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IMMUNOHISTOCHEMICAL INVESTIGATION OF CARTILAGINOUS TUMORS

YOSHIYA INOUE, M. D.

Department of Orthopedic Surgery of Niigata University School of Medicine; and Second Department of Pathology of Niigata University School of Medicine, Niigata, Japan

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Address reprint requests to Dr. Inoue: Second Department of Pathology of Niigata University School of Medicine, I, Asahimachi-dori, Niigata, Japan.

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Twenty-eight cartilaginous tumors and normal bronchial cartilages were investigated immunohistochemically by using the antibodies to S-100 protein, alpha-1 -antitrypsin (AT) and alpha-1-antichymotrypsin (ACT). All lesions and normal control specimens were revealed to have S-100 protein positive chondrocytes in various frequencies. The staining pattern of chondrocytes could be divided into three different types: D (diffusely stained) type, C (cytoplasmic dominant) type and N (nuclear dominant) type. All cartilaginous lesions except two immature teratomas had AT positive chondrocytes and these cells were found much more in malignant tumors than in benign ones. ACT positive chondrocytes were observed in two osteosarcomas, in all chondrosarcomas, and in one of two borderline cartilaginous tumors but no positive cells were seen in benign lesions. From these findings, the appearance of AT positive chondrocytes strongly indicates that the lesions were truely neoplastic. Furthermore the existence of ACT positive chondrocytes in tumors was considered to be a useful marker of malignancy. (Key words; Chondrosarcoma; S-100 protein; Alpha-1-antitrypsin; Alpha-1-antichymotrypsin; Cartilaginous tumor)

FROM ANCIENT TIMES MANY PATHOLOGISTS AND CLINICIANS HAVE done much study on the differential diagnosis of cartilaginous tumors, but even today, we occasionally encounter some cases where it is difficult to judge whether these tumors are benign or low grade malignant. In this immunohistochemical investigation, the author uses the antibodies to alpha-1-antitrypsin (AT), alpha-1-antichymotrypsin (ACT) and S -100 protein as supporting diagnostic indicators to try to determine the specific nature or the grade of malignancy of cartilaginous tumors.

MATERIALS AND METHODS

Twenty-eight cases involving non-decalcified cartilaginous lesions (ten enchondromas, three periosteal chondromas, ten chondrosarcomas, three immature teratomas and two osteosarcomas) seen at the Niigata University School of Medicine and related hospitals from 1971 to 1985 were retrieved from the surgical pathology files. The autopsy materials of one chondrosarcoma were also used. (case 4 in table 3) Our judgement of malignancy for all cartilaginous tumors was based on the macroscopic criteria of chondrosarcoma proposed by O'Neal and Ackerman¹⁴⁾ and on the microscopic criteria of Lichtenstein and Jaffe.¹²⁾ For the normal controls, bronchial cartilages from seven autopsy cases were used. All materials were fixed in 10% formalin and embedded in paraffin. The sections were stained with hematoxylin-eosin (H & E), periodic acid -schiff (PAS) reaction, alcian blue and silver impregnation method (Gomori).

The PAP method of Sternberger¹⁹⁾ for determining S-100 protein, AT and ACT was applied on deparaffinized sections. Rabbit antisera to human S-100 protein, AT and

ACT were all purchased from Dakopatts Ltd., Copenhagen, Denmark. The specificity of the immunohistochemical staining was comfirmed by (1) replacement of the primary antiserum by 0.01M phosphate buffer saline (ph7.4) and (2) absorption with an equal volume of antigen, 50 ng/ml, for both AT and ACT, as previously described by Kodama et al.¹¹ Purified AT and ACT (HPR 03, HPR 052) were purchased from SEROTEC, England. Cells which were positively immunostained by unabsorbed antiserum were not stained at all with AT or ACT absorbed antiserum nor with phosphate buffer saline.

The total number of positive cells for AT and ACT in the immunostained sections were determined by observing vivid tumor areas of 5 high-power fields (10×40) randomly and semiquantitative designations were made (table 1). NAT was the total number of

Table	1.	Semiquantitativ	e Designation	for the	number	of	positively	immunostained	cells
NAT:	Th	e number of AT	positive cells	(10×40)	$\times 5$ field	s			

NACT: The number of ACT positive cells (10×40) $\times 5$ fields

$1 \leq N < 10$ ·····	······
$10 \le N < 100 \cdots$	+
$100 \leq N < 200 \cdots$	······
$200 \leq N$	••••••

AT positive cells in 5 high power fields and NACT was the number with respect to ACT. Both were made up of two components of cellularity and positive cell percentage.

The staining of the S-100 protein was assessed simply as being either negative or positive without counting.

RESULTS

I. Clinical Findings

1. Benign Chondromas

All the cases in table 2 are those dealing with tumors that arose in the small bones of hands. No recurrences nor metastatic lesions could be found but foci of slight cortical destructions were observed in periosteal chondromas by taking X-ray pictures.

2. Chondrosarcomas

Primary sites and age distribution are shown in table 3. Case 1 was an enchondromatous femoral lesion and case 10 was a tumor in the proximal tibia. The surrounding cortical bone had been destroyed without traumatic effect in both cases. The tumors in cases 2, 3, 6 and 9 had developed from pelves and aggressive destruction of the bones was confirmed by X-ray films and operative findings. In cases 3 and 9 recurrent tumors appeared frequently after the initial operations and the patients are still alive with residual tumors after undergoing hemipelvectomy.

The primary site of tumors in case 4 was the first thoracic spine and the patient died 48 months after the initial biopsy due to the metastatic tumors.

Case 5 was a right chest wall tumor involving the 6th, 7th and 8th ribs and 46 months after the initial operation metastatic tumor was noticed in the thoracic spinal canal.

Case 7 involved an 82 year old female with multiple metastatic skin tumors which were found to be a chondrosarcoma by biopsy.

The tumor in case 8 was thought to be malignant because of the clinical finding which showed rapid growth with stubborn bleeding after the excisional biopsy.

II. Histological Findings

1. Benign Chondromas (table 2)

A slight increase of cellularity was observed in each case; however, we rarely found a large chondrocyte with a nucleus larger than the size of an erythrocyte. Binuclear tumor cells were still small and were found occasionally. Irregularity of nuclear shape was uncommon and mitotic figures could not be detected.

2. Chondrosarcomas (table 3)

Microscopic invasions into the surrounding normal bones were observed in cases 1, 2, 3, 9 and 10.

Although mild irregularity of nuclear outline or occasional binuclear cells were seen in both cases 1 and 2, a moderate increase of cellularity being observed in the former case, giant chondrocytes with large single and multiple nuclei or a prominence of nucleoli were not found.

A moderate to marked increase of cellularity and many cells having plump nuclei with pleomorphism were observed in all the tumors of cases 3 to 10, as seen in table 3. In these cases binuclear cells were often found and giant cartilage cells with large or multiple nuclei were also occasionally observed in various frequencies.

Moreover, fibrous septa with abundant blood capillaries perforating the cartilage mass and necrotic foci of cartilage lobules were much more conspicuous in all the tumors of table 3 compared with those of table 2.

3. Immature Teratomas (table 4)

All three tumors were composed of many components, such as nerve, bone, cartilage, glands, hair follicles, chorioid plexus, brain and so on. Cartilage islands in various maturation stages were seen in all tumors, but the chondrocytes were usually rather small and the majority of them had only one nucleus. No giant cartilage cells with large single and multiple nuclei were observed.

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	Enchondroma									Peri. chondro. \ddagger				
Case	1	2	3	4	5	6	,	7	8	9	10	11	12	13
Age	4	7	36	42	34	44	į	3	41	36	33	8	26	25
Location §	II pro p.	III mid p.	IV met	III met	IV pro p.	IV pro p.	II mid p.	II pro p.	II pro p.	V met	IV pro p.	II pro p.	IV mid p.	V mid p.
Cortical destruct.	-	?	-	-	?	-	-	-	?	-	-	+	+	+
S-100	С	С	С	С	С	С	С	С	С	С	C, D	С	С	C, D
АТ %	$\frac{\perp}{3}$	$^{+}_{9}$	$^+_{10}$	$^+_{10}$	+ 15	$^+_{15}$	$^{+}_{11}$	$^{+}_{18}$	$^{+}_{27}$	$^{+}_{51}$	++ 59	$^{+}_{36}$	$^{+\!+}_{40}$	++ 72
ACT	-		-	-		_	-	-	-	-	-	-	-	-

Table 2. Benign Chondromas

§ pro: proximal § mid: middle § met: metacarpus § p.: phalanx

‡ Peri. chondro.: periosteal chondroma

<u>.</u>	case *	age	site #	SSS +	type §	S-100	AT, %	ACT, %	recurr. ‡
grade I	$ \begin{array}{r} 1\\ 2\\ 3 (77)\\ 4 (72)\\ 4 (74) \end{array} $	74 46 48 18 19	femur pelvis pelvis spine spine	Ib Ib Ib Ib III	well well well well well	C C C C D, C	$\begin{array}{rrrrr} + & 14 \\ + & 60 \\ + & 33 \\ + & 72 \\ + & 65 \end{array}$	$egin{array}{cccc} - & 0 \ oldsymbol{\perp} & 16 \ + & 26 \ + & 40 \ + & 65 \end{array}$	$ \begin{array}{c} 0 \\ 0 \\ 0 \\ 1 \\ 4 \end{array} $
grade II	5 6 3 (83) 3 (85) 7 8	41 74 55 57 82 60	rib pelvis trunk m. trunk m. skin m. nasal	Ib Ib IIb IIb III III	well well por. myx. por. myx.	D, C C C C C C C	$\begin{array}{rrrrr} + & 17 \\ + & 23 \\ + & 77 \\ + & 39 \\ + & 32 \\ + & 38 \end{array}$	$\begin{array}{rrrr} + & 26 \\ + & 9 \\ + & 60 \\ + & 17 \\ + & 31 \\ + & 43 \end{array}$	3 0 9 10 ? ?
grade III	$9\\10$	74 18	pelvis tibia	IIb IIb	myx. mod.	C, D C, D	++50 +-52	$^{+\!+}_{+\!-} \begin{array}{c} 38 \\ +\!\!+ 42 \end{array}$	3 ?

Table 3. Chondrosarcomas

 $\boldsymbol{*}$ 3 (77), 3 (83) and 3 (85) are tumors of the same case in different years.

+ SSS: surgical staging system 8 $\$ well: well differentiated

§ por: porrly differentiated § mod.: moderately differentiated

 $myx.: myxomatous \ +m.: metastasis \ \pm recurr.: recurrence$

Table 4.	Tumors	with	Cartilaginous	Component
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case		age ‡	location	S-100	AT	ACT	prognosis #
immature	1	5 days	mediastinum	D, C, N		_	NR
teratoma	2	22 y.	ovarium	N, D	_		NR
	3	10 y.	ovarium	Ν	1	—	NR
osteosarcoma	4	28 у.	sacrum	D	+	+-	death
(chondroid area)	5	35 y.	femur	D, C	+	#	death

‡ y.: years-old # NR: no recurrence



pattern with the antibody to alpha-1-antitrypsin pattern with the antibody to alpha-1-antichymo- $(\times 1000)$

Fig. 1. 3 types of immunoperoxidase staining Fig. 2. 3 types of immunoperoxidase staining trypsin (\times 1000)



Fig. 3. 3 types of immunoperoxidase staining pattern with the antibody to S-100 protein (\times 1000)

4. Osteosarcomas (table 4)

Marked irregular osteoid formation by tumorous osteoblasts and precise chondroid differenciation were seen in both cases. Many cells in cartilaginous tumor areas had a plump and pleomorphic nucleus with prominent nucleoli. Mitotic figures were also occasionally observed. Binuclear chondrocytes or giant cartilage cells with large single and multiple nuclei were fairly common.

- III. Immunohistochemical findings
- 1. AT and ACT
 - 1) Both AT and ACT were negative in normal bronchial cartilages.
 - 2) Benign chondromas, chondrosarcomas and osteosarcomas always had AT positive chondrocytes and NAT of these tumors was much larger in the malignant ones than in the benign ones. (table 2, 3)
 - 3) ACT positive cells were detected in nine chondrosarcomas and in the cartilaginous tumor areas of two osteosarcomas but came up negative in all the benign chondromas and immature teratomas. (table 2, 3, 4)
 - 4) Positive staining of AT and ACT was found only in cytoplasmas and never in nuclei.

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- 5) The staining patterns of chondrocytes were subclassified into three types (small ringed, focal and cytoplasmic diffuse type). These three types were found simultaneously in one tumor; however, the cells of the focally stained pattern were more frequently found in benign tumors whereas cells of the cytoplasmic diffuse type were abundant with tumors of high-grade malignancy. (Fig. 1, 2)
- 2. S-100 protein (table 2, 3, 4)
 - All normal bronchial cartilage and tumorous cartilage cases had S-100 protein positive chondrocytes and the staining pattern was subdivided into 3 types: diffuse (D), nuclear (N) and cytoplasmic (C). (Fig. 3)
 - 2) Benign chondromas and chondrosarcomas belonged mostly to type C, but bronchial cartilages, chondrocytes in osteosarcomas and immature teratomas were found to have a mixed dominancy of N and D.

No staining difference between benign and malignant tumors could be found in specimens stained with PAS, alcian blue and reticulin stain.

DISCUSSION

According to the clinical and histological criteria^{12),14} applied in this study, all enchondromas in table 2 are thoroughly benign from the standpoint of site, size, growth pattern and microscopic findings. Although slight cortical destructions were observed in the three cases presented as periosteal chondromas (case 11, 12, 13 in table 2), the other findings, namely their original site in the hand, their small size, their lack of a metastases history and lack of recurrences for more than 5 years might support the idea that these tumors were of a benign nature. Besides, no large chondrocytes or giant cartilage cells were observed in these three tumors and they were also microscopically judged as being benign. Thus all the cases in table 2 can be regarded as the benign counterparts of completely malignant cartilage tumors.

Among the cases presented as chondrosarcoma in table 3, it was easy to judge all grade II and III tumors as being malignant simply from the histological point of view proposed by Lichtenstein and Jaffe.¹²⁾ As to the tumors of grade I series, cases 3 and 4 were certainly malignant since the atypism of the tumor cells satisfied the histological criteria of Lichtenstein and Jaffe.¹²⁾ Cases 1 and 2 could not be diagnosed as malignant merely from histological findings; however, they completely fulfilled the clinical criteria of chondrosarcoma set forth by O'Neal and Ackerman.¹⁴⁾ Therefore, in this paper, tumors in cases 1 & 2 were included in a borderline malignant group.

Cartilage tumors form a continuous spectrum in terms of both their histological appearance and clinical behavior, ranging from the entirely benign to the profoundly malignant. Although O'Neal and Ackerman's clinical criteria¹⁴⁾ and the histological

criteria of Lichtenstein and Jaffe¹²⁾ have great diagnostic value, we sometimes encounter some diagnostic troubles with cartilaginous tumors. Recently AT positive cells were detected by Dicter⁷⁾ in the chondrosarcomatous area of ovarian malignant mixed mesodermal tumors and AT or ACT positive cells were found by Fukuda et al.⁹⁾ in the cartilaginous tumor area of an extraskeletal chondrosarcoma. But no studies have shown that AT or ACT were contained in nonneoplastic chondrocytes. Our findings suggest that tumorous chondrocytes restrictively have AT or ACT and that, consequently, AT or ACT might be diagnostic indicators of cartilaginous tumors.

AT positive cells appeared in many cartilaginous tumors in this paper, but no AT or ACT positive cells were detected in any specimens of normal bronchial cartilage.

Similarly AT and ACT were detected in epithelial and mesenchymal components of pleomorphic adenomas by Sehested et al.¹⁸⁾ but not in normal parotid tissue.

ACT positive cells were observed in chondrosarcomas of high grade malignancy and in cartilaginous areas of osteosarcomas. As for the borderline cases of the two, only one contained ACT positive cells. The number of positive cells was generally much higher in tumors of high grade malignancy than in those of low grade. (table 3)

The data of this study may indicate that AT and/or ACT positive cells appear restrictively in tumorous chondrocytes and that the existence of ACT positive cells and a large number of AT positive cells in cartilaginous tumors is a useful indicator of the malignant nature of these tumors.

Recently AT and/or ACT positive cells have been found in miscellaneous malignant tumors.^{1),3),4),6),7),9),10),15),16),20),21)} Tahara et al.²⁰⁾ reported that protease inhibitors like AT or ACT are highly positive in advanced gastric cancer cells regardless of histologic type and that AT or ACT are helpful markers of the invasive growth of gastric cancers. Chowdhury et al.⁵⁾ suggested that the accumulation of AT-like material in neoplastic cells could represent a basic process in neoplastic transformation.

There are two possibilities for the positive staining of tumorous chondrocytes. The one is that tumorous chondrocytes synthesize AT or ACT by themselves and the other is that chondrocytes have their own proteases as receptors of AT or ACT like lymphocytes²²⁾ and these proteases can make the aggressive invasion of tumor cells into the surrounding normal tissues possible. In the former case AT or ACT must originate from the tumor cells but in the latter case they must be derived from the serum of the host.

On the other hand, it has been said that S-100 protein is a water-soluble, acidic, calcium¹⁷⁾-and zinc²⁾-binding protein. Recently Weiss et al.²³⁾ proposed the idea that S -100 protein was related to mechanisms of matrix calcification in normal cartilage and cartilaginous tumors. According to this investigation, S-100 protein served no purpose in judging the possibility of malignancy. However, it may nonetheless be useful in judging whether the tumor is truly one of cartilaginous tissue origin or one of the components of composite tumors like immature teratomas.

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