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Commentary

Cell replacement therapy: Lessons from teleost fish

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ABSTRACT

Many disorders of the CNS are characterized by a massive loss of neurons. A promising therapeutic strategy to cure such conditions is based on the activation of endogenous stem cells. Implementation of this strategy will benefit from a better understanding of stem cell dynamics and the local CNS microenvironment in regeneration-competent vertebrate model systems. Using a spinal cord injury paradigm in zebrafish larvae, Briona and Dorsky (2014) have provided evidence for the existence of two distinct neural stem cell populations. One population has the characteristics of radial glia and expresses the homeobox transcription factor Dbx. The other lacks Dbx but expresses Olig2. These results are placed in the context of other studies that also support the notion of heterogeneity of adult stem cells in the CNS. The implication that differences among stem cell populations, in combination with specific factors from the local cellular microenvironment, might have a decisive impact on the fate choices of the new cells, is discussed. Reviewed evidence suggests that rather few modifications in the signaling pathways involved in the control of stem cell behavior have led, in the course of evolution, to the pronounced differences between mammals and regeneration-competent organisms. As a consequence, rather minor pharmacological manipulations may be sufficient to reactivate the hidden neurogenic potential of the mammalian CNS, and thus make it available for therapeutic applications.

Brain and spinal cord injuries: curable through activation of endogenous stem cells?

Brain injuries caused by trauma and stroke, as well as spinal cord injuries, are complex disorders of the central nervous system (CNS) associated with massive loss of neurons. It has been estimated that globally every year over 10 million people suffer a traumatic brain injury (Hyder et al., 2007), approximately 17 million a stroke (Feigin et al., 2014), and up to 500,000 a spinal cord injury (Bickenbach et al., 2013). While current pharmacological treatments and rehabilitative therapies can lead to modest improvements in neurological functions in some individuals (Behrman et al., 2006; El-Kheir et al., 2014; Fehlings and Baptiste, 2005; Young, 2014), no cures exist yet for either of these conditions.

A promising strategy toward restoration of function is based on harnessing the intrinsic potential of the CNS for self-repair through activation of endogenous neural stem/progenitor cells. The feasibility of such a strategy is supported by the observation that after experimental injuries proliferation is stimulated among both active and latent stem/progenitor cells (Greenberg, 2007; Obermair et al., 2008; Ohab and Carmichael, 2008; Panayiotou and Malas, 2013; Parent, 2003; Richardson et al., 2007; Zhang et al., 2005). Cerebral ischemia, for example, evokes the mobilization of endogenous neural stem/progenitor cells in the two neurogenic niches of the adult mammalian brain, the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus of the hippocampus (Arvidsson et al., 2002; Jin et al., 2001; Liu et al., 1998; Rueger et al., 2010; Takasawa et al., 2002; Zhang et al., 2008). In addition, under pathological conditions, including focal and global ischemia, new neurons may arise in certain brain regions from adult stem cells that are quiescent in the healthy brain (Ohira, 2011). Similarly, traumatic injuries to the spinal cord can recruit ependymal cell populations which are normally quiescent (presumably post-mitotic) in the intact spinal cord (Spassky et al., 2005), and induce long-lasting ependymal proliferation within a large area around the lesion site (Lacroix et al., 2014; Lee et al., 2014; Meletis et al., 2008).

An important property of the neural progenitors generated in response to injury is their ability to deviate from a normal pattern of development to migrate to the sites of injury. Such a specific re-routing of migration is commonly observed after stroke, when neuroblasts generated in the subventricular zone (from where they migrate under healthy conditions via the rostral migratory stream into the olfactory bulb) change their path to migrate to the ischemic boundary zone (Arvidsson et al., 2002; Jin et al., 2003; Kojima et al., 2010; Parent et al., 2002; Yamashita et al., 2006). This area, which defines the border between infarcted and non-infarcted tissue, is characterized by pronounced angiogenesis (Ohab et al., 2006; Thored et al., 2007).

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Neurogenesis and angiogenesis in this region are closely linked in that subventricular zone cells increase expression of genes involved in angiogenesis after stroke (Liu et al., 2007), while endothelial cells produce factors that promote neurogenesis, neuronal differentiation, and survival of the new neurons (Jin et al., 2002; Leventhal et al., 1999; Louissaint et al., 2002; Meng et al., 2006).

Despite these encouraging findings of recent years, a cell replacement therapy based on the activation of endogenous stem/progenitor cells is still far from clinical implementation. Major hurdles that need to be overcome toward this goal include the activation of a sufficient number of stem/progenitor cells, the targeted migration of the neuroblasts to areas of focal damage, the controlled development of their progeny into the correct, functional types of cells, and the promotion of long-term survival of the new cells. Neither of these critical issues has been resolved in animal models thus far. For instance, after experimentally induced stroke, immature neurons born in the subventricular zone migrate to the striatal area damaged by the ischemic insult, and even start to express markers for striatal medium-sized spiny neurons (the phenotype most severely affected by ischemia). However, the majority of them die within a few weeks after the stroke (Arvidsson et al., 2002). Thus, it appears that the local environment is unable to support the long-term survival of new neurons. Equally significant, the total number of new neuronal cells is far too low to replace the degenerated ones. In this study, the number of new neurons generated in response to injury totaled just 1600, while an estimated 800,000 such cells were lost. In the mammalian spinal cord, the situation appears even more severe, since although contusion injury promotes massive proliferation of local progenitor cells, their progeny invariably differentiates into astrocytes and, more rarely, into oligodendrocytes, but not into neurons (Lacroix et al., 2014; Meletis et al., 2008).

The importance of better understanding how different aspects of the molecular microenvironment support the development of neural progenitor cells in regeneration-competent organisms is underscored as the number of studies using stem cell implantations increases. A recent study elegantly pointed out current caveats of stem cell replacement therapy in mammals. Kumamaru et al. (2012) performed a detailed analysis of the expression profiles of neural stem/progenitor cells transplanted into the injured spinal cord of mice. They found that, while the grafted stem cells had a positive immediate effect, reducing levels of apoptosis and inflammation, in the long term they failed to properly differentiate. In addition, these grafted cells showed increased expression of brain tumor-specific alternative splicing variants in a number of genes. Interestingly, the microenvironment of the injured spinal cord appeared to have a distinct inhibitory effect on overall stem cell gene expression, much more so than the unlesioned tissue or cell culture environments (Kumamaru et al., 2012).

Teleost fish: models for regeneration competency

The limited success in the implementation of therapies based on endogenous stem cells in the CNS of mammals makes obvious the need for a better understanding of the dynamics of adult stem cells both in normal development and under pathological conditions. Although the mammalian CNS makes clear attempts for self-repair after injury or neurodegeneration, these attempts do not lead to successful structural repair and functional recovery. An attractive research strategy is, therefore, to study non-mammalian model systems with an intrinsic potential for regeneration of nervous tissue. Despite the wide variation in regenerative potential among vertebrates, such a strategy is viable because the capacity for regeneration manifests itself on a continuum, with regeneration competence and regeneration incompetence representing just the two extremes of this spectrum. Most importantly, throughout this range of possibilities, animals share many mechanistic principles that underlie the regenerative processes (Stoick-Cooper et al., 2007). These shared molecular signaling pathways make it possible to extract general principles from the study of regeneration-competent organisms, and, based on the insights obtained, to develop therapeutic strategies for regeneration-incompetent vertebrates, including humans. Among the regeneration-competent organisms, several species of teleost fish are particularly well examined.

Historically, three milestones were critical toward the establishment of teleost fish as a model of regeneration competency: In 1922, Koppányi and Weiss reported that 60 days after spinal cord transection European carp regained normal swimming behavior (Koppányi and Weiss, 1922). In 1935, Tuge and Hanzawa showed that the functional regeneration after spinal cord injury is closely linked to the structural repair of lesioned spinal cord tissue (Tuge and Hanzawa, 1935). Finally, in 1961 Kirsche and Kirsche demonstrated that the successful regeneration critically depends on the presence of 'matrix zones' (Kirsche and Kirsche, 1961), a synonym for neurogenic niches. If these areas of high mitotic activity are destroyed through injury, regeneration fails.

Since these early studies, a much more comprehensive picture of the developmental events underlying the generation of new neurons has emerged, in both the intact and the injured teleostean CNS (Chapouton et al., 2007; Kaslin et al., 2008; Sîrbulescu and Zupanc, 2011, 2013; Zupanc and Sîrbulescu, 2011, 2013). Although only a dozen or so species out of an estimated 30,000 teleosts have been studied in detail thus far, the multitude of features shared among diverse lineages suggests that adult neurogenesis and neuronal regeneration, including their underlying molecular pathways, are evolutionarily conserved traits of many, if not all, teleosts. In each of the species examined, new neurons are generated during adulthood in dozens of proliferation zones of the brain as well as in the spinal cord. These proliferation zones are distinguished by a high concentration of adult stem/progenitor cells. The number of their progeny, relative to the total number of brain cells, is at least one, if not two, orders of magnitude higher than in the mammalian brain or spinal cord. The continuous generation of new cells, combined with the long-term persistence of roughly half of the adult-born cells, results in the growth of the teleostean brain beyond embryonic and juvenile stages of development. Injuries induce the replacement of lost cells by new cells arising both from the neurogenic niches active in the intact brain and from population(s) of quiescent stem cells spread over wide areas of the brain. This structural repair results most often in complete functional recovery.

Dbx-expressing progenitors: stem cells in the zebrafish spinal cord

What is it that enables fish — very much in contrast to mammals — to successfully regenerate CNS tissue? One of the key aspects mentioned above — stem cell dynamics — has been addressed in a report published in a recent issue of *Experimental Neurology* (Briona and Dorsky, 2014). Using a *dbx1a:GFP* transgenic reporter line, the authors showed that this gene was preferentially expressed in a slowly dividing progenitor population in the spinal cord of embryonic and larval zebrafish. *Dbx* genes encode a family of homeodomain-containing proteins of the *Drosophila* H2.0 class (Lu et al., 1992). During mouse embryogenesis, the *Dbx* gene is expressed in many regions of the CNS, including the telencephalon, diencephalon, mesencephalon, cerebellum, and spinal cord. However, within each of these regions, its expression is highly restricted to areas of mitotic activity (Lu et al., 1992, 1994). *Dbx*-expressing precursors generate a subset of interneurons, as well as astrocytes and a subpopulation of oligodendrocytes (Fogarty et al., 2005). In the spinal cord, a discrete subset of commissural interneurons, whose fate is controlled by the activity of *Dbx1*, plays a critical role in the regulation of left-right alternation of firing in motoneurons innervating hindlimb muscles, and thus in the control of proper walking movements (Lanuza et al., 2004).

Co-labeling of the cells expressing the *dbx1a:GFP* transgene in the zebrafish spinal cord with glial fibrillary acidic protein (GFAP) identified them as radial glia. These cells continue to generate neurons beyond embryogenesis during larval stages of development, but are distinct from progenitors expressing Olig2, which have been proposed as neural