Receptor Activator of Nuclear Transcription Factor NF-κB (RANK), Its Ligand RANKL, and Natural Inhibitor of RANKL Osteoprotegerin (OPG) in the Blood Serum of Patients with Primary Bone Tumors


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The content of components of the RANK/RANKL/OPG system, the key regulator of homeostasis in the bone tissue, in blood serum samples from 199 patients with primary bone neoplasms and 131 practically healthy volunteers was measured by ELISA. Borderline giant-cell tumor of the bone with high osteoclastogenic and osteolytic activity is characterized by an increase in the level of all components of this system and highest ratio of sRANKL/OPG in the blood serum. Study indexes in patients with various benign neoplasms and tumor-like bone lesions were lower than in patients with giant-cell tumor. The patients with malignant bone tumors could be divided into 2 subgroups with opposite indexes of the RANK/RANKL/OPG system. The patients with osteosarcoma and Ewing sarcoma had a low level of sRANK, but a high level of sRANKL. The patients with chondrosarcoma and chordoma had a high level of sRANK, but a low level of sRANKL.

Key Words: RANK; RANKL; osteoprotegerin; primary bone tumors

The ligand-receptor system of RANK/RANKL/OPG is a key component in bone tissue homeostasis, which regulates differentiation of osteoclasts and osteolysis [5]. The receptor activator of nuclear transcription factor NF-κB (RANK) is a major part of this system. This transmembrane protein of type 1 belongs to a superfamily of TNF receptors. The only ligand (RANKL) interacting with an extracellular domain of RANK belongs to the TNF family. This transmembrane protein is primarily expressed on the surface of activated T cells, bone marrow stromal cells, and osteoblasts. Soluble forms of RANKL (sRANKL) appear due to proteolytic degradation of the transmembrane protein or alternative splicing of its mRNA. Binding of transmembrane and soluble forms of RANKL to RANK is followed by the induction of osteoclastogenesis from precursor cells and activation of mature osteoclasts. Osteoprotegerin (OPG) serves as a natural antagonist of RANKL, which is named the decoy receptor. This soluble homologue of RANK is primarily secreted by bone marrow stromal cells and osteoblasts and inhibits the interaction of RANK with RANKL (by binding of RANKL).

The RANKL/OPG ratio is differently regulated under physiological and pathological conditions. An imbalance in the RANK/RANKL/OPG system contributes to various pathological processes that are associated with the impairment of bone tissue remodeling [10]. A change in the balance of bone remodeling also results in some processes, which are associated with tumor growth [6]. The RANKL/OPG ratio is regulated by a variety of signals, which depends on the type and nosological characteristics of bone tumor [5].

The increased expression and/or signal activity of RANKL is typical of various solid tumors. RANKL is
produced by tumor cells and in vitro increases osteoclastogenesis. These data suggest that tumor cells in the bone tissue can directly affect this process. Previous studies showed that the RANK/RANKL/OPG system is involved in metastatic dissemination of various tumors into the bone [7]. However, little is known about the effect of this system on the development of primary bone neoplasms. In vitro experiments revealed a high expression of factors that are responsible for osteoclastogenesis by osteosarcoma cell lines Saos-2 and MG63 [4,11]. Clinical observations showed a significant correlation between the increased expression of RANKL in tumor tissues and weak response of osteosarcoma patients to neoadjuvant chemotherapy [9]. Chondrosarcoma [15], Ewing sarcoma [14], and some benign lesions of the bone tissue are also characterized by high expression of components of the RANK/RANKL/OPG system.

Synthetic and natural inhibitors of the RANKL/RANKL interaction were used to suppress osteoclastogenesis and osteolysis in various pathological states accompanied by bone tissue destruction. However, the majority of these attempts failed [1]. Denosumab was most effective in inhibiting the RANK/RANKL signal pathway. This product is a completely humanized monoclonal antibody to RANKL. Denosumab binds RANKL with high affinity and specificity, which prevents RANK activation [2].

It should be emphasized that most researches in this field are devoted to tissue expression of study markers or experiments on tissue cultures. The concentration of components of the RANK/RANKL/OPG system in the peripheral blood during bone tumors was measured only in individual studies [1,8].

This work was designed to compare the level of components of the RANK/RANKL/OPG system in blood serum samples from patients with primary bone neoplasms and practically healthy volunteers. The relationship between these parameters and main clinical-and-morphological characteristics of tumors was evaluated.

**MATERIALS AND METHODS**

The study was performed on 199 patients with various primary bone neoplasms, who received therapy in the N. N. Blokhin Russian Cancer Research Center. We examined 96 women (576 years, median age 43 years) and 103 men (574 years, median age 35 years). The control group consisted of 72 women (575 years, median age 36 years) and 59 men (376 years, median age 28 years). All patients did not receive the specific treatment before a biochemical assay. The clinical and X-ray diagnosis of bone tumor was confirmed by the results of morphological examination.

All patients were divided into the following three groups: group 1 (N=121) with bone sarcomas: osteosarcoma (N=53), chondrosarcoma (N=48), chordoma (N=12), and Ewing sarcoma (ES, N=8); group 2 (N=32) with borderline bone neoplasms (giant-cell tumor of the bone, GCT); and group 3 (N=46) with benign neoplasms and tumor-like lesions of bones (fibrous dysplasia, enchondroma, aneurysmal bone cyst, chondromyxoid fibroma, osteoblastoma, benign fibrous histiocytoma, and bone cartilaginous exostosis).

The concentration of study proteins in blood serum samples (obtained routinely before specific therapy) was measured with ELISA. The results were analyzed with Statistica 7.0 software. The majority of study parameters did not follow a normal distribution. The comparison of these parameters and study of their relationships were performed by nonparametric tests (Mann—Whitney test, Kruskal—Wallis test, and Spearman’s rank correlation test, r). The differences and correlations were significant at p<0.05. The data are presented as the medians and upper and lower quartiles.

**RESULTS**

The detectable levels of sRANK in blood serum samples were revealed in 59% patients with benign bone tumors (0.82 ng/ml) and 54% patients with GCT (1.08 ng/ml). This marker was observed only in 45% patients with malignant tumors and 49% healthy volunteers. No significant between-group differences in this parameter were found (Table 1). The amount of sRANK was measured in patients with various histological types of malignant bone tumors. This protein was revealed in blood serum samples from 39% patients with osteosarcoma, 56% patients with chordoma (0.54 ng/ml), 50% patients with chordoma (0.62 ng/ml), and only 25% patients with ES (Table 1). Our results are consistent with previous data that the level of sRANK is low in patients with bone sarcomas (particularly with osteosarcoma and ES), but highest in patients with GCT.

sRANKL was identified in the blood serum of most patients with benign bone neoplasms and GCT (89 and 90%, respectively; 0.16 and 0.22 pmol/liter, respectively), as well as in 76% volunteers of the control group (0.14 pmol/liter) and 69% patients with bone sarcomas (0.12 pmol/liter; Table 1). The concentration of this marker in blood serum samples from GCT patients was much higher than in volunteers of the control group and patients with malignant tumors (p<0.05). As regards the histological type of malignant neoplasms, the following results were obtained.