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EXPRESSION OF NOTCH IN A CASE OF OSTEOSARCOMA OF THE MAXILLA

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Abstract: In this immunohistochemical examination, the expression of Notch1 peptide was detected in neoplastic cells in a case of osteosarcoma of the maxilla of a 31-year-old Indonesian male patient. Notch1 peptide appeared in the cytoplasm of neoplastic cells of comparatively well-differentiated areas of the osteosarcoma, an osteoblastic area containing osteoid and/or immature bone tissues. The results suggest that Notch1 is closely related to cytological differentiation or acquisition of cytological characteristics in neoplastic cells of osteosarcoma.

Key words: osteosarcoma; Notch1 peptide; cytological characteristics; morphogenesis; cyto-differentiation; morphogenesis regulation factor; immunohistochemistry

INTRODUCTION

Neoplasm development is a cellular process that reflects and is partly driven by alterations in cell determination. We believe that the expression profile of regulation factors of morphogenesis is closely related to cytological characteristics of the neoplasm and its clinical behavior. Notch1 is one of important regulation factor of morphogenesis. Therefore, we have examined the expression of Notch1 intra cellular domain (NICD) in a case of osteosarcoma of the maxilla of a 31-year-old Indonesian male patient.

REPORT OF A CASE

A 31-year-old male Indonesian factory worker was diagnosed as having an osteosarcoma. His medical history in brief is as follows: One month earlier he noticed a chief complaint of a swelling of the left maxilla. The swelling was painless and slowly increasing in size. He saw a general practitioner and was prescribed antibiotics but with no improvement. He was then admitted to the Department of Oral Surgery at Malaya University Hospital. Inspection revealed a single large expansive swelling involving the left maxilla. It extended from the left maxillary second molar to the third molar. The swelling was firm and hard and appeared circumscribed. There was marked buccopalatal expansion. Radiograph OPT and OMV revealed a large radiopaque mass extending from the maxillary first molar to third molar, with involvement of the left maxillary antrum. The clinical diagnosis was osteosarcoma. An incisional biopsy was performed under local anesthesia. Histopathological diagnosis of osteosarcoma was carried out. A left maxillectomy was performed. Samples taken from the margins of the resected specimens were clear of tumor. About 18 months after the operation, the patient returned with a complaint of another swelling in the left mandible, which had been present for one month. It was also painless and slowly growing in size. The mass produced buccal expansion and encroached onto the buccal sulcus, and it measured approximately 3.0 x 2.0 cm. The region was firm and hard, and there was no paraesthesia. Clinically, a recurrence was strongly suspected. An incisional biopsy was performed and the diagnosis of osteosarcoma was taken. However, the patient disappeared and failed to return for definitive surgery.

EXAMINATION METHODS

Immediately after being removed from the patient, the materials were fixed in 10% neutral buffered formalin fixative solution. The materials were then dehydrated by passage through a series of ethanols and embedded in paraffin. After being sectioned, the series specimens were examined by histopathology (hematoxylin-eosin: HE) and by immunohistochemistry. Immunohistochemical examination was carried out using a DAKO EnVision™+Kit-K4006 (Dako Cytomation, Copenhagen) and 2 monoclonal antibodies: anti-human Notch1 (Notch intracellular domain, NICD: 1/20) and anti-Osteopontin (OPN: 1/50). The NICD monoclonal antibody (bTAN20) developed by Artavanis-Tsakonas [1, 2] and the OPN monoclonal antibody (MPIIIB10) developed by Solursh and Franzen [3] were both 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 immunohis-

tochemical activity. We included immunohistochemical staining using PBS in place of the primary antibody as a negative control.

Examination Results

Histopathologically, in HE stained specimens, the osteosarcoma case examined had a sarcomatous stroma directly producing tumor osteoid and coarse immature bone. Comparatively variable histopathological patterns were not observed in the specimens. There were mainly osteoblastic and osteoid and/or immature bone tissues, as well as some spindle-shaped neoplastic cells, such as fibroblastic patterns. Osteoblastic neoplastic cells, around the numerous small osteoid tissues, showed comparatively monotonous, was varying in size and in shape, and showed hyperchromatic nuclei and mitosis (Fig. 1).

Immunohistochemically, expression of OPN peptide appeared in almost all cells in the examined osteosarcoma (Fig. 2). The strength pattern of OPN expression was in the area of comparatively well-differentiated areas of the osteosarcoma, osteoblastic area containing osteoid tissues. Also, expression of NICD was detected only in the cytoplasm of neoplastic cells, and this area was the same as the immunohistochemically strongly stained area by OPN (Fig. 3). No expression of NICD was detected in the fibroblastic and poorly differentiated areas. There was no positive reaction immunohistochemically detected in negative control slides.

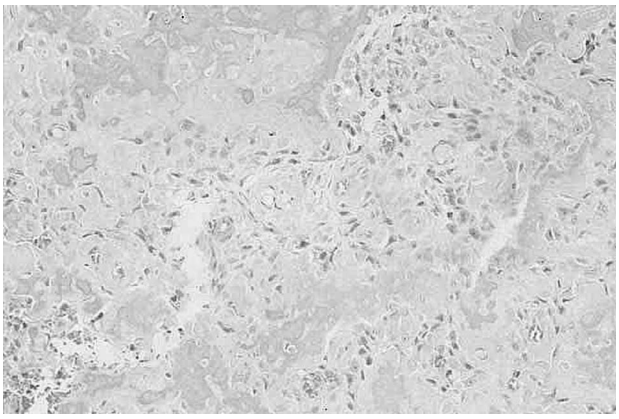

Fig. 1. Histopathological view of examined specimen (Magnification x 100).

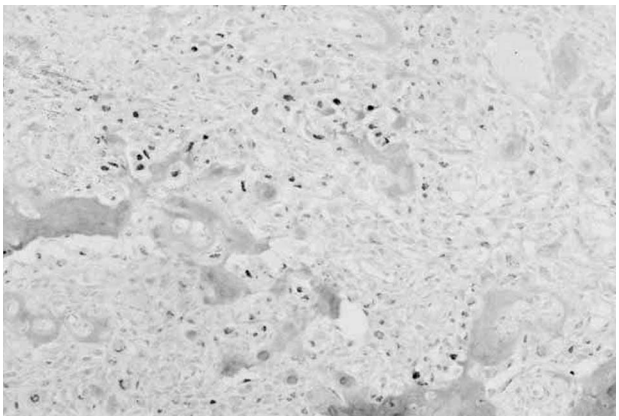

Fig. 2. Positive reactions of OPN in almost all neoplastic cells (Magnification x 100).

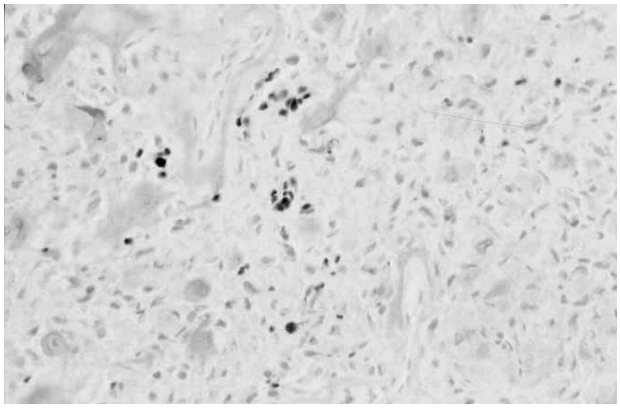

Fig. 3. Positive products of NICD distributed localized in well-differentiated area (Magnification x 200).

Discussion

In the neoplastic region, there are some reports regarding the appearance of Notch signaling: Notch1 is one of the important regulation factors of morphogenesis. Cancer development is a cellular process that reflects and is partly driven by alterations in cell differentiation. Oncogenic/neoplastic mutations in various molecules responsible for cell differentiation have been identified as being oncogenic. But little is known about the involvement of physiological cell fate-differentiation mechanisms in the oncogenic process. Notch pathway defines an evolutionally conserved, general cell interaction mechanism that controls fundamental aspects of cell determination during vertebrate and invertebrate development. Zagouras et al. [2] reported alterations in Notch signaling in neoplastic lesions. In their report, Notch expression is associated with cell populations that are undergoing cell fate changes, and Notch activity can be used to monitor cell fate abnormalities in cervical as well as other epithelial neoplasias. Accordingly, Notch pathway may provide a means to manipulate the developmental fate of malignant cells, thus potentially offering a novel therapeutic approach.

Regarding the relationship between Notch and bone tissue, Tezuka et al. [4] have reported that osteoblastic cell differentiation is regulated positively by Notch, and that Notch could be a unique and interesting target molecule for the treatment of bone lesions, such as osteoporosis. Furthermore, there have been some recently published papers regarding the Notch and osteoblastic/chondroblastic cell differentiation, proliferation and bone formation [5, 6].

In the present investigation, histopathologically, the osteosarcomas examined had a sarcomatous stroma

directly producing tumor osteoid. The NICD was expressed in the area of comparatively well-differentiated areas of osteosarcoma, an osteoblastic area containing osteoid tissues. OPN expression was also detected in almost all cells, while the strength pattern of OPN expression was similar to that of NICD. No expression of NICD was detected in the fibroblastic and poorly-differentiated other osteosarcoma cases in our surgical files, although OPN expression was detected in almost all cells. Therefore, we believe that Notch1 peptide is closely related to cytological differentiation or acquisition of tissue specific characteristics in neoplastic cells in osteosarcomas.

Finally, further immunohistochemical examination of Notch expression in neoplastic osteoblastic and/or chondroblastic cells of collected series cases of osteosarcomas is now in progress, and the results will be reported in the near future.

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