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Leptin Receptor Expression in Parathyroid Cancer

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ABSTRACT

Background:

Epidemiology studies have shown that there is a strong, positive correlation between obesity and the incidence of several types of carcinoma. One of the most important mediators correlating between obesity and an increased risk of cancer is leptin, which is an adipokine whose major functions are regulating appetite and energy homeostasis. However, no previous studies have yet to examine leptin or its receptor expression in parathyroid cancer (PC).

PC is a rare malignancy which accounts about 1% of the cases of primary hyperparathyroidism.

Methods:

In this study, the expression of leptin receptors (OBRs) was examined in 10 parathyroid cancer samples using immunohistochemistry, for which their associations with clinicopathological parameters were also evaluated. Ten patients (six men, four women) underwent operations for parathyroid carcinoma and primary hyperparathyroidism (HPTH) at the Clinical Centre of Serbia. All patients had hypercalcemia (a mean of 3.55 mmol/l) and elevated parathyroid hormone (a mean of 1824 pg/ml).

Results:

The expression of leptin is associated with OBRs, which were observed in the tumor cell membrane and/or cytoplasm of these patients, at a positive rate of 40 %. The Mann-Whitney Test confirmed a statistically significant difference (p=0.033) in the gland's weight which was not possible to find in regard to parathyroid hormone, calcium, gland's size, multifocality or age, due to the size of the sample, although they were increased in this group.

Conclusion:

The results of the researches have found that the expression levels of leptin and OBRs are mutully associated and have a role in the development of a large variety of malignancies promoting angiogenic factors that increase cancer cell survival, proliferation, and migration. The results signify the expression of leptin and/or OBRs in parathyroid cancer to be associated with parathyroid weight and may be a potential target in parathyroid cancer.

This is still the first report. Further research is necessary in order to determine the potential prognostic and thearapeutic implication of the leptin/ OBRs system and whether a correlation exists with other clinicopathological features.

KEYWORDS : Parathyroid carcinoma, obesity, leptin, angiogenesis

Introduction

Parathyroid carcinoma (PC) is an uncommon endocrine malignancy that accounts for 0.005 % for all cancers and about 1% of the cases of primary hyperparathyroidism (HPTH) (1-6).

Interestingly, there appears to be a higher incidence of this disease in the Japanese and Italian populations, reporting an incidence of 5% (7-9).

Obesity is now becoming an epidemic worldwide. Epidemical studies have shown that there is strong, positive correlation between BMI and the incidence of several types of cancer, such as colon, breast, ovarian, prostate, renal cell carcinoma and melanoma. (10). One of the most important mediators correlating between obesity and an increased risk of cancer is leptin (11,12), which is an adipokine whose major functions are regulating appetite and energy homeostasis (13).

Leptin acts predominantly by Ob-R receptor (encoded by db gene). It has several variants from Ob-Ra to Ob-Rf generated by alternative splicing, with Ob-Rb being the predominant isoform responsible for the biological actions of leptin, by activation of the Janus kinase/ signal transducer and activator of transcription (JAK/STAT) pathway, which in turn stimulates phosphatidylinositol 3-kinase (PI3K) which promotes cellular growth, migration and invasion. Suppressor of cytokine signaling-3 (SOCS3) is a leptin-inducible inhibitor of leptin signaling which blocks Ob-R mediated signal transduction, and thus forming a negative feedback mechanism for leptin signaling (14). Leptin induces production of inflammatory cytokines (TNF-α and IL-

6) by macrophages (15) and shifts the T-helper (TH) balance toward a TH1 phenotype, especially in obese individuals. This low grade inflammation in individuals with metabolic syndrome in turn increases the risk of obesity related diseases and cancer (16). Local leptin production through autocrine and paracrine pathways is a better predictor of carcinogenesis than circulating leptin levels (17).

PI3K/AKT pathway has an important role in oncogenesis in various tumors like colorectal cancer, hepatocellular cancer and endometrial cancer (18). AKT has an important role in cancer cell survival by promoting glycolysis and maintaining mitochondrial membrane potential (19). Leptin and leptin receptor in carcinogenesis has been elaborated in XIAP in a member of antiapoptotic proteins and is a physiological substrate of AKT (20). Increased levels of XIAP are associated with increased tumor cell survival due to decreased apoptosis (21).

However, no previous studies have yet to examine leptin or its receptor expression in parathyroid cancer (PC).

The aim of the present study was to detect the expression of OBRs in a group of parathyroid carcinoma patients, and to determine whether his expression correlated with patient and tumor characteristics.

Patients and methods

Patients. In this study, the expression of leptin receptors (OBRs) was examined in 10 parathyroid cancer samples using immunohistochemistry, for which their associations with clinicopathological parameters were also evaluated. Ten patients (six men, four women) underwent operations for parathyroid carcinoma and primary hyperparathyroidism (HPTH) at the Clinical Centre of Serbia. All patients had hypercalcemia (a mean of 3.55 mmol/l) and elevated parathyroid hormone (a mean of 1824 pg/ml).

Hematoxylin and eosin (HE)-stained slides for each patient were reviewed to confirm the diagnosis of parathyroid carcinoma (PC). Representative tissue sections were taken at 4μm and appliedroutineHE immunohistochemical method, AB-PAS histochemical method and immunohistochemical avidin-biotin peroxidase complex (ABC) method.

The study protocol was approved by the local Ethics Committee. Written informed consent was obtained from the patients.

Immunohistochemistry (IHC). Paraffin sections were heated at 55ºC to melt the paraffin, deparaffinized in xylene (3x5 minutes) and then rehydrated trough gradet ethanols. Endogenous peroxidase activity was blocked by 3% H2O2 in methanol for 20 minutes. To reduce nonspecific background, staining of the section was incubated with 10% normal bovine serum albumin for 30 minutes at room temperature. Rabbit polyclonalanti-Leptin Receptor antibody (Abcam; 1:60), were incubated at + 4^0 C overnight. Immunostaining was performed by the avidin-biotin peroxidase complex (ABC) method (Vectastain ABC-*Elite* kit, Vector Laboratories, Burlingame, CA). Staining was visualized with 3.3 diaminobenzidine tetrachloride (DAB). The slides were counterstained with Mayer hematoxylin and mounted in Canada balsam. Negative controls were done by replacing the primary antibody with phosphate buffered solution (PBS).

The expression levels of OBRs were evaluated semiquantitatively by two experienced pathologists.

Expression of the OBRs was determined on 10 visual fields (the mean value is obtained by counting 10 visual fields, and it is the final result for the case), and is classified as follows: 0,<10% of positive cells (negative); 1+, 10-49% of positive cells (moderate); 2+, >50% of positive cells (strong) (Figure 1.).

Patients were sorted into 2 groups; positive expression was defined by final staining scores of 2+, whereas the remaining cases (final scores 0 and 1+) were classified as negative expression.

Statistical analysis. The correlation between the expression of OBRs and clinicopathological features was analyzed. Continuous variables are expressed as the mean ± standard deviation (SD). Data were evaluated for significant differences by the 2-tailed Student's t-test or the Mann-Whitney test using the Statistical Package for Social Sciences, version 15.0 (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate statistically significant differences.

Results

Immunohistochemical detection of OBRs.

Expression levels of OBRs was determined by IHC in 10 PC samples.

OBRs expressionwas observed in the tumor cell membrane and/or cytoplasm with a positive rate of 40 % (4 of 10).

Association of expression of OBRs with clinicopathological parameters.

The Mann-Whitney test confirmed a statistically significant difference ($p=0.033$) in the gland's weight which was not possible to find in regard to parathyroid hormone, calcium, gland's size, multifocality or age, due to the size of the sample, although they were increased in this group.

Discussion

Angiogenesis, the development of new blood vessels from previous blood vessels, is necessary for tumour growth and metastasis. Angiogenesis, measured as tumour microvessel density-MVD, correlates with tumour behaviour. In many human tumours - including breast, bladder, and stomach - increased angiogenesis has been shown to be associated with the development of metastases (22, 23), poor prognosis (24, 25), and reduced survival (26, 27).

Angiogenesis also occurs in parathyroid proliferative lesions, where it has been demonstrated to be increased in comparison to normal glands (28, 29). However, secretory activity and tumour size have also been found to be either related or unrelated to parathyroid angiogenesis (28, 29).

Angiogenesis is required for adipose tissue expansion during weight gain and a direct relationship between obesity and angiogenesis was demonstrated by experiments in which angiogenesis inhibition prevented obesity and caused weight loss in genetically obese mice (30).

Leptin has been identified in several types of human cancers and may also be linked to poor prognosis. In two studies, leptin and leptin receptor expression were significantly increased in primary and metastatic breast cancer relative to noncancerous tissues in women. (31, 32).

Recent study indicates that leptin is overexpressed in human colorectal cancer, which suggests that the hormone might contribute to colorectal cancer development and progression (33).

Serum leptin and leptin receptor expression in renal cell carcinomas was well correlated with progression-free survival, venous invasion and lymph node metastasis (34). Leptin is a melanoma growth factor and the leptin autocrine-loop may contribute to the uncontrolled proliferation of melanolma cells (35). Leptin and OBR expression levels were found to be associated with papillary thyroid cancer (PTC) tumor size (36).

There had been no studies that have examined the relationship betweenleptin/OBRs in PC. The results signify the expression of leptin and/or OBRs in parathyroid cancer to be associated with parathyroid weight and may be a potential target in parathyroid cancer.

This is still the first report. Further research is necessary in order to determine the potential prognostic and thearapeutic implication of the leptin/OBRs system and whether a correlation exists with other clinicopathological features.

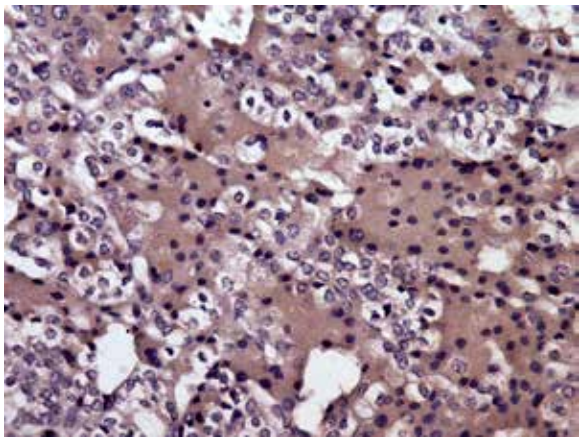

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Figure 1. Immunohistochemical staining. Diffuse expression of leptin receptor in parathyroid carcinoma (ABCx200) (score2+)

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