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Immunohistochemical Examination of Cytological Differentiation in Osteosarcomas*

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Abstract: In this immunohistochemical examination, the expression of Runx2, Notch1, Delta and Osteopontin peptides were detected in neoplastic cells in 10 Japanese cases of osteosarcoma. Immunohistochemically, Runx2 peptide expression appeared in the cytoplasm of almost all neoplastic cells of the 10 cases examined. However, Notch1 peptide expression appeared in the cytoplasm of neoplastic cells in the localized and comparatively well-differentiated area of osteosarcoma, which osteoblastic and chondroblastic containing osteoid and/or chondroid tissues. No expression of Notch1 peptide was detected in the fibroblastic and poorly differentiated areas. Delta peptide appearance was nearly the same pattern of Notch1 peptide. Expression of Osteopontin peptide appeared in almost all cells and the strength expression was shown in the area of comparatively well-differentiated tissues. Therefore, these results suggest that Runx2, Notch1, and Delta peptides are closely related to cytological differentiation or acquisition of tissue specific characteristics in neoplastic cells in osteosarcomas.

Key words: osteosarcoma; Notch1; Runx2; Delta; osteopontin; cytological nature; morphogenesis; differentiation; regulation factor; immunohistochemistry

Introduction

Osteogenesis is a complex biological process, including recruitment of stem cells, proliferation of progenitor cells, differentiation of osteoblasts and production and assembly of bone matrix. The different steps of this process are controlled and regulated by multiple local and systemic factors, such as morphogenesis regulators. Therefore, the complex interaction between these factors and their contribution to the development of neoplastic osteogenesis are nesesray to know the characteristics of the neoplasms.

Previously, we have reported the examination results of immunohistochemical expression of Notch1 intra cellular domain (NICD) in an Indonesian case of osteosarcoma of the maxilla, having examined relationship of the NICD expression and developmental cellular process [1]. Therefore, in this paper we have examined the immunohistochemical expression of some morphogenesis regulation factors, such as Runx2, NICD, and Delta peptides in neoplastic cells in collected series osteosarcoma cases in Japan.

Materials and Methods

Osteosarcoma materials examined in this study were obtained from operation materials, whose diagnosis was carried out in the Department of Pathology, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan. The examination materials are listed in Table 1. The average age of these patients is 17.6 years old. Immediately after removal, the materials were fixed in 10 % neutral buffered formalin solution. The materials were then dehydrated by passage through a series of ethanols, and embedded in paraffin. After sectioning, the series specimens were examined by histopathologically (hematoxylin-eosin: HE).

Immunohistochemical examination was carried out using a DAKO EnVisionTM+Kit-K5006 (Dako Cytomation, Copenhagen) and 4 antibodies: anti-human Notch1 intracellular domain (NICD: 1/20), anti-mouse Runx2 (PEBP2aA-M-70: 1/100: Santa Cruz Biotechnology, Inc. USA), anti-mouse Delta (C594.9B: 1/5) and anti-Osteopontin (OPN: MPIIIB10: 1/50). The NICD monoclonal antibody (bTAN20) developed by Spyros Artavanis-Tsakonas [2, 3], the Delta by Spyros Artavanis-Tsakonas [4] and the OPN by

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Table 1. Cases Examined.

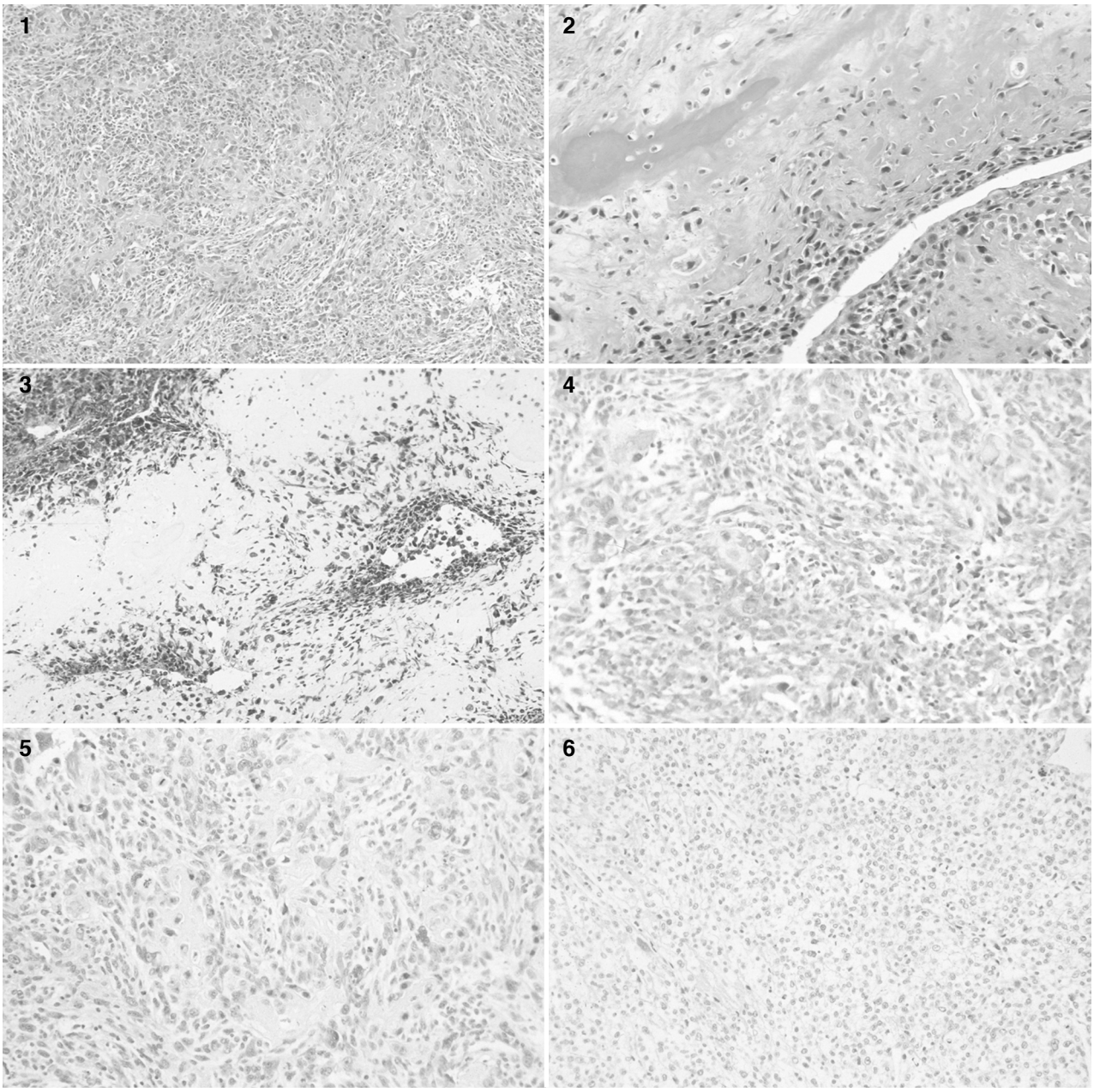
Case No	Age	Sex	Location
01 (#R490695)	19	M	Femur
02 (#R491429)	10	M	Tibia
03 (#R510448)	15	M	Fibula
04 (#R512734)	13	F	Femur
05 (#R495583)	14	M	Femur
06 (#R495484)	15	M	Femur
07 (#R500510)	17	M	Femur
08 (#R524098)	12	F	Femur
09 (#R532004)	05	M	Femur
10 (#R507597)	56	M	Tibia

Solursh and Franzen [5] were all obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development (NICHD) of the National Institute of Health (NIH) and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA, USA. DAB was applied for the visualization of immunohistochemical activity. We included immunohistochemical staining using PBS in place of the primary antibody as a negative control.

Examination Results

Histopathology

Histopathologically examined of specimens of all examined serious10 cases, these osteosarcomas had spindle-shaped sarcomatous cells proliferating mesenchy-



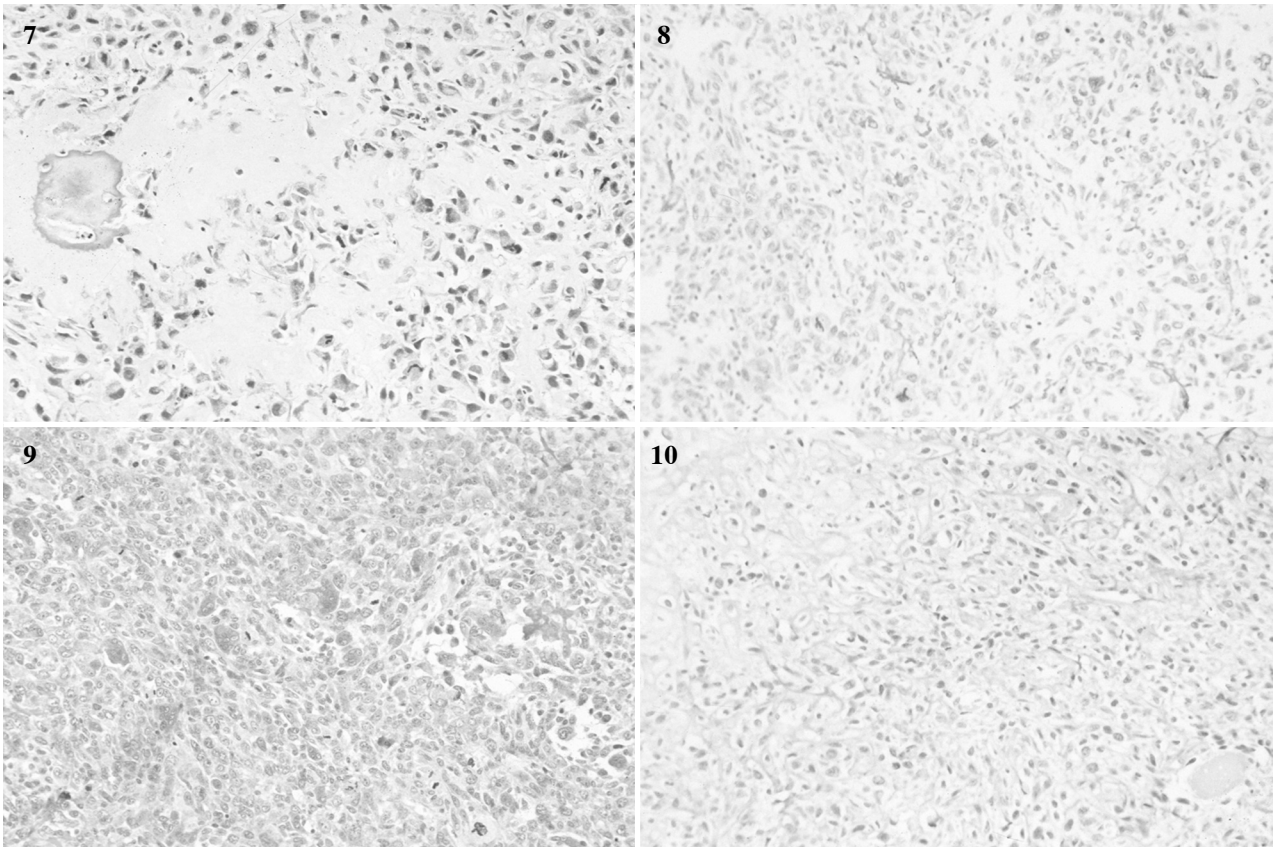


Fig. 1. Fibroblastic cell proliferation is dominant (Case 4, HE, Magnification x 100).
Fig. 2. Osteoblastic cells and bone/osteoid materials (Case 3, HE, Magnification x 100).
Fig. 3. Positive products of OPN in well-differentiated area (Case 3, Magnification x 100).
Fig. 4. OPN positive products in fibroblastic area (Case 4, Magnification x 200).
Fig. 5. Positive reaction of Runx2 in well-differentiated area (Case 4, Magnification x 100).
Fig. 6. Runx2 weakly positive products in poorly-differentiated area (Case 8, Magnification x 100).
Fig. 7. NICD is strongly detected in well-differentiated area (Case 3, Magnification x 100).
Fig. 8. No-immunohistochemical reaction of NICD in poorly-differentiated area (Case 7, Magnification x 100).
Fig. 9. Weak reaction of Delta appearing in well-differentiated area (Case 4, Magnification x 100).
Fig.10. No-Positive products of Delta in fibroblastic region (Case 5, Magnification x 100).

mal tissue directly producing neoplastic osteoid and/or coarse immature bone tissues. In these observations, variable histopathological patterns were seen in specimens of some cases. There were mainly osteoblastic and osteoid and/or immature bone matrices (Fig. 1), as well as some spindle-shaped fibroblastic neoplastic cells (Fig. 2). Osteoblastic neoplastic cells, located around the numerous small osteoid tissues, were comparatively monotonous, varying in size and in shape, and showed hyperchromatic nuclei and mitosis.

Immunohistochemistry

Immunohistochemically, OPN peptide, as control, was expressed in almost all cells of the examined osteosarcoma. The strong expression area of OPN was in the comparatively well-differentiated regions of the osteosarcoma, that is the osteoblastic area containing osteoid tissues (Fig. 3). In contrast, weak reaction products were detected in the monotonous spindle-shaped cell proliferation area (Fig. 4).

Runx2 peptide expression appeared in the cytoplasm of almost all neoplastic cells of examined 10 cases. The expression pattern showed uniformly in the proliferating cells of almost all cases. At the bone and/or osteoid forming region as well differentiated area, the positive reactions of Runx2 were slightly strong in compared of other poorly differentiated regions (Figs. 5, 6). Regarding the expression of NICD peptide, the peptide was detected in the cytoplasm of neoplastic cells of the comparatively well-differentiated areas of osteosarcomas, which osteoblastic and chondroblastic containing osteoid and/or chondroid tissues, and this area was the same as the immunohistochemically strongly stained area by OPN (Fig. 7). No expression of NICD peptide was detected in the fibroblastic and poorly differentiated area (Fig. 8). Delta peptide appearance was nearly the same that of NICD peptide. The positive products of Delta were appeared

in the cytoplasms of osteoblastic and chondroblastic cells (Fig. 9), but there were no positive reaction in the poorly differentiated fibroblastic cell proliferation regions (Fig. 10). On the other hand, there was no positive reaction immunohistochemically detected in negative control slides.

Discussion

In general, it is important to examine the expression or localization of morphogenesis regulators to the neoplastic proliferating conditions, such as benign and malignant tumors. Previously, we have reported the expression of NICD in a Indonesian male case of osteosarcoma of the maxilla [1]. As mentioned above, we consider that the expression situation of regulation factors of morphogenesis is closely related to the neoplastic cytological nature of the neoplasm and its clinical behavior. Thus, we examined regulation factors in this paper.

Regarding the relationship between these regulation factors and bone tissue, there are some reports in the literature. First, NICD is one of the important regulation factors of morphogenesis. NICD has been reported as a unique and interesting regulator for treatment of osteoporosis by Tezuka et al. [5]. Furthermore, some papers have considered the NICD and bone tissue, especially the differentiation of bone forming cells [6, 7, 8]. Functional involvement of NICD in osteoblastic cell differentiation has been also reported. However, it is unclear whether Notch1 ligand Delta also induce an identical cellular response in these differentiations. Nobta et al.[9] reported the critical regulation of osteoblastic cell differentiation by Delta-activated Notch1 signaling.

The molecular basis for inverse relationship between differentiation and oncogenesis is unknown. However, regarding Runx2, a master regulator of osteoblast differentiation belonging to that runt family of tumor suppressor genes, is consistently disrupted in osteosarcomas. Thomas et al. [10] have described that physiological coupling of osteoblast differentiation to cell cycle withdrawal is mediated through Runx2, and the process are disrupted in osteosarcoma. Furthermore, Andela et al. [11] have reported that Runx2 was expressed constitutively in all pathology specimens of human osteosarcoma. In this examination, expression of Runx2 appeared in the cytoplasm of almost all neoplastic cells of examined 10 cases, and the expression pattern showed uniformly in the proliferating cells of almost all cases. At the bone and/or osteoid forming region, as well at the differentiated area, the positive reactions of Runx2 were slightly strong in comparison with other poorly differentiated regions. These immunohistocemical results are consistent with the above mentioned discussion.

In the present investigation, the NICD peptide was expressed in the area of comparatively well-differentiated areas of osteosarcoma, osteoblastic and chondroblastic area containing osteoid and/or chondroid tissues. The results are also similar to those of our previously published Indonesian case [1]. With OPN as control peptide in this examination, expression was also detected in almost all cells, the strength pattern of OPN expression was similar to that of NICD. Therefore, we believe that Notch peptide is closely related to cytological differentiation or acquisition of tissue specific characteristics in neoplastic cells in osteosarcomas.

In summary, the expression of Runx2, NICD, Delta and OPN were examined in neoplastic cells in 10 Japanese cases of osteosarcoma and the immunohistocemical expression of Runx2 appeared in the cytoplasm of almost all neoplastic cells of examined 10 cases. However, NICD appeared in the localized comparatively well-differentiated areas. No expression of NICD peptide was detected in the poorly differentiated area. Delta showed nearly the same as NICD. Expression of OPN as control appeared in almost all cells and the strength of expression was shown in the area of comparatively well-differentiated tissues. Therefore, these results suggest that Runx2, Notch1, and Delta are closely related to cytological differentiation or acquisition of tissue specific characteristics in these neoplastic cells of osteosarcomas.

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