ONCOLOGY

Calmodulin Content in Chondrosarcomas

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The content of calmodulin in chondrosarcomas was notably lower in patients with early relapses or generalized tumor process in comparison with patients without postoperation relapses. The mean level of calmodulin in chondrosarcomas virtually did not depend on patient's sex and age and tumor location in bones. There was a tendency to an inverse correlation between calmodulin level in chondrosarcoma and the degree of tumor differentiation. The highest level of calmodulin was detected in the cytosolic fraction of chondrosarcomas containing no estrogen and androgen receptors.

Key Words: *calmodulin; chondrosarcoma; steroid receptors*

Multiple effects of hormones, polypeptide growth factors, and other bioactive substance modifying bone tumor growth are realized through intercellular and intracellular mediators [2,6]. One of such modulators is calmodulin (CM), a universal messenger of Ca^{2+} effects in the cell [5]. CM participates in transmission of apoptotic signal in cells, in particular it activates DAP-kinase responsible for changes in cytoskeleton during apoptosis [5]. CM mediates signal of tumor necrosis factor-α by activating CM-dependent type 2 kinase responsible for DNA fragmentation during apoptosis [10]. Numerous functions of CM are regulated by many factors, and structural changes in this protein caused by various mutations modify its activity [4].

We previously showed that high concentration of CM in osteogenic sarcoma (above than 6000 ng/mg) is associated with low incidence of metastases during the first 5 years of observation.

In this study we measured the content of CM in chondrosarcoma (CS) homogenates and investigated the relationship between CM concentration in the tumor and the main clinical and morphological characteristics of the disease and the duration of relapse-free period.

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MATERIALS AND METHODS

Thirty patients (16 men and 14 women) aged 16-70 years with CS in tubular bones (n=20) and flat bones (n=10) were examined.

The morphological variants of CS were as follows: (24.1%) with the first (n=7, 24.1%), second (n=12, 41.4%) and third CS of typical histological structure with the first degree of differentiation, 12 cases (41.4%) with the second degree of differentiation, 4 cases (13.8%) with the third degree of differentiation and mesenchymal CS (n=4, 13.8%) and 3 cases with dedifferentiated CS.

Calmodulin in tumor homogenates was measured by radioimmunoassay using Amersham kits.

The results were processed using standard statistical software for medical data.

RESULTS

The content of CM varied from 1114 to 8235 ng/mg total protein. The mean level of tumor CM in men was

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TABLE 1. Mean CM Content in Histological Variants of CS with Different Degree of Differentiation (*M±m*)

Histological variant of CS and degree of differentiation		Mean CM level, ng/mg protein
CS with typical histological structure	I (n=7)	5510.9±1075.3
	II (n=12)	3744.5±375.3
	III (*n*=4)	2802.8±401.4
Mesenchymal CS (*n*=4)		2529.6±540.1
Dedifferentiated CS (*n*=3)		3363.0±608.1

TABLE 2. Content of CM in CS and Duration of Relapse-Free Period (*M±m*)

Parameters	Group 1	Group 2
Number of cases	14	13
Number of tumors with CM content>4000 ng/mg total protein, %	21.5	58.3
Range of CM values, ng/mg protein	1114-4580	1200-8235
Mean CM content, ng/mg total protein	3002.5±261.6	4913.2±561.6*

Note. *$p<0.05$ *vs.* group 1.

3534±493.8 ng/mg protein, which did not differ significantly from the level in women (4387.8±506.0 ng/mg protein).

Patients were divided into two groups by the age when bone CS was diagnosed: before 30 years (*n*=7) and older (*n*=23). The mean tumor CM level in the younger group was 3306.0±650.3 ng/mg protein and in the older group 4123.1±419.9 ng/mg protein (the difference is insignificant).

The content of CM in CS of different histological structure is presented in Table 1. This parameter tended to decrease in highly differentiated CS of typical histological structure in comparison with poorly differentiated tumors. The level of CM in mesenchymal CS was comparable with that in CS of the third degree of differentiation.

The mean level of CM in tumors was 4059.1± 458.4 ng/mg protein in tubular bones and 3679.2± 575.1 ng/mg protein in flat bones (the difference is insignificant).

TABLE 3. Content of CM in CS and Expression of Steroid Receptors in the Tumor (*M±m*)

Tumor receptor phenotype	Number of cases	Mean CM level in tumor, ng/mg protein
AR^+ER^+	16	3909.0±492.8
AR^+ER^-	6	2869.8±354.2
AR^-ER^+	3	3332.0±965.5
AR^-ER^-	5	5642.8±1011.0*

Note. AR: androgen receptors, ER: estrogen receptors. *$p<0.05$ *vs.* AR^+ER^-.

The relationship between CM content in CS and delayed outcomes of treatment was studied in 27 (90%) patients.

For detecting the relationship between CM level in CS and the time of metastasis development or tumor relapse, the patients were divided into 2 groups (Table 2). In group 1 metastases developed in the lungs or CS grew in the bone after surgery. This group also included 2 patients with primarily generalized process. In group 2 no distant metastases or CS relapses in the bone were detected after surgery.

We found a relationship between CM content and receptor phenotype of CS (Table 3). The level of CM in the cytosolic fraction was higher in CS containing no estrogen and androgen receptors in comparison with CS expressing one of these receptors. The content of CM in CS with both receptors was lower than in receptor-negative CS, but higher than in tumors with only one type of steroid hormone receptors.

Hence, the content of CM in CS was significantly lower in patients with early relapses or generalized tumors than in patients who had no relapses after surgical removal of the primary tumor. The mean level of CM in CS did not depend on patient's age and sex or tumor location. A tendency to an inverse relationship between CM level in CS and the degree of tumor differentiation was detected. The highest levels of CM were detected in the cytosolic fraction of CS containing no estrogen and androgen receptors.

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