signaling, reducing the transcription of classic Notch targets including Hes1 and Deltex in T cells (Calzavara et al., 2007). There is no information on the effects of Notch signaling on cell proliferation in these experiments but a similar outcome has been observed in HEK cells where Akt inhibition promotes Notch-1 intracellular domain- and RbpSuh-mediated canonical Notch activity (Baek et al., 2007). These results are also consistent with a role for canonical Notch signaling in differentiation rather than self renewal.

The protein Numb inhibits canonical Notch signaling and controls asymmetrical cell fate decisions in Drosophila (Roegiers and Jan, 2004). In mice, Numb and Numblike also inhibit canonical Notch signaling (Guo et al., 1996; Spana and Doe, 1996). Deletion of both genes in Nestin$^+$ precursor cells in the developing SVZ induces loss of the immature precursor cells and leads to premature neuronal differentiation (Kuo et al., 2006). The Nestin$^+$ cells that escape Numb/Numblike deletion eventually regenerate the SVZ (Kuo et al., 2006). These results further suggest that the canonical Notch pathway opposes precursor cell renewal in the developing SVZ.

Numb/Numblike activity may not always lead to self-renewal and regeneration, as Numb prevents ubiquitination of the p53 protein, increasing the activity of this tumour suppressor (Colaluca et al., 2008). Elevated p53 activity is strongly associated with reduced proliferation and increased cell death in adult neural stem cells (Meletis et al., 2006). However, a nucleolar protein that negatively regulates p53, nucleostemin, is specifically expressed in stem cells but not in transit amplifying progenitors (Tsai and McKay, 2002; Tsai and McKay, 2005). Nucleostemin is a component of the signals required for cell growth in pluripotent cells of the blastocyst and in somatic stem cells, including NSCs (Tsai and McKay, 2002; Tsai and McKay, 2005; Beekman et al., 2006). In amphibian systems where de-differentiation leads to tissue regeneration, the early up-regulation of nucleostemin is consistent with an early stem-cell specific switch in the control of the p53 system (Maki et al., 2007).

These results showing a concerted switch in signaling when NSCs differentiate offer an unusual opportunity to define how interactions between pathways leads to biologically meaningful outcomes. Here we illustrate this point with interactions between Notch, Akt, mTOR, STAT3, Shh and p53. As we acquire improved control over NSCs and other stem cells, we suggest the predictive power of these signaling models will increase with important implications for our understanding of self-renewal and cancer.

5. Stem cell regulation by controlling protein localization

Regulating processes such as membrane trafficking, cytokeletal re-organization, and protein shuffling provide an alternative approach for controlling stem cell proliferation and survival. Treatment with the entertoxin protein cholera toxin, which has long been known to inhibit the growth of numerous cancer cell lines (Coffino et al., 1975; Cho-Chung et al., 1983; Pessina et al., 1989; Viallet et al., 1990; Allam et al., 1997; Pessina et al., 1998), interferes with membrane trafficking by binding to GM1 gangliosides on a subset of lipid rafts on cell membranes (Sahyoun and Cuatrecasas, 1975). This results in increased recycling of the Tie2 receptor and nuclear shuttling of Hes3 in cultures of fetal and adult neural stem cells. Nuclear Hes3 following cholera toxin treatment correlates with the proliferative state of neural stem cells (Androutsellis-Theotokis et al., 2010). In contrast, cultured neural stem cells that are induced to differentiate by mitogen removal quickly lose nuclear Hes3 expression while retaining cytoplasmic Hes3 pools, before they fully lose Hes3 expression altogether. Like self renewing neural stem cells, cancer stem cells in glioblastoma biopsies also show nuclear Hes3 staining (along with a distinct cytoplasmic pool, co-localized in prominin+ particles) (Androutsellis-Theotokis et al., 2010). These findings raise the possibility that Hes3 localization is a common mechanism by which both normal and cancer neural stem cells regulate their expansion.

As discussed previously, driving CSCs to differentiate is a viable mechanism for inhibiting tumor growth. Interestingly, cholera toxin can sometimes induce differentiation of malignant cells as has been observed with cell lines from lymphoma, glioblastoma, medulloblastoma, and melanoma, and appears to involve disrupting tyrosine kinase-dependent mechanisms by which neurotrophic factors stimulate malignant stem cell proliferation (O'Keefe and Cuatrecasas, 1974; Houghton et al., 1982; Olsson and Breitman, 1982; Dufay et al., 1994; Shaw et al., 2002; Li et al., 2007; Xu et al., 2009). It is intriguing to think of cellular responses to cholera toxin as a distinguishing factor between stem cells and more mature cells.

6. Stem cells and the vasculature

The similarities between normal and cancer stem cells extend beyond the intracellular signals they use in common. Both cell populations associate tightly with the vasculature, a central component of the stem cell niche, and are affected by factors such as cell-cell cell-extra cellular matrix interactions, as well as soluble ligands produced by cells comprising blood vessels. An important signal is vascular endothelial growth factor (VEGF), a major activator of angiogenesis in both embryos and tumors (Coultas et al., 2005; Jain et al., 2006). The VEGFR-2 receptor (Flk1/KDR) plays a role in adult angiogenesis and VEGFR-1 and VEGFR-2 are upregulated in the injured brain (Beck et al., 2002). Indeed, VEGF directly promotes the self-renewal of stem/progenitor cells in vitro (Maurer et al., 2003; Schanzer et al., 2004; Meng et al., 2006) and in vivo (Jin et al., 2002; Schanzer et al., 2004). Notch signals are downstream of VEGF and vascular growth is inhibited by Notch signaling (Siekmann et al., 2008). During normal development of the retina, the tip-cell state is favored in endothelial cells that lack Notch signaling (Hellstrom et al., 2007). Similar results have been obtained in developing fish embryos that lack Delta-like ligand 4 (Dll4) or Rbpsuh (Siekmann and Lawson, 2007). In a reverse experiment, Dll4 up-regulation inhibited the proliferation of endothelial cells in culture (Williams et al., 2006). Taken together, these results establish that Notch signaling regulates vascular development. Consistent with this notion and in the context of cancer, two recent papers indicate that inhibition of Notch signaling disrupts the vascular supply causing tumors to shrink (Noguera-Troise et al., 2006; Ridgway et al., 2006). These studies suggest that the therapeutic benefit of Dll4 inhibition is achieved by generating vessels that are poorly perfused. However, the persistence of this effect will determine the utility of Dll4 as a cancer therapeutic. Another consideration is the dual effects of Dll4 on blood vessels and neural stem cells which pose a challenge for dissociating the effects of treatments between the vasculature and the stem cell compartment.

Angiopoietin 2, the soluble ligand of the Tie2 receptor was found to have a similar effect as Dll4 on neural stem cells, both in vitro and in vivo, but the opposite effect on blood vessels (Androutsellis-Theotokis et al., 2009). In fact, Angiopoietin 2 has been known to be a potent pro-angiogenic factor. These results established Angiopoietin 2 as a pro-angiogenic soluble factor that increases the number of neural stem cells in vitro and in vivo.

When Dll4 and Angiopoietin 2 were mixed into a cocktail that also contained insulin and a JAK inhibitor (all of which are known to increase neural stem cell numbers), the effects on the vasculature were significantly reduced. These results demonstrate the potential to separately regulate neural stem cells and blood vessels through combinations of pharmacological treatments, a concept that has important implications in addressing the growth of tumor tissue containing a cancer stem cell compartment.

Targeting the neurovascular niche is at the center of many anti-cancer and pro-regenerative therapeutic approaches, as it contains blood vessels that often feed tumors and stem cells that may be stimulated to proliferate and replenish damaged tissue. This tight association between blood vessels and stem cells is complicating efforts to specifically affect vessels or stem cells in the context of degenerative disease or cancer. For example, in the case of regenerative therapy, one can envision treatments that increase the numbers of stem cells in the tissue without affecting the vasculature. In the case of chemotherapeutics, one may aim at a reduction of both the cancer stem cell compartment and inhibition of angiogenesis.
Advances in stem cell biology suggest possible treatment strategies to specifically target cell sub-populations in the normal and neoplastic tissue. Notch signaling pathway activation by Delta4 increases the number of endogenous neural stem cells in the brain, and at the same time reduces vascular coverage. Tie2 activation by Angiopoietin2 has a similar effect on endogenous neural stem cells but the opposite effect on blood vessels. VEGF, a secreted cytokine, promotes angiogenesis as well as neural stem cells survival and neurogenic potential. Combination treatments that include Delta4, Angiopoietin2, insulin, and a JAK inhibitor maximize the effects on endogenous neural stem cell increases but significantly reduce the angiogenic effects. VEGF inhibition and Dll4 inhibition are currently being exploited in cancer research in an effort to disrupt the blood supply to a tumor. VEGF inhibition reduces tumor vascularization by opposing angiogenesis. Dll4 inhibition decreases blood flow to the tumor by enhancing non-productive angiogenesis, i.e. the formation of new blood vessels from existing ones that are not able to efficiently carry blood to the tissue. VEGF and Dll4 also promote the survival of neural stem cells. As a result, VEGF inhibition and Dll4 inhibition may also reduce the number of stem cells in a tumor, and this effect may be partly responsible for the anti-cancer functions of these treatments (Figure 3).

7. Implications and conclusions

Current therapeutic approaches for cancer are based on the decades old concept of targeting the proliferative state of the cells in a tumor. This strategy is effective at killing those cells that proliferate fast and which therefore comprise the bulk of the tumor. As a result, many tumors can be shrunk in size. However, certain tumors contain a resistant cell population, the cancer stem cell, which is often spared and can regenerate the disease as they are able to produce more CSCs (self renewal) along with more differentiated cells which will make up the bulk of a tumor (potential). In cancer, regeneration manifests itself as both recurrence and metastasis (regeneration in a new location).
Accumulating knowledge suggests that CSC resistance to therapy is partly due to the fact that they operate variations of normal proliferative and survival signaling pathways. These cells can be isolated and cultured in order to study their signal transduction requirements. However, CSCs contain vast numbers of mutations and exhibit great genomic instability, making it difficult to work with. The heterogeneity of the mutations found in CSCs from different patient samples further hinders drawing general conclusions. It is becoming appreciated that many of the properties of CSCs are also found in primary non-tumor stem cells. For example, neural stem cells use similar signaling pathways as their cancerous counterparts (e.g. from brain tumors). This distinguishes normal and cancerous stem cells from more differentiated tumor cells. Primary neural stem cells from non-cancerous tissue, therefore, are a valuable model to study the signaling requirements of CSCs from brain tumors, as they avoid the problem of heterogeneous mutations and genomic instability.

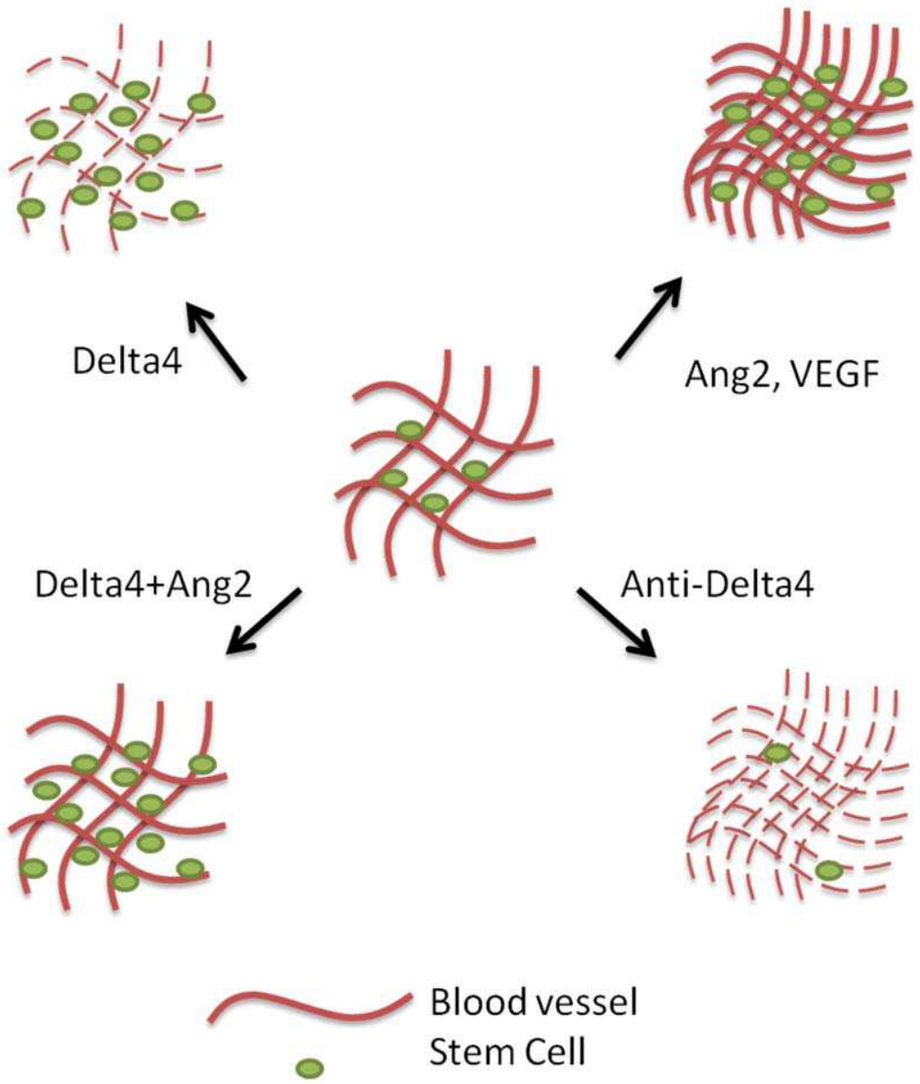

Fig. 3. Distinct modulation of stem cell and vascular biology. Combinations of pro- and anti-angiogenic factors that also influence neural stem cell numbers offer the potential to distinctly regulate the vasculature and the endogenous neural stem cell population in tissues to best address anti-cancer or regenerative medicine applications

Understanding these pathways not only provides us with novel targets for chemotherapeutics, but allows us to consider the impact current and future therapies may have on underlying malignancies. For example, VEGF is being used on a trial basis for treatment of peripheral vascular disease, chronic skin ulcers and coronary artery disease by stimulating growth of new blood vessels, but VEGF also can stimulate proliferation of malignant glioblastoma stem cells (Huang et al., 2010; Kim et al., 2004; Lekas et al., 2004; Bao et al., 2009). Trophic factors in the central nervous system such as nerve growth factor, brain-derived neurotrophic factor and others have long been investigated as a potential cure for devastating neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (Aron and Klein, 2010; Rangasamy et al., 2010). There are many technical obstacles

to perfecting a workable therapeutic regimen for trophic factors in the central nervous system, such as achieving adequate delivery to large numbers of target neurons over a large absolute space within a human brain (Alisky and Davidson, 2000). However, once these technical issues are resolved, those contemplating clinical use of neurotrophic factors will have to consider their potential to stimulate cancer cells. Receptors for neurotrophic factors, and growth stimulated by these factors, can be demonstrated for a diversity of malignancies that run the gamut from solid organ to epithelial, lymphoid and hematopoietic (Dudas et al., 2011; Pearse et al., 2005; Thiele et al., 2009). Stimulation of tyrosine kinase receptors by neurotrophins is probably the molecular basis by which trophic factors proliferate malignant stem cells (Dudas et al., 2011; Zhang et al., 2010).

Evidence that a trophic factor can produce malignancy by stimulation of malignant stem cells is the occurrence of a clinically significant cancer that did not exist until a trophic factor was given, that regressed when the factor was withdrawn, and that demonstrated trophic-factor dependent growth in vitro. This was indeed true for a patient with epogen-driven acute myelogenous leukemia (Bunworasate et al., 2001), but to the best of our knowledge, this is the only case history where the cause and effect relationship between trophic factor and stimulation of malignant cells is so iron clad. Epogen is a synthetic form of erythropoietin, a polypeptide hormone produced mainly in the kidneys which stimulates production of red blood cells from erythroblast precursors in the bone marrow. Recombinant erythropoietin is frequently employed for treatment of anemia from chronic kidney disease, bone marrow failure and cytotoxic cancer chemotherapy (Spivak et al., 2009). Thus, a systemic search is needed to find cases and compile a registry for epidemiological and clinical data, and then a tumor bank could be set up, dedicated to store issue samples for further investigation, especially for isolating and purifying malignant stem cell populations from more differentiated neoplastic cells. We would in essence be seeking to extend Koch's postulates of infectious disease (proving a particular pathogen causes a specific disease) to the realm of oncology, by proving trophic factor stimulated malignant stem cells are the cause of malignancy (Garcion et al., 2009).

The cancer stem cell hypothesis will likely guide the thinking that brings about important future breakthroughs in cancer treatments. The wealth of information generated from ongoing studies of somatic stem cell biology will provide critical insight into how uni- and multimodal therapeutic approaches are applied to maximize the benefits to patients while minimize side effects. These include the establishment of extensive gene expression profiles that allow for more precise identification of CSC populations and detailed signal transduction analyses like those described in this review that define novel pharmacological targets. This understanding will also have important consequences that shape how endogenous stem cells and exogenous trophic factors are utilized as the field of regenerative medicine continues to grow.

8. References

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Cancer Stem Cells - The Cutting Edge

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Over the last thirty years, the foremost inspiration for research on metastasis, cancer recurrence, and increased resistance to chemo- and radiotherapy has been the notion of cancer stem cells.The twenty-eight chapters assembled in Cancer Stem Cells - The Cutting Edge summarize the work of cancer researchers and oncologists at leading universities and hospitals around the world on every aspect of cancer stem cells, from theory and models to specific applications (glioma), from laboratory research on signal pathways to clinical trials of bio-therapies using a host of devices, from solutions to laboratory problems to speculation on cancersâ€™ stem cellsâ€™ evolution. Cancer stem cells may or may not be a subset of slowly dividing cancer cells that both disseminate cancers and defy oncotoxic drugs and radiation directed at rapidly dividing bulk cancer cells, but research on cancer stem cells has paid dividends for cancer prevention, detection, targeted treatment, and improved prognosis.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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