# ONCOLOGY

# Leptin and Apoptosis Inhibitor Soluble Fas Antigen in the Serum of Patients with Osteosarcoma and Neuroectodermal Bone Tumors

**N. E. Kushlinskii, Yu. N. Solov'ev, I. V. Babkina, S. G. Abbasova,* I. A. Kostanyan,* V. M. Lipkin,* and N. N. Trapeznikov**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 129, No. 5, pp. 583-586, May, 2000
Original article submitted April 24, 2000

Serum contents of leptin and soluble Fas antigen were measured in 28 patients with osteosarcoma, 7 with neuroectodermal bone tumors, and 17 healthy subjects. The incidence and levels of soluble Fas antigen in patients with osteosarcoma and neuroectodermal bone tumors were higher than in healthy subjects and did not depend on sex and age in both healthy subjects and patients. Serum concentration of leptin in women was higher than in men (both in healthy controls and patients) and was lower in healthy subjects compared to patients with osteosarcoma and neuroectodermal bone tumors. A trend to a negative correlation between the concentrations of leptin and soluble Fas antigen in female patients with osteosarcoma and male patients with neuroectodermal bone tumors was revealed. The role of leptin and soluble Fas antigen in the pathogenesis of primary bone tumors is discussed.

**Key Words:** *leptin; sFas; osteosarcoma; Ewing's tumor*

Tumor growth, including tumor growth in the bones, results from imbalance between cell proliferation and apoptosis [1,11]. Apoptosis is an extremely important mechanism maintaining homeostasis of multicellular organism, ensuring removal of damaged, aged, and "undesirable" cells without damaging cell microenvironment [7,10]. Impairment of apoptosis mechanisms is associated with pathological states, in particular, oncological diseases [1,11]. Many factors are involved in the mechanisms of apoptosis in normal and transformed cells. One of such factors is the key apoptosis receptor Fas/Apo-1/CD95, a glycoprotein from the tumor necrosis factor receptor family, and its ligand FasL [7,8]. Fas is expressed in virtually all human organs and tissues, including tumors, while FasL is expressed mainly in activated lymphocytes and natural killers. Soluble Fas (sFas), an alternative splicing product of full-length Fas mRNA, inhibiting Fas-dependent apoptosis, is intensely expressed in disease [1,9].

Malignant bone tumors characterized by aggressive course, rapid hematogenic dissemination (most often into the lungs), and extremely unfavorable prognosis [2,4,5] attract special attention. A new item has been distinguished in histological classification of primary bone tumors and bone-like formations because of discovery of their neuroectodermal origin: Ewing tumor (sarcoma) family" [4]. This group includes Ewing's tumor and primitive neuroectodermal tumor. The histogenesis of bone tumors is different; the most common is osteosarcoma, occurring mainly in children during the puberty, when sex glands start active functioning [2,5]. Some scientists believe that the patho-

N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences; *M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

0007-4888/00/0005-496$25.00 

genesis of osteosarcoma can be associated with metabolism of sex steroid hormones, specifically androgens, actively stimulating the growth of these tumors [2]. An inverse relationship was noted between serum concentrations of testosterone and leptin in adolescent boys [14,15]. The main target organ of leptin is the central nervous system; leptin decreases appetite, stimulates lipid utilization in energy metabolism, and decreases fat depositions. Leptin plays an important role in the regulation of the reproductive function. Leptin gene is expressed in gonads, and therefore gonads can also be the target for leptin [3]. In addition, leptin is secreted not only in white and brown fatty tissue, but also by placental trophoblasts, embryonal cardiac, bone/cartilagenous tissues, and amnion cells [12,13]. On the other hand, leptin and sFas levels were never measured in patients with bone tumors whose growth and development are closely connected with sex steroid hormones.

We compared the incidence and levels of sFas and leptin in the sera of healthy subjects and patients with osteosarcoma and neuroectodermal bone tumors.

## MATERIALS AND METHODS

Thirty patients with primary bone tumors (21 men and 9 women) aged 14-30 years were examined. Clinical and X-ray diagnosis was confirmed histologically in all patients. According to histological classification of bone tumors [4,5], the following variants were identified: primary osteosarcoma ($n$=23), Ewing's tumor ($n$=5), and primitive neuroectodermal tumor ($n$=2). The diseases were first diagnosed in all patients, and no specific treatment were administered before.

The sFas control were serum specimens from 49 healthy subjects aged 14-23 years. Leptin was measured in 8 healthy men and 9 women aged 14-21 years with body weight index 17.4-22.1 kg/m$^2$, which corresponded to that of patients (17.1-22.3 kg/m$^2$).

Serum concentration of sFas was measured by enzyme immunoassay [1], leptin by enzyme immunoassay with a DRG kit. Leptin and sFas levels in women were measured on days 21-24 of the cycle (cycle length 22-28 days).

## RESULTS

No relationships between the incidence and the levels of sFas and patient's age and sex were detected in healthy subjects (Table 1).

Blood leptin level in healthy women was 3.9 times higher than in men (Table 1), which is in line with other reports [14,15].

A trend to an inverse correlation ($r$=-0.5) between serum leptin and sFas levels was noted in adolescent girls with osteosarcoma.

In 14 men and all 7 women with osteosarcoma, long tubular bones were involved. The tumor was most often located in bone metaphyses forming the knee joint. In men the mean serum leptin level was 2.36± 0.85 ng/ml; sFas was detected in 13 of 14 patients, its mean content was 2.79±0.45 ng/ml.

Osteosarcoma seldom occurs in flat bones [2,4,6]. In our study the tumor was located in the clavicle and iliac bone in only 2 patients; leptin concentrations in them were 2.12 and 3.84 ng/ml, respectively. No sFas was detected in one patient, while in the other its level was 4.75 ng/ml.

Osteosarcoma metastases in the lungs were found in 6 patients (3 men and 3 women), in 4 of them during the first 3 months of treatment. Serum leptin concentration in men was 0.54-1.48 ng/ml, sFas 0.8-1.8 ng/ml; these values were lower than in osteosarcoma without metastases and closer to the control. In women blood leptin level was 8.22-14.5 ng/ml, and sFas was detected in 2 of 3 patients (1.5 ng/ml in each).

The following morphological variants of osteosarcoma were identified: osteoblastic ($n$=18), small-cell ($n$=2), chondroblastic ($n$=2), and giant-cell osteosarcoma ($n$=1).

The mean concentration of leptin in patients with osteoblastic osteosarcoma was 3.72±1.07 ng/ml; sFas was detected in 15 patients (2.97±0.45 ng/ml). In two

**TABLE 1.** Serum Concentrations of sFas Antigen and Leptin in Healthy Subjects and Patients with Osteosarcoma and Neuroectodermal Bone Tumors ($M$±$m$)

| Parameter | Healthy subjects | | Patients with osteosarcoma | | Patients with neuroectodermal bone tumors | |
|---|---|---|---|---|---|---|
| | men ($n$=15) | women ($n$=15) | men ($n$=16) | women ($n$=7) | men ($n$=5) | women ($n$=2) |
| Age, years | 18.3±0.4 | 18.0±0.2 | 19.3±1.9 | 19.8±1.9 | 17.5±0.9 | 15 |
| Leptin, ng/ml | 1.69±0.52 | 6.58±1.19 | 2.36±0.85 | 9.0±1.70 | 8.54±4.04 | 15.20 and 3.61* |
| Incidence of sFas, % | 36 | 32 | 87 | 86 | 80 | 100 |
| sFas, ng/ml | 0.86±0.30 | 0.82±0.25 | 2.93±0.44 | 2.70±0.75 | 7.29±4.71 | 8.18 and 7.28* |

**Note.** *Individual values for 2 women are presented.

patients (man and woman) with chondroblastic tumors blood leptin concentrations were 3.84 and 8.22 ng/ml, and sFas 4.75 and 1.50 ng/ml, respectively. Small-cell variant of osteosarcoma was characterized by blood leptin levels 1.48 and 11.0 ng/ml and sFas levels 5.7 and 3.2 ng/ml, respectively. In a 22-year-old female patient with giant-cell osteosarcoma of the tibial bone, the concentrations of leptin and sFas were 10.5 ng/ml and 1.5 ng/ml, respectively.

In the majority of male patients with osteosarcoma, blood leptin concentrations were 0.026-3.84 ng/ml. The highest leptin levels (11.05 and 8.20 ng/ml) were observed in 2 patients aged 15 and 17 years, with small-cell and osteoblastic tumors of the tibial bone. The concentration of sFas in one of them was 3.2 ng/ml, while in the other no sFas was detected. In women blood leptin concentrations were virtually the same as in healthy women.

In men with neuroectodermal bone tumors, the mean blood levels of sFas and leptin were 7.29±4.71 ng/ml and 8.54±4.04 ng/ml, respectively. A trend to a negative correlation ($r$=-0.43) between the concentrations of leptin and sFas was observed (Table 1).

No regularities could be traced in two women with Ewing's tumor of the iliac bone (Table 1).

Tumors of the neuroectodermal origin are usually located in long tubular bones and pelvic bones, but generally, any bone can be involved. In our study the tumor primarily involved long tubular bones (in 4 of 7 patients): femur ($n$=2), humerus (1), and fibula (1). Ewing's tumor was detected in two flat bones: iliac and ischial.

Ewing's tumor is characterized by early fulminant metastases. In this study a 22-year-old patient with tumor of the ischial bone hospitalized in the generalized stage of disease, had brain metastases. Blood leptin concentration was 24.39 ng/ml, sFas content was 0.9 ng/ml.

In two patients with primitive neuroectodermal tumor of the tibia (metastases in the liver, parietal bone, and rib) and VI-VIII ribs, serum leptin levels during generalization of the process were 7.26 and 4.86 ng/ml and sFas concentrations 4.20 and 2.95 ng/ml, respectively.

Hence, the incidence and the concentrations of serum sFas are higher in patients with osteosarcoma and neuroectodermal bone tumors than in healthy subjects. Serum leptin concentrations are higher in women than in men. In addition, osteosarcoma, Ewing's tumor, and primitive neuroectodermal tumor of the skeleton are characterized by increased levels of leptin in both men and women. These data suggest that the expression of sFas and leptin is associated with the pathogenetic mechanisms of these diseases, but further studies are needed to confirm this hypothesis.

## REFERENCES

1. S. G. Abbasova, N. E. Kushlinskii, V. M. Lipkin, and N. N. Trapeznikov, *Vopr. Biol. Med. Farmatsevt. Khim.*, No. 3, 3-17 (1999).
2. N. E. Kushlinskii and Yu. N. Solov'ev, *Arkh. Patol.*, **61,** No. 1, 1-13 (1999).
3. Yu. A. Pankov, *Biokhimiya,* **68,** No. 12, 1600-1614 (1998).
4. Yu. N. Solov'ev, *Arkh. Patol.*, **60,** No. 4, 57-61 (1998).
5. N. N. Trapeznikov, Yu. N. Solov'ev, N. E. Kushlinskii, *et al., Ros. Onkol. Zh.*, No. 3, 21-25 (1998).
6. W. T. Ambrosius, J. A. Compton, R. R. Bowsher, *et al., Horm. Res.*, **49,** No. 5, 240-246 (1998).
7. M. Boldin, I. Mett, E. Varfolomeev, *et al., J. Biol. Chem.*, **270,** 387-391 (1995).
8. P. J. Cohen and R. A. Eisenberg, *Apoptosis and the Immune Response,* Ed. C. D. Gregory, Oxford (1995), No. 4, pp. 169-186.
9. T. Hasunuma, N. Kayagaki, H. Asahara, *et al. Arth. Rheum.*, **40,** 80-86 (1997).
10. Y. F. R. Kerr, A. H. Wyllie, and A. R. Currie, *Brit. J. Cancer,* **26,** No. 2, 239-257 (1972).
11. Y. F. R. Kerr, C. M. Winterford, and B. V. Harmon, *Cancer,* **73,** 2013-2026 (1994).
12. S. Loffreda, S. Q. Yang, H. Z. Lin, *et al., FASEB J.*, **12,** No. 1, 57-65 (1998).
13. H. Masuzaki, Y. Ogawa, N. Sagawa, *et al., Nat. Med.*, **3,** No. 9, 1029-1033 (1997).
14. M. R. Palmert, S. Radovick, and P. A. Boepple, *J. Clin. Endocrin. Metab.*, **83,** No. 4, 1091-1096 (1998).
15. M. Wabitsch, W. F. Blum, R. Muche, *et al., J. Clin. Invest.*, **100,** No. 4, 808-813 (1997).