ONCOLOGY

Receptors for the Epidermal Growth Factor and Estrogens in Primary Bone Tumors

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The content of receptors for the epidermal growth factor and estrogens is studied in primary human bone tumors: osteogenic sarcoma, chondrosarcoma, malignant fibrous histiocytoma, and giant cell tumor. In all of these tumors, radioligand analysis reveals receptors for epidermal growth factor in the membrane fraction and receptors for estrogens in the cytosolic fraction without any statistical differences in the mean receptor content. The vast majority of osteogenic sarcomas, chondrosarcomas of the 2nd-3rd degree of anaplasia, and one third of giant cell tumors are characterized as growth factor receptor-positive and estrogen receptor-negative, which probably reflects the common tendency of receptor-positive malignant tumors towards rapid growth.

Key Words: *epidermal growth factor receptors; estrogen receptors; osteogenic sarcoma; chondrosarcoma*

Malignant bone tumors have not been well investigated. They cause severe disorders in the organism, are highly resistant to modern therapies, and produce early metastases. This calls for further investigation of their pathogenesis (specifically, of the mechanisms responsible for metastasizing) to establish reliable prognostic criteria and develop new pathogenetic approaches to their therapy.

Impaired regulation of proliferative processes is one of the major characteristics of malignant tumors. In malignant growth, both the spectrum of regulators of cell proliferation and differentiation and their role in these processes change. Local control over the growth of tumor cells is exerted by auto- and/or paracrine mechanisms, when growth factors produced by tumor cells themselves (autocrine regulation) or by stromal cells (paracrine regulation) act on the tumors via functionally active membrane receptors.

Epidermal and α-transforming growth factors, which interact with a common membrane receptor, are well known auto- and paracrine regulators of tumor growth. Disorders in the mechanisms responsible for tumor cell proliferation are associated with changes in the sensitivity to epidermal growth factor: it increases as a result of enhanced expression of the epidermal growth factor receptors (EGFR), their affinity, and degree of phosphorylation and dimerization and, probably, due to ectopic expression of EGFR in tissues which do not express them in healthy subjects [8]. For example, normal adult human osteoblasts do not express EGFR. However, these receptors were detected in cultured mammalian and human osteoblasts and chondrocytes [9,13]. Receptors for epidermal growth factor were

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TABLE 1. Specific Binding of EGFR in Primary Bone Tumors (*M±m*)

Tumor type	Number of samples	Occurrence of EGFR, %	Mean content of EGFR, fmol/mg protein
Osteogenic sarcoma	26	65	247±39
Chondrosarcoma	12	40	252±69
MFH	4	75	185±60
GCT	6	50	47±8*

Note. *$p<0.05$ compared with other types.

identified by hybridization techniques in bioptates from patients with osteogenic sarcoma and chondrosarcoma [6].

The binding sites for epidermal growth factor are located predominantly on the membranes of colony-forming, rapidly dividing young cells. High-affinity EGFR are expressed by cultured cells of the osteogenic sarcoma OST-1-RF, but not by cultured cells of the highly differentiated ROS 17/2.8 osteogenic sarcoma [14]. It was demonstrated that epidermal growth factor stimulates the mitogenic activity of cultured G292 osteoblasts in a dose-dependent manner, the effect being suppressed by the inhibitors of tyrosine kinase activity [15]. The growth of CFK 1 osteoblasts is inhibited by anti-EGFR and anti-α-transforming growth factor antibodies and by nonspecific inhibitors of the epidermal growth factor-receptor binding, for example, suramin [5]. In addition to other factors, the expression of EGFR is stimulated by epidermal growth factor [12]. Based on these data, we studied EGFR and related their content and that of estrogen receptors (ER) to morphological characteristics of primary bone tumors.

MATERIALS AND METHODS

Four groups of patients with untreated primary osteosarcomas were included in the study: 26 of them had osteogenic sarcoma, 12 patients had chondrosarcoma, 4 patients had malignant fibrous histiocytoma (MFH), and 6 patients had giant cell tumor (GCT). The mean age of patients with osteogenic sarcoma was 19.5 years, with chondrosarcoma 45 years, with MFH 41.7 years, and with GCT 23 years. Males predominated among patients with osteogenic sarcoma, chondrosarcoma, and MFH, the male:female ratio being 18:8, 7:5, and 4:0, respectively.

Tumor samples (300-500 mg) were frozen in liquid nitrogen, and the contents of EGFR and ER were then determined.

To prepare the cytosolic fraction, frozen samples were ground and homogenized in TED buffer (pH 7.4 at 4°C) containing 10 mM Tris-HCl (Merck), 1.5 mM EDTA (Sigma), 0.5 mM dithiothreitol (Koch-Light Lab.), and 10% (v/v) glycerol (chemically pure grade). The cytosolic fraction was obtained by centrifugation of the homogenate in a Beckman centrifuge (105,000*g*, 30 min at 4°C). The content of ER in this fraction was determined by the standard radioligand method recommended by EORTC with small modifications [3], which included separation of the hormone-receptor complexes and unbound ligand on Dextran-coated charcoal. Radioactivity was measured in an LS 6500 scintillation β-counter (Beckman). The cytosolic protein content was determined by the method of Lowry in a DU 650 spectrophotometer (Beckman). Cells with receptor content higher than 10 fmol/mg cytosolic protein were considered to be ER-positive.

The pellet was used for the preparation of crude tumor cell membrane fraction, in which the EGFR content was determined. For this purpose it was rehomogenized at 4°C in phosphate-salt buffer (pH 7.4) containing 1 g/liter bovine serum albumin (Sigma) and 0.07 g/liter bacitracin and centrifuged (2000*g* for 10 min at 4°C). The EGFR content was determined in a radioligand assay [4] of the supernatant (crude membrane fraction) using murine epidermal growth

TABLE 2. Occurrence and Content of ER in Primary Bone Tumors (*M±m*)

Tumor type	Number of samples	Occurrence of ER, %	Mean content of ER, fmol/mg protein
Osteogenic sarcoma	26	19	20.5±8.0
Chondrosarcoma	12	25	55.5±43.8
MFH	4	75	18.8±4.1
GCT	6	33	11.5±1.5

TABLE 3. Distribution of ER and EGFR in Bone Tumors, %

Tumor type	ER^+EGFR^-	ER^-EGFR^+	ER^+EGFR^+	ER^-EGFR^-
Osteogenic sarcoma	13	63	4	20
Chondrosarcoma	16	32	8	44
MFH	0	0	75	25
GCT	17	33	17	17

factor as a ligand (Sigma). The ligand was iodinated with ^{125}I-Na (St. Petersburg) in the presence of chloramine T. The specific radioactivity of labeled ligand was 100 Ci/mmol. Membrane samples (protein content 0.2-1.0 mg/ml) were incubated for 1 hour with 3.5 nmol ^{125}I-labeled epidermal growth factor in the presence or absence of a 200-fold excess of unlabeled growth factor. The reaction was terminated by the addition of 75% hydroxyapatite suspension (Sigma) in phosphate-salt buffer (pH 7.4) on ice. After three successive washings at 4°C, the radioactivity of the samples was measured in an LKB γ-counter. Tumors containing not less that 10 fmol EGFR/mg membrane protein were considered to be EGFR-positive ($EGFR^+$).

The results were statistically analyzed using Student's *t* test.

RESULTS

The receptors for epidermal growth factor were present in 67% osteogenic sarcomas, 40% chondrosarcomas, in 50% GCT, and 75% MFH. There were no statistically significant differences in the mean EGFR content in the membrane fraction of osteogenic sarcomas, chondrosarcomas, and MFH, which was 247±39, 252±69, 185±60 fmol/mg membrane protein, respectively. In GCT, the EGFR content was significantly lower (47±8 fmol/mg protein, $p \leq 0.05$). The maximum variation range of the EGFR content, from 64 to 605 fmol/mg protein, was recorded in osteogenic sarcomas. It was minimal in GCT: 30-50 fmol/mg protein.

The EGFR levels in osteogenic sarcomas, chondrosarcomas, and MFH were comparable to those in the epidermal growth factor-dependent malignant tumors: breast cancer, ovarian cancer, and lung cancer [10]. Presumably, the presence and different levels of EGFR in osteogenic sarcomas, chondrosarcomas, and MFT are not the specific feature of these tumors, but reflect the common tendency of all $EGFR^+$ epidermal and mesenchymal [6] tumors towards rapid growth.

ER were found in 17% osteogenic sarcomas, 25% chondrosarcomas, 75% MFH, and 34% GCT (Table 2). The mean content of ER (fmol/mg cytosolic protein) was 20.5±8.0 in osteogenic sarcomas, 55.5±43.8 in chondrosarcomas, 18.8±4.1 in MFH, and 11.5±1.5 in GCT. The maximum variation range for the ER content was recorded in chondrosarcoma (10.0-143.2) and the minimal in GCT (10.0-12.9).

The presence of cellular hormone receptors is one of the main indicators of hormone sensitivity. Some strains of osteogenic sarcomas and human chondrocytes [1,7] contain ER. Direct, receptor-mediated effects of estrogens in osteoblasts and osteogenic sarcomas were demonstrated *in vitro* [11]. However, the controversy over the mechanisms responsible for estrogen effects during blastomogenesis in the bone tissue still exists.

Previously, we revealed specific binding of 17β-estradiol in the cytosolic fraction of human osteogenic sarcoma [2]. The occurrence and content of ER in this sarcoma depend on sex and age of patients, morphological variant of tumor, and degree of tumor differentiation. Although exogenously administered 17β-estradiol had no effect on the growth of the HOS strain osteogenic sarcoma grafted into thymus-free male rats, it was specifically bound in the cytosol prepared from HOS cells [1]. This strain was isolated from osteogenic sarcoma of the femur of a 13-year-old girl by Dr. McAllister and coworkers in 1971.

In the present study ER were found in less that one third of tumors with the exception of MFH, in which the receptors were identified in 75% of the studied tumors (3 out of 4). From the low occurrence of ER in most of the studied bone sarcomas, the absence of significant differences in the ER content between these tumors, and the low ER content in them, it can be assumed that some bone sarcomas are probably sensitive to estrogens, which may be associated with their histological structure and degree of differentiation.

The occurrence of both receptors (EGFR and ER) was the highest in MFH (75%) and the lowest in osteogenic sarcomas (4%). The occurrence of EGFR alone was higher in osteogenic sarcomas (63%), being 32% and 33% in chondrosarcomas and MFH, respectively (Table 3). It is noteworthy that $EGFR^+ER^-$ chondrosarcomas were represented only

by histologically dedifferentiated tumors and those of the 2nd-3rd degree of anaplasia (the most malignant variants). The presence of EGFR in the epidermal growth factor-dependent tumors and the absence of ER imply bad prognosis for some tumor types [10].

The ER^-EGFR^- phenotypes (both receptors are absent) prevailed among chondrosarcomas of the 1st-2nd degree of anaplasia (44%). In osteogenic sarcomas, MFH, and GCT this phenotype was revealed in 20, 25, and 17% of cases, respectively.

The occurrence of the ER^+EGFR^- phenotype was practically the same in osteogenic sarcomas, chondrosarcomas, and GCT (13, 16, and 17%, respectively). None of the studied MFH had this phenotype. All ER^+EGFR^- chondrosarcomas were of the 1st-2nd degree of anaplasia, i.e., had the highest degree of histological differentiation. Some researchers regard this combination of ER and EGFR receptors as the most favorable prognostic factor, specifically, in breast cancer [2]. The low occurrence of the ER^+EGFR^- phenotype in osteogenic sarcomas, chondrosarcomas, and GCT is probably associated with the highly malignant growth of bone sarcomas.

We managed to characterize the receptor status only of the osteoblast variant of osteogenic sarcoma (50% of the studied tumors). Most of them (62%) were EGFR-positive and ER-negative, none contained both ER and EGFR, while 15 and 23% had ER^+EGFR^- and ER^-EGFR^- phenotypes, respectively.

Thus, EGFR and ER were present in all four types of untreated primary bone sarcomas, their contents being similar. The mean EGFR content is comparable to that in other epidermal growth factor-dependent tumors. The $EGFR^+ER^-$ phenotype prevailed among osteogenic sarcomas (the most malignant neoplasm). This combination was revealed in the most malignant variant of chondrosarcoma and in one third of the studied GCT.

Osteogenic sarcomas, chondrosarcomas, and MFH had virtually the same low occurrence of ER^+EGFR^- phenotype, which is prognostically most favorable for other tumors. This probably reflects the highly malignant growth of these neoplasms. Our findings lead to a preliminary conclusion that epidermal growth factors and estrogens are involved in the regulation of the bone tumor growth, and the clinical validity of combined EGFR and ER analysis should be confirmed by long-term clinical observations, in which the levels of ER and EGFR will be compared with the response to chemotherapy, duration of remission, and time of metastasizing.

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