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Note

SRY-box Transcription Factor 6 Is Expressed Not Only in the Dorsal but Also in the Ventral Zone of the Neural Tube and Is Highly Expressed in the Notochord and Chordoma

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In the course of SRY-box transcription factor 6 (SOX6) expression profiling in human embryonic tissue, SOX 6 was found to be highly expressed in the notochord, based on the findings of immunohistochemistry (IHC). Sox6 is also expressed in the neural tube and the distribution of SOX6 is located in the ventral and dorsal zones of the neural tube. In contrast to the findings that SOX6-positive cells were located on the floor plate of the neural tube, OLIG2- and NKX2.2-expressing cells were lacking on the floor plate of the neural tube, and their expression was restricted only to the ventral zone of the neural tube. The expression patterns of SOX9 were similar to those of OLIG2 and NKX2.2 in the neural tube. NKX2.2 and OLIG2 are not expressed in the notochord, but SOX9 and SOX6 are. Because Sox6 is highly expressed in the notochord, the present study investigated whether or not SOX6 is an immunohistochemical marker for the pathologic diagnosis of chordoma, a neoplasm derived from the notochord. IHC revealed that chordoma was strongly positive for SOX6 in two cases of chordoma, one of which occurred in the sacrococcygeal region and another that developed at the base of the skull, suggesting that SOX6 is a useful marker for the histopathologic diagnosis of chordoma.

Key words: SOX6, chordoma, notochord, SOX9, NKX2.2

I. Introduction

Chordoma is a lesion of the midline of the spinal column that originates from the primitive notochord. Approximately half of the tumors occur in the lumbosacral spinal region, and approximately 40% occur at the base of the skull in the spheno-occipital region, particularly in the clivus. Through immunohistochemical analyses using SRY-box transcription factor 6 (SOX6) antibodies in the human embryos, it has been revealed that SOX6 is strongly expressed in the notochord. This observation prompted my interest in analyzing SOX6 expression in chordoma.

SOX6 is a DNA-binding transcription factor that specifically binds the 5'-AACAAT-3'DNA motif, and in embryogenesis, SOX6 plays a key role in multiple organ development processes, such as chondrogenesis and neurogenesis [1, 5]. SOX6 influences the differentiation of chondrocytes and neuronal cells, thereby causing diseases such as Tolchin-Le Caignec syndrome (TOLCAS) and osteochondroma. TOLCAS (OMIM 618971) is characterized as an intellectual developmental disorder with behavioral abnormalities and variable bone defects [10].

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II. Materials and Methods

Tissue specimen

A human embryo was incidentally identified in a surgical specimen that was resected in a case of an ectopic tubal pregnancy. No detailed information about the stage of embryo was available. Patient 1 was a 75-year-old Japanese woman with sacral chordoma, while patient 2 was a Japanese woman who had been 65 years old at the first

surgery for clivus chordoma, which thereafter recurred 3 times during the next 6 years.

Antibody and immunohistochemistry

Primary antibodies against the following antigens were used: SOX6 (clone A-4: Santa Cruz Biotechnology, Mouse monoclonal, 1:100 dilution), NKX2.2 (clone SPM564, MyBioSource, Mouse monoclonal, 1:100), OLIG2 (ab109186, Abcam, Rabbit monoclonal, 1:100), SOX9 (ab185966, Abcam, Rabbit moloclonal, 1:1000) and Brachyury (ab209665, Abcam, Rabbit monoclonal, 1:1000).

Immunohistochemistry (IHC) was performed on formalin (10% buffered formalin)-fixed overnight at room temperature, paraffin-embedded tissue sections at 4°C overnight after antigen retrieval at 120°C for 20 min. As a secondary antibody, a mixture of peroxidase-labeled goat anti-mouse and anti-rabbit IgG (Histofine simple stain MAX-PO: Nichirei Bioscience, Japan) was used. Finally, a Histofine DAB substrate kit was utilized for color detection. No inactivation of endogenous peroxidase was performed because the activity of endogenous peroxidase is low in the neural tube, notochord and chordoma. No carrier proteins were used. PBS (phosphate buffered saline, pH 7.4) was used as a washing solution and no surfactant was utilized [3].

This study has been reviewed and approved by the ethics committee for clinical research at Showa University. The number for the approval is 22-258-A. Informed consent was obtained. This investigation was conducted in accordance with the Declaration of Helsinki of 1975.

III. Results

Expression of SOX6 in human embryonic tissues

Fig. 1 shows a human embryo with a neural tube and a notochord. IHC revealed that SOX6 was extensively positive in the notochord, as indicated by an arrow (upper left panel). SOX6-positive cells were also observed in the neural tube and predominantly located in the floor plate. SOX6-positive neuroepithelial cells were evenly distributed in the neural tube, namely not only in the ventral but also in the dorsal zone of the neural tube. In contract, NKX2.2-positive cells and OLIG2-positive cells were located in the ventral zone of the neural tube, and both cell types were lacking on the floor plate of the neural tube, as shown in the lower left and lower right panels, respectively. NKX2.2-positive cells were fewer in number than OLIG2-positive cells and they were located close to the floor plate. SOX9-expressing cells were predominantly observed in the ventral zone of the neural tube (upper right panel). Another characteristic feature in the context of the distribution of SOX6-positive cells is that SOX6-positive cells were mostly scattered in the ventricular zone, with a few in the mantle zone and marginal zone, which could be postmitotic neurons. SOX9, on the other hand, was unevenly distributed in the ventricular zone, suggesting that SOX9-positive cells are neuroepithelial cells (neural stem cells and immature progenitor cells). NKX2.2 and OLIG2 are not expressed in the notochord, but SOX9 and SOX6 are.

Expression of SOX6 in chordomas

Fig. 2 shows the histopathology of case 1 on hematoxylin-eosin (HE) staining, indicating that large neoplastic cells possess rich and eosinophilic cytoplasm, and a few chordoma cells have gigantic nuclei (upper left panel). These cells were positive for brachyury (lower left panel) and cytokeratin (data not shown), enabling the diagnosis of chordoma. Nuclei of chordoma cells were strongly positive not only for SOX6 (upper right panel) but also for SOX9 (lower right panel). The nuclei of stromal cells were negative for brachyury, SOX6 and SOX9. The exactly same expression profiles obtained in case 1 were observed in case 2 (data not shown).

IV. Discussion

SOX6 expression in the neural tube prompted me to investigate the expression profiles of other transcription factors expressed in the neuronal tissue. OLIG2 is one of the candidates to be investigated because OLIG2-expressing astrocytes show region-specific distribution in the adult mouse brain and spinal cord [8, 11]. Another transcription factor is NKX2.2, which regulates oligodendrocyte differentiation [13]. Sonic hedgehog (SHH) is secreted from the notochord and the floor plate of the neural tube and induces neurons in the ventral neural tube. SHH acts as a morphogen, such that high concentrations induce ventral neurons and the lowest concentrations induce dorsal neurons [2]. Interestingly, OLIG2-expressing cells as well as NKX2.2-expressing cells were lacking in the SHH secretion area but were located adjacent to the SHH secretion area. The distribution of these cells was restricted to the ventral zone of the neural tube that differentiates into motoneurons and forms the ventral motor column. Luppi *et al.* reported that Sox6 expression distinguishes dorsally and ventrally biased dopamine neurons in the substantia nigra [6]. The present study also demonstrated the unique expression of SOX6 which might be under direct or indirect influences of SHH.

Chongrogenesis begins as mesenchymal cells condense, which is characterized by the expression of Sox9, therefore Sox9 plays an essential role in the chondrocyte differentiation pathway and it is required for the expression of Sox5 and Sox6 [1]. Similar to SOX6, SOX9 was highly expressed in the notochord; however, the distribution of SOX9-expressing cells was different from that of SOX6-expressing cells. After completing the present immunohistochemical analysis, it remains to be elucidated precisely how dynamically SOX6 is involved in the differentiation of neural tubes and the development of motor neurons. The expression of brachyury is extremely restricted and

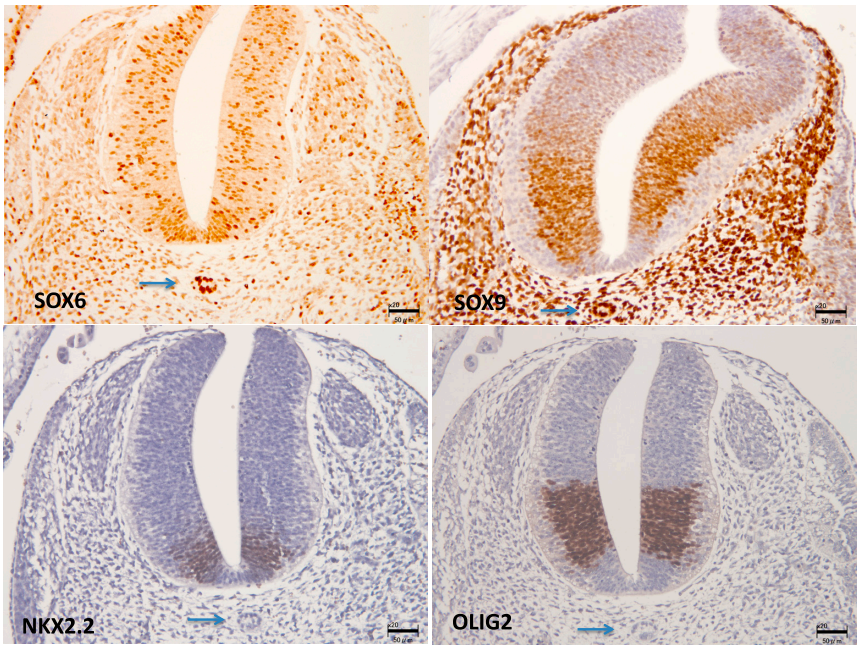

Fig. 1. Hematoxylin-eosin (HE) staining of a human embryo identified incidentally in an ectopically pregnant specimen, showing the morphology of the neural tube as well as the notochord. The sky blue arrow indicates the notochord. SOX6 immunohistochemistry (IHC) shows that SOX6 is highly expressed in the notochord and also expressed in several neuronal cells in the neural tube. SOX6-expressing neuronal cells were densely distributed on the floor plate (FP) of the neural tube and were loosely distributed not only in the ventral, but also in the dorsal region of the neural tube (upper left panel). In contrast, FP of the neural tube lacked NKX2.2 expression, and NKX2.2-expressing cells were located in a limited area of the ventral region of the neural tube (lower left panel). Similar to SOX6 expression in the notochord, SOX9 is highly expressed in the notochord but the distribution of SOX9 in the neural tube is different from that of SOX6. SOX9-positive cells were compactly located in the ventral area of the neural tube but absent in the FP of the neural tube (upper right panel). The distribution pattern of OLIG2-expressing cells is similar to that of NKX2.2-expressing cells, but different from the area of the distribution. OLIG2-positive cells were located in a broader area of the ventral region of the neural tube (lower right panel). The notochord dose not express either NKX2.2 or OLIG2. Bars = 50 μm.

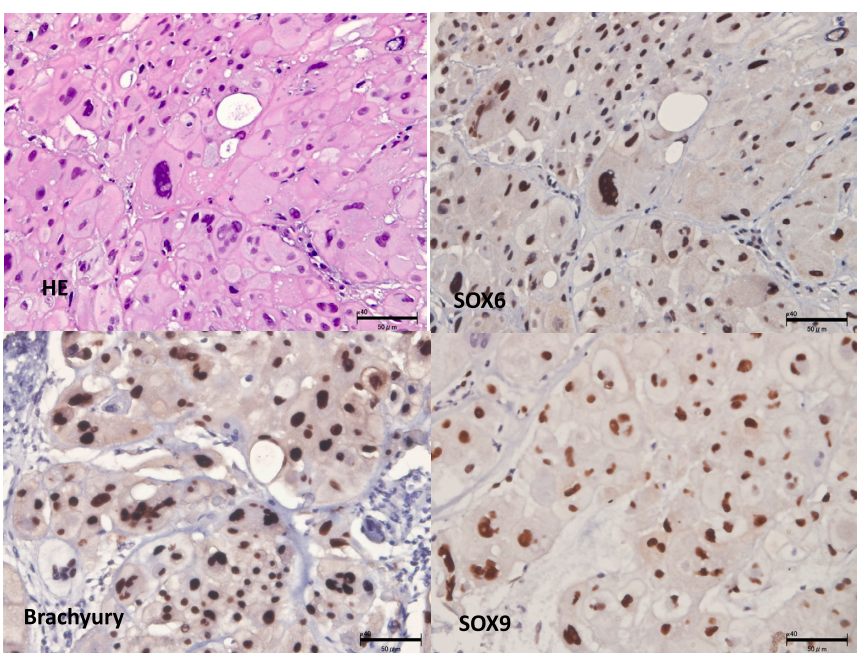

Fig. 2. Histopathology of HE staining in case 1, showing that the chordoma cells are rich in cytoplasm and contain bizarre nuclei (upper left panel). IHC revealed that chordoma cells were strongly positive for brachyury (lower left panel), SOX6 (upper right panel) and SOX9 (lower right panel). Bars = 50 μm.

detected only in the notochord, thus brachyury is superior to SOX6 and SOX9 as a specific marker for chordoma.

The management of chordoma is typically surgery; however, most chordomas recur. Thus, molecular targets for therapy against chordoma are needed, although little is known about the molecular profile of chordoma at present [9]. Several recent reports have revealed the involvement of SOX6 in tumor progression. Lv *et al.* reported that SOX6 suppresses the development of lung adenocarcinoma [7], and Wang *et al.* documented the role of the p14ARF-HDM2-p53 axis in SOX6-mediated tumor suppression [12]. Therefore, molecular targeting against SOX6 might be possible, although further studies will be required to clarify the molecular pathogenesis of the initiation and progression of chordoma.

The SOX6 expression in chordoma presented in this report provides a possible differential diagnostic marker to distinguish chordoma from bone metastasis of lung adenocarcinoma, as Kambara *et al.* reported that the SOX6 expression is restricted in lung adenocarcinomas, with only 7% of cases of lung adenocarcinoma being positive for SOX6, whereas 98% of cases of epithelioid mesothelioma expressed SOX6 [4].

In conclusion, the present report revealed that the distribution pattern of SOX6-positive neural cells is different from that of NKX2.2-, OLIG2- and SOX9-expressing cells in the neural tube. The current study also showed that SOX6 is highly expressed in the notochord as well as in chordoma, a neoplasm originating from the notochord. This finding highlights the potential applicability of SOX6 for the immunohistochemical differential diagnosis of chordoma from metastatic bone tumors.

V. Conflicts of Interest

The author declares that there are no conflicts of interest.

VI. References

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