대 한 골 관 절 종 양 학 회 지
J. of Korean Bone & Joint Tumor Soc.
Volume 13, Number 2, December, 2007

양성 및 악성 연골 종양의 Cyclooxygenase-2 발현

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목적: 최근 골 및 연부조직 종양을 포함한 각종 악성 종양에서 cyclooxygenase-2 (COX-2)의 발현 증가가 보고되고 있다. 그러나 연골 종양에서의 COX-2 발현에 대해서는 별로 알려진 바가 없다.

대상 및 방법: 내연골종 10예, 연골모세포종 11예, 연골점액양섬유종 5예, 통상적 연골육종 17예, 투명세포 연골육종 7예, 간엽성 연골육종 6예를 대상으로 COX-2에 대한 면역조직화학법을 시행하였다.

결과: 양성 연골 종양 중 연골모세포종 11예 중 6예(54.5%)에서 특징적인 강한 양성 반응을 나타내었다. 내연골종과 연골점액양섬유종은 단 1예를 제외하고는 모든 증례에서 음성이었다. 통상적인 연골육종에서 3예(17.6%)는 COX-2에 대해 강한 양성 반응을 보였는데 이러한 양성 증례는 모두 조직학적 등급 3의 분화가 나쁜 연골육종이었다. 투명세포 연골육종 중 2예(28.5%)는 국소적인 양성 반응을 나타내었고 모든 간엽성 연골육종은 음성이었다.

결론: 이러한 결과는 통상적인 연골육종에서 COX-2 과발현이 조직학적 등급 진행과 관계된 소견임을 시사한다. 연골모세포종의 COX-2 발현은 특별히 이 종양에 동반되는 종양 주변부의 염증성 변화를 유발하는 중요한 요소로 생각된다.

색인 단어: 연골 종양, COX-2

INTRODUCTION

Cyclooxygenase (COX) is a key enzyme that catalyzes the synthesis of prostaglandins from arachidonic acid[1]. COX-2 is not detected in most normal tissues. However, it can be rapidly induced by both inflammatory and mitogenic stimuli, to increase prostaglandin synthesis in inflamed and neoplastic tissues[1]. COX-2 also plays an important role in carcinogenesis because it can promote angiogenesis by stimulating the production of proangiogenic factors such as vascular endothelial growth factor[2,3], inhibit apoptosis

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*This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant Funded by the Korea government (MOST) (No. R13-2002-020-02001-0).

and immune surveillance[4,5], and increase invasion and metastatic potential[1,6,7].

COX-2 is expressed in a variety of human cancers, including prostate, gastric, bladder, cervical, colon, ovarian, pancreatic, lung, melanoma, and glioma[8-17]. In most of these cases, COX-2 expression correlates with tumor grade or prognosis. Most recently, cyclooxygenase-2 inhibitors have shown promise in terms of its chemotherapeutic effect on cancers. However, little is still known regarding its effects and role with sarcomas. In musculoskeletal tumors, the overexpression of COX-2 was observed in osteosarcomas, Ewing's sarcomas, and rhabdomyosarcomas[18-21]. There are also some reports on COX-2 overexpression in osteoid osteomas[22] and chondroblastomas[23], suggesting that the COX-2 activation of eicosanoid synthesis is biologically important for these tumors.

In the present study, we performed immunohistochemical staining for COX-2 in various benign and malignant chondroid tumors and analyzed the staining results with respect to both tumor type and histological grade.

MATERIALS AND METHODS

1. Pathology material

This study was approved by the Institutional Review Board at Kyung Hee University Hospital. We collected enchondromas (n=10), chondroblastomas (n=11), chondromyxoid fibromas (n=5), conventional chondrosarcomas (n=17), clear cell chondrosarcomas (n=7), and mesenchymal chondrosarcomas (n=6) from the pathology files at Hallym University Sacred Heart Hospital (Anyang, Korea), Kyung Hee University Hospital (Seoul, Korea), and Mayo Clinic (Rochester, MN, USA). Two pathologists (HR Park and YK Park) reviewed all histologic slides for confirmation of histologic diagnosis. The histologic grade of the conventional chondrosarcomas was determined based on the nuclear size, nuclear hyperchromasia, and cellularity according to the 2002 World Health Organization Classification of Bone Tumors[24].

2. Immunohistochemistry

The immunoperoxidase method was performed on 4 μm thick tissue sections for immunohistochemical analysis using Envision ChemMate kit (DAKO). Sections were deparaffinized with xylene for 15 min and treated in a microwave oven using 0.01 M citrate buffer (pH 6.0) for 30 min. Sections were incubated(1:100 dilution) with the rabbit polyclonal antibody directed against COX-2(Neomarkers, Fremont, CA, USA) for 30 min at room temperature. Colorectal adenocarcinoma tissue known to express COX-2 was used as the positive control. Primary antibody was replaced with buffer to serve as the negative control. The results were expressed according to a semiquantitative scale according to the following criteria: 0= complete absence of tumor cells stained positive; 1+ =1~30% of the cells stained positive; 2+ =31~100% of the cells stained positive. In each case, 10 high power fields of representative areas were counted.

3. Statistical analysis

Correlation between the COX-2 expression and histological grading of conventional chondrosarcomas was tested by the Kruskal Wallis test and Spearman's correlation coef-

ficient by rank test. Statistical significance was defined as $p<0.05$.

RESULTS

The results are summarized in Table 1. The expression of COX-2 was noted in the cytoplasm of tumor cells. Among benign chondroid tumors, chondroblastomas revealed characteristic strong positivity in 6 of 11 cases(54.5%). The expression of COX-2 was found in both the tumor cell cellular sheets and tumor cells embedded in the chondroid matrix. Osteoclast-like giant cells were consistently negative for COX-2. All enchondromas and chondromyxoid fibromas were negative except in one enchondroma case with positive focal staining (Fig. 1).

Among the conventional chondrosarcomas (n=17), three cases(17.6%) were strongly reactive with COX-2 in a diffuse pattern. The histologic grade of these three COX-2 positive cases was grade III, representing 50% of the grade III samples studied. All the grade I and II cases were negative for COX-2. So, COX-2 expression was significantly correlated with the histologic grade in conventional chondrosarcomas. (Spearman's rank correlation coefficients was 0.55 with corresponding p-value of 0.02) Clear cell chondrosarcomas were focally positive in two cases(28.5%) and all mesenchymal chondrosarcomas were negative (Fig. 2).

DISCUSSION

Recent studies have shown increased levels of cyclooxygenase-2 in various human malignancies, including some bone and soft tissue tumors, but there is little information about its expression pattern in chondroid tumors[23,25,26]. In our study, 17.6% of conventional chondrosarcomas, were strongly reactive with COX-2. Interestingly, 50% of our grade III cases were positive. As a result, COX-2 overexpression in conventional chondrosarcomas may be a useful predictor of patient outcome and may have strong association with the histologic grade in chondrosarcomas. Clear cell chondrosarcomas were focally positive in 2 cases(28.5%) and all mesenchymal chondrosarcomas were negative. In contrast, all enchondromas and chondromyxoid fibromas were negative

Table 1. COX-2 expression in variable chondroid tumors

Type of tumors	COX-2 expression		
	None	1+	2+
Benign			
Enchondroma (n=10)	9	1	
Chondroblastoma (n=11)	5		6
Chondromyxoid fibroma (n=5)	5		
Malignant			
Conventional chondrosarcoma (n=17)*			
Grade I (n=6)	6		
Grade II (n=5)	5		
Grade III (n=6)	3		3
Clear cell chondrosarcoma (n=7)	5	2	
Mesenchymal chondrosarcoma (n=6)	6		

* p=0.04 according to the histologic grade (Kruskal Wallis test)

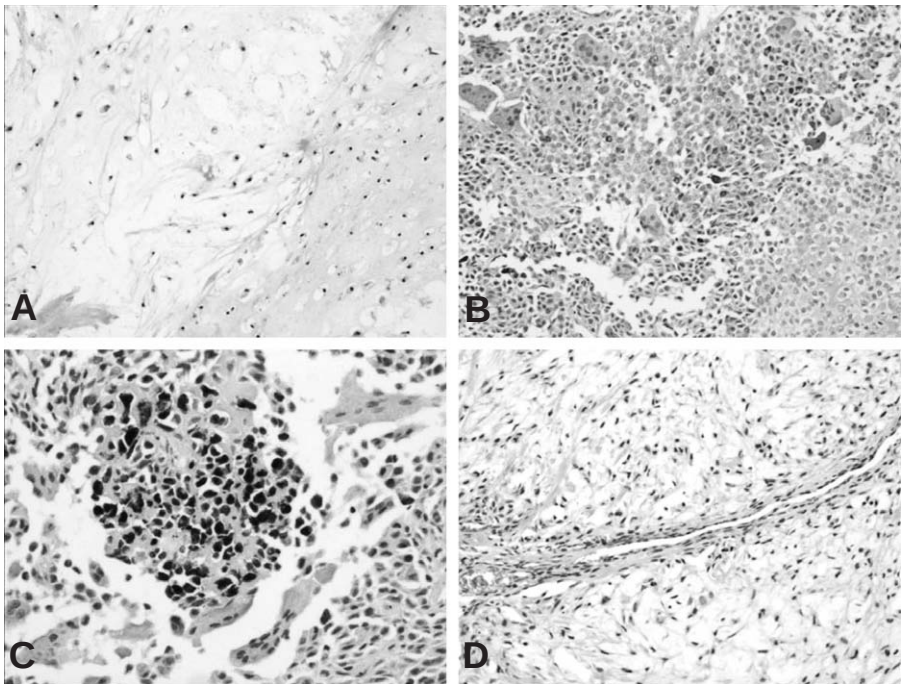

Fig. 1. COX-2 expression in benign chondroid tumors. (**A**) Enchondroma was negative. (**B**, **C**) Chondroblastomas revealed characteristic positivity in 54.5%. (**D**) Chondromyxoid fibroma was negative (IHC for COX-2, × 100).

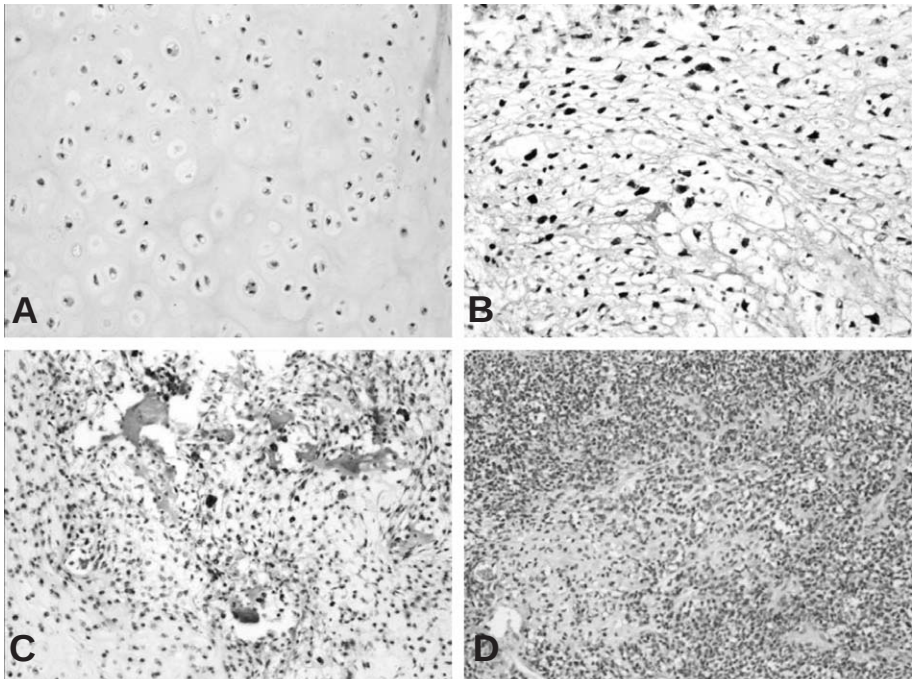

Fig. 2. COX-2 expression in malignant chondroid tumors. (**A**) Conventional chondrosarcoma, grade I was negative. (**B**) Conventional chondrosarcoma, grade III was strong reactive. (**C**) Clear cell chondrosarcomas were focal positive in 28.5%. (**D**) Mesenchymal chondrosarcoma was negative (IHC for COX-2, ×100).

except in one case. Our data strongly supports the concept that COX-2 protein is upregulated in chondrosarcoma of the bone. Based on these results, it is apparent that further investigation is needed to define COX-2's biologic role in malignant sarcomas which may assist in determining whether selective inhibitors of COX-2 may be useful in altering or ameliorating some of its malignant attributes in chondrosarcomas or other connective tissue tumors.

Endo et al. provided the immunohistochemical evidence that COX-2 overexpression was significantly associated with decreased survival in conventional chondrosarcomas[25]. Sutton et al. also demonstrated by Western blotting that detectable levels of COX-2 protein were expressed in 54% of malignant chondrosarcomas while none in benign enchondromas[26]. However, they stated that no statistically valid correlation could be found between the presence of COX-2 and the clinical data to include age, sex, staging, anatomical site, presence of metastases, and death rate[26].

Several different mechanisms could provide an important link between COX-2 and the biologic behavior of chondrosarcoma. COX-2 is expressed in a variety of human cancers, including prostate, gastric, bladder, cervical, colon, ovarian, pancreatic, lung, melanoma, and glioma[8-17]. In most of these cases, COX-2 expression correlates with tumor grade or prognosis. The mechanism of activation by which COX-2 supports tumor growth and metastasis appears to be multifactorial. COX-2 activity is known to promote angiogenesis[3] and matrix metalloproteinase production[27], as well as inhibit apoptosis[28] and natural killer cell activity[29]. Singh et al[30]. reported that COX-2 overexpression in human breast cancer cells enhanced cell motility and invasiveness thus suggesting a mechanism of COX-2 mediated metastasis. Ito et al[21]. reported that fibronectin-induced COX-2 mediated MMP-2 expression and invasiveness in rhabdomyosarcoma.

Recent studies have shown increased levels of cyclooxygenase (COX)-2 in other bone and soft tissue tumors. Dickens and his colleagues[18,19] reported COX-2 expression in osteosarcoma, rhabdomyosarcoma, and Ewing sarcoma. However, COX-2 expression did not vary with any clinical or pathologic features and was not predictive of prognosis in these cases. Their study did not support the use of COX-2 expression as an upfront prognostic variable in patients with osteosarcoma or rhabdomyosarcoma. Raspollini et al[31]. and Mullins et al[20]. also reported COX-2 expression in uterine leiomyosarcomas and canine appendicular osteosarcomas.

Although there have been several trials investigating the role of COX-2 inhibitors sarcomas in animal species[32], there has not been any reported trials COX-2 inhibitors in patients with sarcomas. Naruse et al[33]. evaluated the antitumor activity of meloxicam, a preferential COX- inhibitor, in osteosarcoma. Meloxicam may have both COX-2-dependent and independent inhibitory actions on osteosarcoma. Its effects were more prominent in osteosarcoma cells that have relatively high levels of COX-2. Klenke et al[34]. reported that the selective COX-2 inhibitor Celecoxib was a potent inhibitor of tumor growth of secondary bone tumors in vivo which could be explained by its anti-angiogenic and pro-apoptotic effects.

In our study, chondroblastomas revealed strong positive characteristics for COX-2 staining in 54.5% of cases. In contrast, all enchondromas and chondromyxoid fibromas

were negative except in one case. Shinmura et al[23]. also reported COX-2 expression in 71% of chondroblastoma. The intensity of COX-2 immunoreactivity was correlated statistically with the presence of periosteal reaction, bone marrow edema, soft tissue edema, and synovitis. They indicated that activation of eicosanoid synthesis by COX-2 expression in the tumor itself was probably an important factor, inducing peritumoral inflammatory changes in chondroblastomas. The mechanism of inflammatory reactions that are sometimes observed in benign bone tumors remains unclear. Recent studies suggest, however, that high levels of prostaglandins within the lesion, such as an osteoid osteoma or chondroblastoma, may play an important role for the development or maintenance of inflammatory reactions[22,23]. Mungo et al[22]. also found that tumor osteoblasts had strong immunohistochemical staining for COX-2 in osteoid osteomas, while staining in the surrounding host osteoblasts in the reactive bone was scant.

In conclusion, our study suggests that COX-2 overexpression in conventional chondrosarcoma may have an association with higher histologic grade. Interestingly, expression of COX-2 in chondroblastomas may play an important factor for inducing peritumoral inflammatory changes.

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Abstract

Cyclooxygenase-2 Expression in Benign and Malignant Chondroid Tumors

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Purpose: Recent studies have shown increased levels of cyclooxygenase-2 (COX-2) in various human malignancies to include various bone and soft tissue tumors. However, little is known with regard to COX-2 expression patterns in chondroid tumors.

Materials and Methods: Immunohistochemistry assays were performed for COX-2 in enchondromas (n=10), chondroblastomas (n=11), chondromyxoid fibromas (n=5), conventional chondrosarcomas (n=17), clear cell chondrosarcomas (n=7), and mesenchymal chondrosarcomas (n=6).

Results: Among the benign chondroid tumors, chondroblastomas revealed characteristic strong positivity in 6 of 11 cases(54.5%). All enchondromas and chondromyxoid fibromas were negative except in one case. In conventional chondrosarcomas, three cases(17.6%) were strongly reactive with COX-2 and all positive cases represented grade III chondrosarcomas. Clear cell chondrosarcomas were found to be focally positive in two cases(28.5%), while all mesenchymal chondrosarcomas were negative.

Conclusions: These findings suggest that COX-2 overexpression in conventional chondrosarcoma may represent an advanced histologic grade. Interestingly, expression of COX-2 in chondroblastomas could be an important factor for inducing peritumoral inflammatory changes in these specific tumors.

Key Words: Chondroid tumors, COX-2

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