

Manipulating Midbrain Stem Cell Self-Renewal

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In this issue of *Cell Stem Cell*, Falk and colleagues (Falk et al., 2008) demonstrate that differential responsiveness to TGF- β signaling selectively modulates self-renewal of dorsal midbrain stem cells. This observation may lead to strategies for expanding specific neural stem cell subtypes.

Studies that elucidate regulatory mechanisms that impact endogenous stem cell function offer important insights into normal developmental pathways and also provide clues that may be applied to in vitro protocols designed to differentiate and/or expand populations with potential clinical relevance. In this issue, Falk and colleagues (Falk et al., 2008) demonstrate that TGF- β signaling is part of a regionally specific mechanism for regulating the size of the dorsal midbrain. The authors propose that TGF- β released systemically into the cerebral spinal fluid can have selective effects due to the localized expression pattern of TGF- β receptors and signaling machinery. The choroid plexus is the presumed source of TGF- β 1 ligand, based on strong immunolabelling, and the TGF- β target is likely the neuroepithelial cells lining the ventricles, as they express the activated TGF- β mediators Smad2/3. To examine the role of TGF- β signaling during midbrain/hindbrain development, the *Cre/LoxP* system was used to conditionally delete the TGF- β receptor, *Tgfr2*, using *Emx1-Cre* to localize effects to the midbrain/hindbrain. Deletion of *Tgfr2* in the midbrain led to selective expansion of the dorsal neuroepithelium. The expansion was due to an increase in neuroepithelial cell proliferation and was characterized by a shorter cell-cycle time and decreased cell-cycle exit.

The authors note that an identical phenotype was observed previously, in that *Wnt1* overexpression leads to an expanded caudal midbrain by enhanced neuroepithelial cell proliferation and is also associated with shortening of the cell cycle (Panhuysen et al., 2004). Based on this similarity, Sommer and colleagues suggest that the primary function of TGF- β may be to repress

Wnt signaling. The canonical Wnt signaling pathway appears to be involved, as ectopic *Wnt1* expression was observed in the mutant dorsal midbrain, along with nuclear accumulation of β -catenin in the affected region, as would be expected following β -catenin activation. The expanded number of neuroepithelial cells in the *Tgfr2*-deficient midbrain region is also reminiscent of the effect of β -catenin overexpression in the forebrain that promotes self-renewing symmetric divisions and expansion of the neuroepithelial population (Chenn and Walsh, 2002). Furthermore, inactivation of TGF- β signaling enhanced expression of the cell-cycle-promoting factors *CyclinD2* and *CyclinD1*, known Wnt targets, as well as the cyclin interaction partners *Cdk4* and *Cdk2*, supporting the concept that TGF- β acts to repress Wnt signaling. In addition to involvement of Wnt signaling, Falk et al. (2008) show that *fgf8* mRNA was ectopically expressed in the *Tgfr2*-deficient midbrain region. This ectopic expression is consistent with the authors' interpretation that regulated FGF signaling may play a role in dorsal midbrain expansion. Taken together, the expansion of neuroepithelial cells by self-renewal appears dependent upon FGF and Wnt signaling, and TGF- β antagonizes canonical Wnt signaling and represses *fgf8* expression to negatively regulate self-renewal and limit the size of the midbrain proliferative zone.

The findings of this paper have implications both for understanding developmental patterning and for propagation and selection of regionally specified neural stem cells. From a developmental perspective, it is known that areal patterning of the neural tube occurs by a series of localized inductive signals, often released from nonneural tissues (for review, see

Jessell and Sanes, 2000). The inductive signals then combine with largely undefined intrinsic programs to determine subsequent region-specific growth and proliferation rates of the instructed, regionalized progenitor populations. Differences between individuals or species in either induction or intrinsic proliferation patterns may account for differences in the relative sizes of brain regions. The current results of Falk et al. (2008) suggest that the relative size of the midbrain to forebrain region is determined in part by the differential response of dorsal midbrain progenitors to TGF- β released into the ventricle from the choroid plexus. Of note, the authors also demonstrate a kinetic specificity of the effects of TGF- β signal repression, in that the earliest patterning events during midbrain and hindbrain development are unaffected by deletion of the TGF- β receptor. This observation raises the possibility that the neuroepithelium is regionalized by both early and late events, whereby initial local specification of receptor expression leads to subsequent cell selection and patterning in response to nonlocalized signals.

Importantly, Falk et al. (2008) reveal a differential response to TGF- β signaling among neuroprogenitors that may have important implications for the propagation and selection of mixed neural stem cell populations. The authors demonstrate that TGF- β signals specifically induce midbrain progenitors to re-enter the cell cycle, whereas forebrain progenitors were unaffected by either inactivation or activation of TGF- β signaling. In addition, cell-cycle induction was shown to correlate with the capacity to undergo multiple rounds of self-renewal, as assessed using neurosphere assays. Combined, these observations suggest

that in mixed populations of neural progenitors, such as those that typically arise during neural differentiation of embryonic stem cells, regionally fated progenitors may be selectively sensitive to factor(s) that shorten the cell cycle and enhance self-renewal. Over several passages, a significant increase in one population relative to another may result. This observation may permit researchers to perform negative and positive selection to enrich specific progenitor subtypes. For example, according to the Falk et al. study, addition of TGF- β to mixed forebrain/midbrain neurosphere cultures would favor forebrain-derived relative to midbrain-derived progenitors due to reduced midbrain progenitor self-renewal. These results also underscore the idea that culture conditions that appear to direct stem cell differentiation toward a particular lineage may, in fact, lead to the enrichment of a specific population by differen-

tial expansion of a pre-existing progenitor instead.

Several important questions, both developmental and practical, are raised by the results of Falk et al. (2008). First, do physiological or pathophysiological alterations in TGF- β levels alter the relative size of the midbrain? Second, do species differences in the responses to or release of TGF- β correlate with the relative size of midbrain structures such as the superior and inferior colliculi? Third, are all progenitors within the dorsal midbrain equally capable of enhanced self-renewal upon removal of TGF- β signaling, or are specific subsets of the population sensitive to TGF- β ? And fourth, are there other region-specific signaling cascades that might be manipulated in neural stem cell cultures to selectively expand particular progenitors? For example, do populations with therapeutic potential, such as forebrain or ventral midbrain

progenitors, exhibit differential sensitivity to other factors that stimulate or inhibit self-renewal? If so, it may be possible in the future to select different neural stem cell subtypes from mixed populations by taking advantage of definitive region-specific responses such as those described by Falk et al.

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Cutaneous Cancer Stem Cells: β -Catenin Strikes Again

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Cancer stem cells (CSCs) are a subpopulation of tumor cells that retain properties of tissue-specific stem cells, including the ability to self-renew. In a recent article in *Nature*, Malanchi et al. (2008) identified a population of CD34⁺ cells in epidermal tumors that require β -catenin signaling to maintain a CSC phenotype.

Tissue-specific stem cells maintain a strictly regulated balance between self-renewal and differentiation to preserve the stem cell pool and give rise to all the cell lineages in the organ. Stem or progenitor cells may serve as cancer-initiating cells, following deregulation of the self-renewal process (Wicha et al., 2006). It has been hypothesized that cancer-initiating stem cells give rise to cancer stem cells (CSCs), although there is a lack of direct evidence that these are the same cell population. CSCs are a subpopulation of cells within tumors that retain stem cell proper-

ties, undergoing long-term self-renewal and driving tumor growth by giving rise to the differentiated cells that comprise the majority of the tumor mass. CSCs have been well-characterized in hematopoietic tumors, where serial transplantation studies in immunocompromised mice demonstrate that grafted CSCs give rise to tumors that maintain the complexity of the primary tumor. More recently, CSCs have been identified in solid tumors including breast, central nervous system, prostate, colon, and pancreas (Ailles and Weissman, 2007; Al-Hajj et al., 2003).

Mammalian epidermis is a rapidly renewing tissue and is maintained by stem cells that give rise to the differentiated lineages of the interfollicular epidermis, sebaceous glands, hair follicles, and sweat glands. The bulge region of the murine hair follicle contains a population of multipotent stem cells capable of giving rise to all of the epithelial cell lineages within the hair follicle during normal hair growth and contributing transiently to the interfollicular epidermis in response to wounding. Whereas most epidermal cells are lost via a process of terminal differentiation,