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Immunohistochemical c-fos Expression in Osteosarcoma

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- Abstract -

The products of c-fos and c-jun proto-oncogenes form the heterodimeric complex activator protein 1 (AP-1), which plays an important part in the control of bone cell proliferation and differentiation, as well as in the development of bone tumors. The expression of c-fos protein was examined in 35 cases of human osteosarcomas as formalin-fixed paraffin-embedded tissue sections using a monoclonal antibody. The expression of c-fos was restricted to bone-forming lesions, while low grade cartilaginous tumors were devoid of immunoreactivity. The highest levels of c-fos expression were detected in osteoblastic osteosarcoma (13 of 17 cases with grade one on two) while two chondroblastic osteosarcomas, one fibroblastic osteosarcoma, and two parosteal osteosarcomas were negative. Two cases of telangiectatic osteosarcomas were positive for c-fos protein. However, since there is a tendency of high c-fos protein expression at the higher histological grade, significant differences were not present in the expression of c-fos protein. Thus c-fos expression may be implicated in the development of osteosarcomas, but they appear to have little or no relevance in the development of low grade cartilaginous neoplasms.

Key Words : Osteosarcoma, c-fos, Immunohistochemistry

INTRODUCTION

c-fos and c-jun are part of a family of transcription factors that form dimers necessary for biding to regions of DNA termed AP-1 (activator protein 1) sites[9,21,24].

Modifications in the levels of fos and jun family members that result in the presence of a specific subset of dimers within the cell influence the transcription of classes of genes with appropriate AP-1 flanking regions[1,12]. An upregulation and maintenance of c-fos expression has been reported to precede programmed cell death in vivo and observed in osteoblasts in vitro[3,16,23].

The fos/jun family of transcription factors exhibit complex and functionally relevant changes in cellular representation during differentiation, as well as in transactivation capability. Striking developmental modifications in expression of various fos and jun

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proteins are found in osteoblasts reflected by both proteins and mRNA levels[16]. Antisense strategies demonstrate consequential effects of c-fos on development of mature osteoblast phenotypic properties and establishment of bone tissue organization.

Bone is physiologic target for the action of c-fos and c-jun. The expression of c-fos proto-oncogene has been demonstrated in developing bone and teeth, elevated levels of c-fos have been found in osteoblasts, osteocytes, osteoclasts, periosteal cells, articular and growth plate chondrocytes by mRNA in situ hybridization studies and immunohistochemistry[4,5,13,15,18,22]. The role of c-fos proto-oncogene expression in skeletal development and remodeling processes has been investigated using in vivo approaches employing mice with both loss of function and gain of function of the gene. Mice lacing c-fos are affected by a severe form of osteopetrosis owing to lack of osteoclast activity[11,31]. Over expression of c-fos in transgenic mice results in increased formation of woven bone, increased resorption, and the development of osteosarcomas[8,20,30].

Subsequently, an association between c-fos overexpression and human osteosarcoma has been postulated on the basis of the results of immunohistochemical studies showing significantly higher oncoprotein expression in osteosarcoma than in normal tissues or nonosteosarcoma lesions[32].

In order to clarify the possible role of c-fos expression in the development of skeletal neoplasms as well as to compare the histological grade, we analyzed the immunohistochemical expression of c-fos in osteosarcomas.

MATERIALS AND METHODS

We collected 35 cases of osteosarcomas from the department of Pathology, Kyung Hee University and Hallym University. There are 17 osteoblastic osteosarcomas, 7 chondroblastic osteosarcomas, 6 fibroblastic osteosarcomas, three parosteal osteosarcomas and two telangiectatic osteosarcomas.

Immunohistochemical c-fos expression

c-fos protein expression was determined by the automated immunoperoxidase immunohistochemical technique (Ventana 320 ES, Ventana Medical Systems, Tucson, AZ, USA) as recommendation of the manufacture. Briefly, formalin-fixed, paraffin-embedded 5-㎛ tissue block sections were deparaffinized in xylene and graded alcohols. The deparaffinized sections were loaded onto the Ventana ES Automated Slide Stainer, and incubated with Protease 1 (8 minutes), 1 : 50 diluted c-fos primary antibody (32 minutes, 37℃ with rabbit polyclonal K-25; Santa Cruz), biotinylated secondary antibody, avidin-streptavidin-enzyme conjugate, and chromogenic enzyme substate (8 minutes, 37℃ each) according to the automated protocol. This was followed by application of a copper diaminobenzidine enhancer, hematoxylin counter staining, and liquid cover-slip as part of the automated process. All reagents and secondary antibody were obtained from Ventana. A negative control reaction was carried out with irrelevant isotype-matched primary rabbit polyclonal antibody. Positive controls included reactions with paraffin-embedded sections of normal tonsil with documented c-fos expression. The sections were examined by light microscopy. c-fos expression in tumor-involved areas was graded as negative(-), weak(+), or strong positive(++).

RESULTS

Osteoblastic osteosarcoma

Among the 17 cases of osteoblastic osteosarcomas, 13 cases (76%) were positive for c-fos antigen. The staining intensity were varied from grade one to two. Histological grading of these positive staining cases ranged from grade 1 to grade 3. One grade 1 osteosarcoma showed grade one staining intensity. Among the seven grade two osteosarcomas, four showed grade one and three showed grade two staining intensity. Among the five grade three osteosarcomas, three revealed grade one and two revealed grade two staining intensity. There is no specific correlation between staining intensity and histological grading. The positive staining cases showed tumor cell nuclear staining pattern(Fig. 1). Lots of giant cells among the tumor tissue showed also characteristic nuclear staining pattern(Fig 2). In some area, there are also cytoplasmic staining pattern. Four cases were negative for c-fos immunostaining. The histological grading of these negative cases ranged from grade 1 to 4. Two cases showed heavily osteoid formation cases.

Chondroblastic osteosarcoma

We had seven cases of chondroblastic osteosarcomas. Five cases (71%) were positive for c-fos antigen. Staining intensities of these cases ranged from grade + to grade ++. Histological grade of the positive cases ranged from grade 2 to 3. There is no specific correlation between histological grade and staining intensity. Most of the positive cases showed cytoplasmic staining pattern. The positive cells were spindle cells peripheral zone of the neoplastic chondroid tissue (Fig. 3). Most of the chondroid tissue were negative and only some of the chondroid tissue were positive.

Fibroblastic osteosarcoma

There were six fibroblastic osteosarcomas. Five cases (83%) were positive for c-fos antigen and one case was negative. Staining pattern of these five positive cases were cytoplasmic staining of the tumor cells. Giant cells among the tumor tissue were also nuclear positive pattern. Histological grade of these positive cases ranged from grade 2 to 3.

One case of parosteal osteosarcoma showed positive for c-fos immunostaining. Spindle cells between the newly formed trabecular bones were grade + positive. The cartilage cap of the tumor tissue were consistenly negative for c-fos antigen. We had two cases of telangiectatic osteosarcomas. Tumor giant cells showed nuclear positive staining. High grade tumor cells were negative.

DISCUSSION

The results of this study confirm that the c-fos gene is frequently overexpressed in human osteosarcomas[6,32]. We observed higher percentage (84%) of osteosarcomas with diffuse expression of c-fos than in the studies of Wu et al. or Franchi et a[16,32].

This is probably differences used sample. We performed this investigation on formalin-fixed, paraffin-embedded materials, while Wu et al. used fresh tumor sections. Also Frianchi et al. used paraffin tissue and they counted only nuclear staining. In our study, we counted not only nuclear staining but cytoplasmic staining as positive. Indeed, nearly exclusive nuclear staining has been obtained in studies on fresh tissue sections[28], while both cytroplasmic and nuclear staining

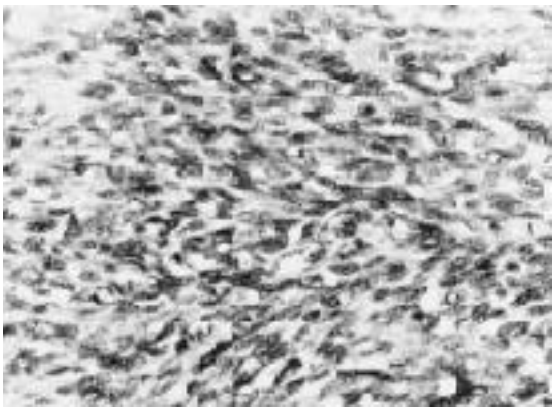

Fig. 1. Photomicrograph of the spindle tumor cells show intense grade ++ nuclear staining for c-fos antibody (ABC, ×200).

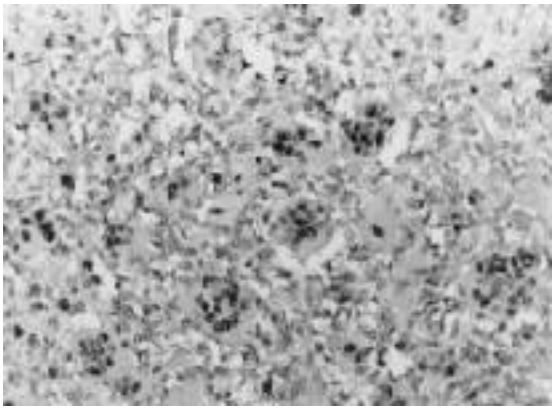

Fig. 2. Lots of tumor giant cells show intense nuclear staining (ABC, ×200).

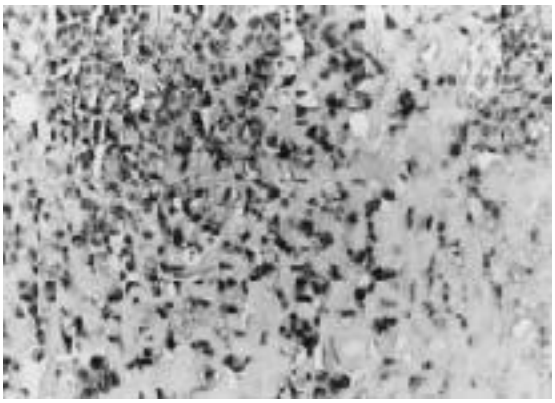

Fig. 3. Some spindle cells peripheral to the neoplastic cartilage show nuclear staining for c-fos antibody (ABC, ×200).

has been observed in fixed material[10,14]. However, the study of bone-forming lesions is extremely difficult in fresh tissue sections owing to the presence of calcified matrix, which is particularly abundant in benign neoplasms and in some variants of osteosarcoma.

However, benign and low grade malignant cartilaginous tumors do not express immunohistochemically detectable levels of c-fos in others, suggesting that these oncogenes are not primarily involved in the development of these neoplasms[6]. This is apparent contrast to the observations that ectopic expression of c-fos chimeric mice is associated with frequent development of cartilaginous tumors[29]. However, it should be noted that these experimentally induced tumors do not reproduce the same histological profile of human chondrosarcomas exactly, as they also contain foci of bone-forming neoplastic cells and undifferentiated mesenchymal spindle cells[29]. In this present study, we observed seven chondroblastic osteosarcomas. More than 70% of the tumor showed positive staining either intranuclear as well as intracytoplasmic c-fos reactivity. However these positive cells were mainly spindle cells periphery to the neoplastic chondroid tissue. Most of the chondroid lesions were negative for c-fos immunostaining. This kind of reaction could be also obtained in the parosteal osteosarcomas. In parosteal osteosarcomas, there were low grade neoplastic cartilage cap. It clearly showed negative reaction for c-fos immunostaining.

Elevated levels of c-fos has been described in several tumor types[14,26,28] and it has been suggested that elevated c-fos and c-jun expression is an important event in tumorigenesis, because it may determine an increased proliferation rate[14]. With specific

reference to osteosarcomas, it is of interest that the expression of both c-fos and c-jun is significantly higher in high-grade osteosarcomas (characterized by aggressive growth with tendency to systemic spread) than in low grade osteosarcomas (locally aggressive lesions with infrequent metastases), suggesting that these oncogenes may be involved in determining the clinical behavior of these neoplasms[6]. In this present studies, we could observe higher expression tendency of the high grade osteosarcomas than low grade osteosarcoma. However, there is no statistically significant correlation between histological grade versus immunohistochemical expression of c-fos proto-oncogene.

The elevated levels of c-fos oncoproteins in high-grade osteosarcomas may be the result of the alteration of several pathways that ultimately control cell proliferation. First, c-fos and c-jun are under the regulation of other oncogenes, such as the retinoblastoma tumor suppressor gene (RB), whose product can down regulate c-fos transcription and AP-1 activity[19]. The RB gene is frequently altered in human osteosarcomas[27], and loss of RB activity could be involved in determining increased levels of c-fos and c-jun in these tumors. In addition, the expression of c-fos appears to be regulated by transforming growth factor beta, one of the major growth factors for bone tissue. Recent studies have shown that TGF-β induces an increase of c-fos mRNA levels in culture normal and transformed human osteoblast-like cells[25], and this may be responsible for an increase in proliferative activity, since the uptake of antisense c-fos oligonucleotide abolishes the mitogenic effect of TGF-β on osteoblast-like cells[13]. Franchi et al. 's study supports the existence of a strong direct relationship between the expression of c-fos and TGF-β in human osteosarcomas, since high-grade osteosarcomas show significantly higher levels of c-fos and of TGF-β1 than low-grade lesions[6,7]. The observation that high--grade osteosarcomas have a significantly higher proliferative activity than low-grade osteosarcomas suggests that in these variants of osteosarcoma the elevated expression of TGF-β1 and c-fos may sustain a higher proliferative activity and may contribute substantially to establishment of an aggressive phenotype[17]. Conversely, in low-grade osteosarcomas lower levels of TGF-β1 and c-fos may result in lower proliferative activity and ultimately in less aggressive growth[7]. Taken together, these data indicate that the control pathways of the expression of c-fos and c-jun could play an important part in determining the clinical behaviour of osteosarcomas. Further studies with larger series are needed to determine whether the evaluation of c-fos and c-jun expression may be useful in predicting of clinical outcome in these neoplasms.

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= 국문초록 =

골육종의 c-fos 발현에 관한 면역조직화학적 검색

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c-fos와 c-jun은 암 유전자의 하나이며, 이 유전자의 단백질 산물은 여러 가지의 다른 활성화 단백 (activator protein 1, AP-1)으로 골 종양에서 골세포의 증식과 분화를 조절하는 중요한 역할을 하는 인자 중 하나로 알려져 있다.

본 연구에서는 단클론 항체를 이용하여 포르말린에 고정된 파라핀 포매조직을 이용하여 35례의 사람 골육종에서 c-fos 단백의 발현을 연구하였다. c-fos의 발현은 골 형성 병변에서 주로 발현되며, 저등급의 연골형성 병변에서는 발현이 관찰되지 않았다. 높은 빈도의 c-fos 단백의 발현이 골아성 골육종에서 발현되었으나 (17례 중 13례에서 1등급 내지 2등급으로 발현), 2례의 연골형성 골육종, 1례의 섬유아세포성 골육종, 2례의 방골성 골육종에서는 음성으로 나타났다. 2례의 혈관확장성 골육종에서는 양성으로 c-fos 단백의 발현되었다. 비록 조직학적으로 고등급의 골육종에서 면역조직화학적 염색상 고 빈도의 c-fos 단백의 발현이 관찰되나, 조직학적 등급과, 면역염색상 발현사이에 통계적인 유의성은 관찰되지 않았다. 이상의 결과로 c-fos 단백이 골육종의 발생에 관여할 것으로 추론되며, 저등급의 연골형성 육종에 이 단백의 역할에는 추후 연구가 필요할 것으로 사료된다.

중심어 : 골육종, c-fos, 면역조직화학 검사