Diverse Functions of Sox2 in the Peripheral Nervous System

Taro Koike* and Hisao Yamada
Department of Anatomy and Cell Science, Kansai Medical University, Hirakata-City, Osaka, Japan

Abstract:
The Sox2 protein is a transcription factor and a member of the SoxB1 family. The distribution and functions of Sox2 have been well studied in neural stem cells of the central nervous system (CNS). In the neural stem cells of the CNS, the Sox2 protein helps to maintain the stem cell state. Some recent reports have shown that the Sox2 gene is also expressed in the cells of the peripheral nervous system (PNS) throughout life. During PNS development, the Sox2 protein regulates the epithelial-to-mesenchymal transition, neurogenesis and myelination. In the adult PNS, our recent reports using immunohistochemistry showed that Sox2 is found in some types of rat glial cells and promotes satellite glial cell survival. This review outlines the distribution and functions of Sox2 in the PNS.

Key words: Sox2, Peripheral nervous system, Glial cell, Cell-survival, Myelination

Introduction

The Sox family includes transcription factors that are encoded by the Sox (SRY-related-HMG-box) gene. Recently, 20 different Sox genes have been identified, and they are divided into groups according to the similarity of the High-Mobility-Group DNA binding domain. Sox2 is classified into the SoxB1 family and plays essential roles in directly controlling gene expression along with co-transcription factors.

Sox2 is a key and sufficient factor to regulate the proliferation, differentiation and survival of neural stem cells of the central nervous system (CNS). In the peripheral nervous system (PNS), Sox2 gene expression are reported in the
neural crest stem cells and immature Schwann cells. Furthermore, matured glial cells in the adult animals also express the Sox2 gene, and some roles for Sox2 have been investigated in the glial cells. Here, we described the distribution and functions of Sox2 in the PNS.

Sox2 in Embryonic Neurogenesis

During embryogenesis, the Sox2 mRNA is found in the inner cell mass of the blastocyst and in the neuroectoderm that gives rise to neural stem cells by in situ hybridization\(^3\). When the neural plate converges at dorsal midline, the neural crest cells leave the neural fold. Next, the neural crest cells arrive at the appropriate position and differentiate into primary sensory neurons, post-ganglionic neurons of the autonomic nerves and glial cells. In early stages of PNS development, both the Sox2 mRNA and protein are histochemically observed in the neural plate, but not in neural crest cells. This suppression is maintained during migration, followed by upregulation after reaching the primordium of the dorsal root ganglion (DRG) (Figure 1)\(^4\). This result indicates that Sox2 inhibits the epithelial-to-mesenchymal transition (the epithelial cells change own character and then leave epithelium to enter the mesoderm). In addition, the functions of the Sox2 protein during PNS development were also investigated using a human neural crest-forming culture system\(^5\). This experiment showed that downregulation of Sox2 gene expression increased the epithelial-to-mesenchymal transition in the presence of Bone Morphogenetic Protein4 (BMP4), indicating that the Sox2 protein acts as a downstream protein in BMP4 signaling to regulate the epithelial-to-mesenchymal transition.

It has been shown that the Sox2 protein regulates CNS development. Using electroporated chick embryos, the Sox2 gene inhibits neuronal differentiation and promotes neural stem cell proliferation. In contrast, forced expression of a dominant negative Sox2 protein caused the neural stem cells to differentiate into neurons and glia\(^6,7\). These results indicate that the Sox2 protein preserves the neural progenitor characteristics. It is reported that Sox2 overexpression upregulated the mRNAs for Notch1 and its downstream signaling intermediate, Hes5, in cultured murine neural stem cells\(^8\). Hes family proteins maintain the neural stem state to inhibit proneural basic-loop-helix-loop (bHLH) genes such as Mash1, Mmath and Neurogenin\(^9\). Thus, the Sox2 protein regulates their differentiation through Notch and bHLH genes.

Some researchers have demonstrated that neurogenesis in the PNS is also regulated by the Sox2 protein. Forced expression of the Sox2 gene using electroporation decreased the number of mature neurons during chick DRG development\(^10\). Cimadamore et al.\(^5\) also showed that forced expression of the Sox2 gene inhibits neurogenesis using a human neural crest-forming culture system. In addition to this result, they found that an shRNA for the Sox2 mRNA also decreased the number of neurons and that the Sox2 protein directly binds to the proneural bHLH genes, such as Neurogenin1 and MASH1, to upregulate their mRNA levels at the early stages of neurogenesis. Thus, the Sox2 protein promotes the neuronal commitment of the neural crest cells at the early stage of neurogenesis in DRG neurons.

Sox2 in the Adult Peripheral Nervous System

Recently, we revealed the existence of the Sox2 protein in some types of glial cells in the adult rat PNS. The perisomatic satellite glial cells, axonic satellite glial cells, non-myelinating Schwann cells, terminal Schwann cells of lamellar corpuscle and terminal Schwann cells of the lanceolate ending showed Sox2 immunoreactivity, but the DRG neurons and myelinating Schwann cells did not (Figure 2).
Sox2 in the PNS

The intensity was different among cell types (Table 1). Furthermore, the presence of the Sox2 mRNA was also showed with reverse transcription PCR in the DRG, sciatic nerve, foot pad and auricular skin. These results suggest that the Sox2 gene is expressed at different levels in the glial cells of the adult PNS.

In the adult rodent brain, the Sox2 protein was found in neural stem cells located in the adult neurogenic regions of the brain, such as the periventricular germinal zone of the lateral ventricle and the subgranular layer of the hippocampal dentate gyrus. Deletion of the Sox2 gene inhibited neurogenesis in the brain, indicating that Sox2 is involved in adult neurogenesis. In the adult DRG, it is believed that neurogenesis does not occur because BrdU-positive neurons were not observed in the adult following BrdU injections in neonatal rats. Therefore, it is thought that the satellite glial cells are not stem cells/progenitors in the adult DRG. Recently, our study revealed one function of the Sox2 protein in the satellite glial cells. We established a cultivation method to enrich the perisomatic satellite glial cells, and knocked down the Sox2 gene using siRNA. As a result, ErbB2 and ErbB3 were downregulated, as confirmed by quantitative PCR, and TUNEL-positive satellite glial cells were also observed. These results suggest that Sox2 promotes satellite glial survival through ErbB2 and ErbB3 signaling.

Sox2 gene expression and its roles have also been investigated in Schwann cells. Schwann cells are derived from immature Schwann cells, which give rise to both myelinating and non-myelinating Schwann cells. During Schwann cell development, immunohistochemical and quantitative PCR experiments revealed that the Sox2 gene is expressed in immature Schwann cells in mice. They also investigated the functions of the Sox2 protein in the immature

Fig. 2. Sox2-positive glial cells in the adult rat.
a, b) Microscopic pictures of the DRG. a) Glial cells were stained with anti-S100 antibody (green), the myelin sheaths were stained with anti-myelin basic protein antibody (magenta), and the nuclei were stained with Hoechst (cyan). b) Sox2 immunoreactivity. Perisomatic satellite glial cells surround neuronal somata (N) with their thin cytoplasm. These glial cells are Sox2-positive (arrows). The neuronal processes (P) are covered by axonic satellite glial cells, which do not form the myelin sheath. These glial cells are also Sox2-positive (arrowheads). c–e) Microscopic images of the auricular skin. c) The terminal Schwann cells of lanceolate ending were stained with anti-S100 antibody. d) Sox2 immunoreactivity. e) Merged image of c, d and the Hoechst-stained nuclei. The lanceolate ending surrounds a hair follicle (HF). The nuclei of terminal Schwann cells of the lanceolate ending show Sox2 immunoreactivity. f–h) Microscopic images of the foot-pad skin. f) The terminal Schwann cells of lamella corpuscle were stained with anti-S100 antibody. g) Sox2 immunoreactivity. h) Merged image of f, g and the Hoechst-stained nuclei. The lamella corpuscle is located in the dermal papilla (DP). The nuclei of the terminal Schwann cells of the lamella corpuscle exhibit Sox2 immunoreactivity. Scale bar, 20 μm
Schwann cells. Overexpression of the Sox2 gene by an adenovirus inhibits myelination and promotes neuregulin-dependent proliferation. Moreover, they also observed the presence of the Sox2 protein and mRNA in de-differentiated Schwann cells, which are thought to be in an immature state because the de-differentiated Schwann cells re-enter cell cycle and re-obtain migratory competence. Therefore, they concluded that the Sox2 protein maintains the undifferentiated state of Schwann cells by inhibiting myelination. This conclusion is consistent with our result that non-myelinating Schwann cells were weakly immunopositive for Sox2.

Parrinello et al. demonstrated that the Sox2 protein regulates Schwann cell sorting during axonal regeneration in vitro. While Schwann cells align to guide the regenerating axons after nerve injury, ephrinB/EphB2 signaling between fibroblasts and Schwann cells upregulates the Sox2 protein in Schwann cells. In addition, overexpression of the Sox2 gene with an adenovirus changes N-cadherin localization without altering the of N-cadherin mRNA and protein levels, which causes the Schwann cells to fail to sort. Therefore, these results indicate that EphB2 is certainly upstream of the Sox2 protein and that the Sox2 protein is thought to regulate the post-translational modification of N-cadherin for Schwann cell sorting.

The enteric nervous system includes Sox2 immunopositive cells. The Sox2-positive cells are derived from the neural crest cells, similar to the DRG, and the Sox2 protein is not found in the migrating neural crest cells. All Sox2-positive cells were immunohistochemically identified as glial cells that were located in the enteric nerve plexus. The glial cells had the ability to de-differentiate into neural stem cells in vitro; however, it is not known whether these Sox2-positive cells have neural stem cells ability in vivo.

### Conclusions

The Sox2 gene is expressed in the cells of the PNS. In the embryo, the Sox2 gene is expressed in the neural crest stem cells and regulates neurogenesis in the DRG. In the adult PNS, the Sox2 gene is expressed in some types of glial cells. In each glial cell, the Sox2 protein plays some cell type-specific roles.

This review includes a summary that was presented at the 14th Igakkai Award of the Kansai Medical University. The authors won the incentive award at the award ceremony.

### References

7. Bylund M, Andersson E, Novitch BG, Muhr J.

### Table 1. Intensity of Sox2 immunoreactivity.

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<thead>
<tr>
<th>Type of glial cells</th>
<th>Intensity</th>
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<tbody>
<tr>
<td>Peri-somatic satellite glial cells</td>
<td>++</td>
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<tr>
<td>Axonic satellite glial cells</td>
<td>++</td>
</tr>
<tr>
<td>Myelinating Schwann cells</td>
<td>–</td>
</tr>
<tr>
<td>Non-myelinating Schwann cells</td>
<td>+</td>
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<tr>
<td>Terminal Schwann cells</td>
<td>Lamella corpuscle Lanceolate ending ++</td>
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<td>Free nerve ending –</td>
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Sox2 intensities were compared among cell types using immunohistochemically stained sections. The immunoreactivity is different among cell types. ++, strongly positive; +, weakly positive; –, negative.


