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Neural Stem Cells of CNS and PNS and Their Regulation by Sox Genes

Jaesang Kim

Division of Molecular Life Sciences, Ewha Womans University,
Seoul 120-750, Korea

ABSTRACT

Neural stem cells function as the cellular source for the nervous system construction during embryonic development and for the continuous turnover of neurons in adults. Neural stem cells may also serve ultimately as the source material for the treatment of degenerative neural diseases and neural traumas. Much needs to be learned, however, about the neural stem cells before such practices can be realized. Particularly important is understanding the molecular mechanism of self renewal and proliferation which is not only necessary for generating a sufficient quantity of neural stem cells but also critical for safe application of them. Several recent studies suggest that members of Sox transcription factor family play critical roles in specifying key attributes of neural stem cells of central and peripheral nervous systems. Their function in inhibiting premature neurogenesis and maintaining cellular proliferation is just beginning to be understood providing the basis of further molecular characterization of neural stem cells. This review will provide up-to-date background information about neural stem cells and discuss the significance and implication of recent functional analyses of Sox genes.

Key words: Regeneration, Neural stem cells, CNS, PNS, Sox

Neural Stem Cells of CNS and PNS

The vertebrate nervous system can be divided into the central nervous system (CNS) and the peripheral nervous system (PNS). Such division reflects the cellular origin of the tissues as well as the hierarchy of functional relationship. The CNS originates from the dorsal ectoderm which under the influence of the underlying notochord and surrounding epidermal ectoderm develops into the neural plate (Nicholls et al., 1992). The subsequent cellular movements and morphological changes collectively known as the neurulation lead to neural tube formation. It is the enlargement and structural elaboration of the neural tube that give rise to the brain and spinal cord. The PNS originates from the neural crest, a vertebrate unique tissue transiently present during embryogenesis (Le Douarin and Kalcheim, 1999). Neural crest cells arise from the ectoderm at the border region between the neural plate and epidermal ectoderm. Rather than becoming a part either of the neural tube or the epidermis, neural crest cells undergo an epithelial to mesenchymal transition and migrate extensively into the body proper and differentiate into a variety cell types.

The role of neural stem cells during embryogenesis is well defined. They build the neural

*To whom correspondence should be addressed.
TEL: 02-3277-3414, FAX: 02-3277-3760
e-mail: jkim1964@ewha.ac.kr

tissues and organs by serving as the source of cells. In CNS, active proliferation of cells is found throughout the developing brain and the spinal cord (Gage, 2000; Pevny and Rao, 2003). The so called ventricular zone (VZ) next to ventricles of the brain and central canal of the spinal cord is where undifferentiated cells continue to divide and initiate their lateral migration. The relative size of the ventricular zone varies among the regions of the brain, and in all areas gradually diminishes in relative terms as the development progresses. Neural crest cells continue to divide after their detachment from the ectoderm and migrate along defined pathways to their predestined sites (Le Douarin, 1986; Anderson, 1997). They generate neurons and glial cells of PNS organs including dorsal root ganglia and sympathetic and parasympathetic ganglia. They also differentiate into many non-neural cell types including melanocytes, smooth muscle cells, endocrine cells, and chondrocytes.

The operational definition of neural stem cells is that they must possess self-renewal capacity and multipotency (Morrison et al., 1997; Gage, 2000). In CNS, this implies that a stem cell should generate more stem cells and be able to differentiate into neurons, astrocytes and oligodendrocytes (Reynolds and Weiss, 1992; Reynolds and Weiss, 1996; Rietze et al., 2001). A rigorous proof that a single cell can satisfy all these conditions requires a clonal analysis. This has been difficult in vivo as labeling and following the fate of a single cell in CNS during embryogenesis represent a major technical challenge. In this regard, a breakthrough was the establishment of in vitro culture of CNS stem cells. Reynolds and Weiss reported the isolation of self-renewing and multipotent cells from mammalian embryonic CNS that grows in vitro as an aggregation of cells known as the neurosphere (Reynolds and Weiss, 1996). When dissociated, a certain fraction of single cells could regenerate neurospheres, and when induced to differentiate, cells within a single neurosphere could differentiate into neurons, astrocytes, and oligodendrocytes. In PNS, although somewhat more successful attempts have been made in terms of labeling single neural crest cells in vivo for fate analyses, in vitro approaches using avian and mammalian neural crest cells have been more resounding in demonstrating the existence of self-renewing and multipoint stem cells. In particular, Stemple and Anderson have isolated rat neural crest stem cells from 10.5 d old embryos that could be cultured at a clonal density and generate neurons, glial cells and smooth muscle cells (Stemple and Anderson, 1992; Stemple and Anderson, 1993).

Regenerative Potential of Neural Tissues

Organs of the nervous system including brain, spinal cord, and peripheral ganglia have long been thought to be static tissues once the development is completed. This implies that once the maximal number of cells is reached in each of these organs, the only change in them is the loss of cells through the aging process. This notion has been challenged by many recent evidences against it obtained mainly from mice and rats. Chief among them is bromodeoxyuridine (BrdU) labeling of cells in the adult mouse brain which clearly showed that newly dividing population of cells was present in the adult brain (Nilsson et al., 1999). Another is the isolation of stem cells and establishment of neurosphere culture from the adult mouse CNS again clearly showing that a population of stem cells is present (Rietze et al., 2001). In fact, many different subregions of adult brain have been shown to contain neurosphere-generating stem cells (Garcia-Verdugo et al., 1998; Seaberg and van der Kooy, 2002). Furthermore, certain region-specific characteristics in terms of gene expression are seen in thus-derived neurospheres suggesting that diverse regeneration programs may exist in the brain and that regeneration is a much more common phenomenon than thus far confirmed (Hitoshi et al., 2002). Neurospheres have been generated even from a fetal human brain as well attesting to the generality of the presence of stem cells (Uchida et al., 2000). Efforts to isolate stem cells from adult PNS have not been as extensive as those from CNS. Nevertheless, it is now clear that long after the migration to peripheral ganglia by neural crest cells is completed, undifferentiated multipotent neural cells are present there. This has been clearly demonstrated by isolation of multipotent stem cells and neurosphere generating cells from enteric ganglia of rodent models. (Kruger et al., 2002; Molofsky et al.,

2003).

Are the neurons generated in the adult stage functional? This is the key question in that only if the neurons are functional, any efforts to manipulate the "regeneration" process using either endogenous or exogenous stem cells would be meaningful. Several circumstantial evidences suggest that they are. First, environmental stimuli and exercise, both of which have been causally associated with improved brain function, promote neuronal generation in the brain (Nilsson et al., 1999). Second, injuries to brain promote cellular proliferation in the vicinity of injured sites (Arvidsson et al., 2002; Hallbergson et al., 2003). Third, new born neurons in the adult dentate gyrus make synaptic contacts and display action potential (van Praag et al., 2002). Though not direct proofs that new born cells are involved in brain function or injury response, these data suggest that these new born cells and neurons derived from them are functional. A more direct test has been recently made by Santarelli and co-workers using the novelty-suppressed feeding test which is used to assess the effect of antidpressants in mouse models (Santarelli et al., 2003). Their insight was based on the fact that although the biochemical activity of many antidepressants is seen immediately after their application, the actual effect of the therapy requires over 3 to 4 weeks to be detectable. This happens to be the time required for the burst of neuronal birth stimulated by antidepressants. They tested their hypothesis that the new born neurons are required for the recovery from depression by blocking the cell proliferation with X-ray exposure. Indeed, the recovery from the depression was effectively blocked by X-ray thus supporting the idea that new born neurons have real functions.

There are also evidences from applying exogenous cells into the system. That neural stem cells, when introduced into neural tissues, can differentiate into a neuron with essential properties including neuronal polarity and synaptic activity has been shown (Song et al., 2002). Plus, using "neural-differentiated" embryonic stem cells McDonald and co-workers showed that introduced cells differentiated into astorcytes, oligodendrocytes, and neurons (McDonald et al., 1999). In this case, however, a direct cause and effect relationship has not been demonstrated between the neurons and the change in motor behavior which was being tested.

Sox Genes and Their Role in Neural Stem Cells

The interest in neural stem cells is in large part based on the hope of using them for therapeutic purposes. There are many diverse and complex issues including the source, safety, efficacy and ethical concerns. From a scientific view, much progress needs to be made in terms of regulating their expansion prior to and after transplantation, inducing their differentiation into desired neuronal or glial cell types, and integrating them into the existing neural networks. In short, we need to learn more about the neural stem cells especially at the molecular level. Studies about genes that control proliferation and differentiation are of particular importance in this regard.

Sox (Sry-type HMG box) genes make up a family of transcription factors (Soullier et al., 1999; Bowles et al., 2000; Wilson and Koopman, 2002). They share a common DNA binding motif called HMG (High Mobility Group) box which is known to be a minor groove binding domain causing DNA bending (Bewley et al., 1998). Sox genes are found in all metazoan species, and over 20 members are believed to exist for a given mammalian species. They show diverse and overlapping expression patterns and play critical roles during embryonic development. This family of transcription factors is often divided into several subfamilies. The subfamilies of interest for neural stem cell biologists are SoxB1 family which includes Sox1, Sox2, and Sox3 and SoxE family which includes Sox8, Sox9, and Sox10. SoxB1 family members are expressed in developing CNS while SoxE family members are expressed in neural crest derivatives (Bowles et al., 2000; Pevny and Rao, 2003).

The functional roles of the SoxE and SoxB1 family members in neural stem cells have been reported in a series of recent publications. SoxB1 family members are expressed in precursor populations of developing CNS and are apparently down-regulated during terminal differentiation and the accompanying cell cycle arrest (Bylund et al., 2003; Graham et al., 2003). Importantly, by following

the fate of a single Sox2 expressing cell, it was shown that at least Sox2 was expressed in multipotent stem cells that give rise to neurons, astrocytes and oligodendrocytes. Functional analyses carried out by two independent research groups took advantage of the in ovo expression system in which expression plasmid constructs are injected into the lumnen of the chicken spinal cord and electroporated into one side of the spinal cord (Bylund et al., 2003; Graham et al., 2003). The effect of introducing Sox B1 gene derivatives were examined by comparing the cells that incorporated the construct and the cells that did not as well as by comparing the two sides of the spinal cord. Neruogenesis as detected by the expression of proneural markers and terminal neuronal differentiation markers was inhibited among the cells that expressed the exogenous full length SoxB1 family members indicating that these genes are not just markers of stem cells but rather an active inhibitor of neuronal differentiation. Importantly, a dominant negative derivative of Sox2, generated by fusing the repressor domain of Engrailed transcription factor to Sox2 HMG domain, not only caused a premature neurogenesis but also significantly lowered BrdU incorporation. Additionally, the loss of Sox2 function mediated by the dominant negative Sox2 could be rescued by Sox1 indicating that Sox1 has similar functios and that the two act in a redundant manner. Thus, these data strongly suggest that SoxB1 genes are critical determinants of "stemness" of CNS neural stem cells maintaining the proliferative state of the cells and preventing premature differentiation into neurons.

As mentioned above, it is the neural crest that gives rise to PNS. Although Sox9 is expressed in much of the trunk derived neural crest cells, the key regulatory SoxE subfamily member that controls PNS development appears to be Sox10 (Herbarth et al., 1998; Kuhlbrodt et al., 1998). Expression in vivo starts early during neural crest migration and apparently persists in glial and melanocyte lineages but not in neuronal lineage. Functional analyses of Sox10 have been aided by a well established in vitro culture system and the availability of loss-of-function mutant. Neural crest stem cells derived from embryonic d 10.5 trunk spinal cord of rat can be cultured in a defined medium at the clonal density (Stemple and Anderson, 1992). The differentiation assay has conclusively shown that most of the thus-derived stem cells possess self-renewal capacity and multipotency. Importantly, Sox10 is expressed in most of the cells at the single cell stage and is down-regulated as neural crest stem cells differentiate into neurons and smooth muscle cells (Kim et al., 2003). The result conclusively demonstrates that Sox10 is expressed in multipotent neural stem cells of PNS. The Sox10 loss-of-function mutant known as the Dom (for Dominant megacolon) mouse has a frame-shift mutation that results in a truncated form of Sox10 with no apparent activity (Herbarth et al., 1998). The phenotype of the mutation includes Hirschsprung symptom showing extended colon in heterozygotes and early embryonic lethality in homozygotes. The megacolon phenotype results from the lack of peristalsis in the colon which in turn results from the lack of neural crest derived enteric ganglia. A forced expression of Sox10 inhibited differentiation of neural crest stem cells into neuronal and smooth muscle lineages (Kim et al., 2003). Furthermore, the neural crest cells which normally stop proliferation when exposed to TGFβ continued to incorporate BrdU as long as Sox10 level was maintained thorough ectopic expression. In vivo, homozygous Dom mutant embryos showed a premature emergence of neuronal markers, consistent with the proposed role of Sox10 as a neuronal differentiation inhibitor. In short, the expression pattern and function unmistakably demonstrate that SoxB1 and SoxE subfmaillies play comparable and central roles in the development of CNS and PNS respectively.

Concluding Remarks

The molecular mechanism that govern the proliferation and differentiation of neural stem cells is just beginning to be elucidated. An interesting scientific issue in itself, understanding the molecular mechanism also has important practical values as the success of regenerative medicine using stem cells as therapeutic reagents likely depends on understanding regulatory mechanisms of these cells. In this regards, isolating and functionally characterizing key molecular factors is the priority re-

search agenda for the whole field of stem cell biology. Sox genes have surfaced as such factors in neural stem cells. Their expression in the stem cells but not in neurons and their function in cellular proliferation and differentiation show a striking conservation in the CNS and PNS. It is thus highly likely that the factors regulating the expression of SoxB1 and SoxE genes and the transcriptional targets of SoxB1 and SoxE genes are also conserved to a large extent. The biochemical and genetic networks of genes with Sox genes at the center may then be what we need to understand in order to utilize neural stem cells down the road.

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