Leptin receptor expression in neoplastic and normal ovarian and endometrial tissue

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Summary

Purpose: The objective of this study was to investigate the expression of leptin receptors in benign and malignant tumors of the ovaries and endometrium and its association with body mass index (BMI). *Methods:* Histological uterine and ovarian samples of normal and neoplastic tissue from 35 patients aged 37-72 years were examined for the expression of leptin receptors with the method of RT-PCR. T. *Results:* A BMI > 30 was correlated with increased expression of leptin receptors. Both Ra and Rb receptors were expressed in normal and neoplastic tissues. A statistically significant difference in leptin receptor expression was detected between normal and neoplastic tissue, with expression being around 5-fold higher in neoplatic tissue. *Conclusion:* Endometrial neoplasms and long leptin isoform receptor expression were associated with an increased BMI. A role of long isoform in endometrial carcinogenesis is proposed.

Key words: Leptin; Receptor; Cancer; Endometrium; Ovary.

Introduction

Leptin receptor expression is decisive for much of neoplastic cell growth. Leptin acts as a mitogenic factor in normal, as well as in cancer cells [1-3]. There are two identifiable leptin receptors (ObR), the short (ORa) and the long (ObRb) isoforms. Few data regarding leptin receptors exist on female reproductive system tumors in humans [4]. Leptin induces the proliferation of cancer endometrial cells and increases their aggressiveness, as was shown in the drug matrigel in the laboratory [5]. Petridou *et al*. found a strong positive correlation between leptin levels and endometrial cancer in 84 women with histologically proven endometrial carcinoma [6]. In the study of Koda *et al*. both expression of leptin and leptin receptors (long isoform), was found in 30% and 57% of the sample, respectively. Even though no statistically significant correlation was established, there was a tendency of cross-correlation of leptin with local spread and moderately differentiated tumors [4].

The purpose of this study was to investigate the expression of leptin receptors (Ra & Rb isoforms) in benign and malignant ovarian and endometrial tissue.

Materials and Methods

Histological samples from 37 patients aged 37-72 years were collected between the years 2004 and 2007. During scheduled surgery, two samples, 1 cm each, were collected from pathological and physiologic tissue, respectively. The samples revealed 16 cancers of the ovaries, 15 cancers of the endometrium and six uterine fibromyomas. Patients with previous new-adjunct chemotherapy/radiotherapy were excluded. The examination of preparations for the expression of leptin and its receptors was made with the method of RT-PCR. Results are expressed as values related to endogenous expression of standard h-HPRT gene coding the enzyme used in the method. The study included 35 samples, which were eventually suitable for evaluation. Unfortunately, demographic data and BMI values were not available for all patients.

Statistical Analysis

Coding and processing: Initially the variables were coded and derivative variables were also created, e.g., BMI was dichotomized and epidemiologic characteristics of samples were constructed as well as for histological characteristics and serologic markers. The Student's t-test was used for comparisons of parametric variables and the Mann-Whitney U test for ordinal ones. Normality was tested with the Smirnov-Kolmogorov test. Percentiles were used to present non quantitative data. The level of importance was set at 0.05. Statistics were processed with the program SPSS for Windows, v. 13.0.

Results

Subgroups of patients were studied according to BMI distribution, histological type, age, as well as stage and grade of differentiation. The results showed expression of leptin and its receptors (Ra > Rb) in the preparations examined. Mean patient age was 54.80 ± 10.35 and BMI was 31.49 ± 6.43. Mean leptin Ra and Rb concentrations were 0.98 ± 0.72 and 0.02 ± 0.03, respectively (Table 1).

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Table 1. — *Epidemiologic characteristics, serologic and tissue markers of women's samples*.

Variables	Mean ± SD	Max - Min
Age (n = 31)	54.80 ± 10.35	32-75
BMI (n = 28)	31.49 ± 6.43	18.94-50.20
Ra (n = 22)	0.98 ± 0.72	0.05-2.50
Rb (n = 19)	0.02 ± 0.03	0.00-0.43

Table 2. — *Histological classification of neoplasms*.

	Serious	Adeno-carcinoma	Endo-metrioid	Fibro-myoma	Borderline	Other
Uterus	1	9	2	2	0	5
Ovarian origin	9	0	2	0	5	0
Total (n = 35)	10	9	4	2	5	5

Table 3. — *Body mass index (BMI) and ovarian/uterine neoplasms*.

Variables	Mean ± SD
BMI - Ovary (n = 13)	28.73 ± 4.90
BMI - Uterus (n = 14)	33.98 ± 7.03
p	0.035

T-test.

Table 4. — *Differences in the expression of leptin receptors, depending on the size of obesity*.

Receptor	BMI > 30 N	25^{th}-50^{th}-75^{th}	BMI ≤ 30 No.	25^{th}-50^{th}-75^{th}	*p*
Rb	11	0.01 - 0.06 - 0.15	5	0.002 - 0.004 - 0.009	0.052
Rb-n*	14	0.00 - 0.01 - 0.04	7	0.000 - 0.001 - 0.004	0.007

* Normal tissue.

Table 5. — *Differences in expression of leptin receptors in normal and neoplastic tissue*.

Receptor	Neoplastic tissue N	25^{th}-50^{th}-75^{th}	Normal tissue No.	25^{th}-50^{th}-75^{th}	*p*
Rb	19	0.004 - 0.024 - 0.083	26	0.001 - 0.008 - 0.022	0.044
Ra	22	0.36 - 0.97 - 1.25	29	0.06 - 0.17 - 0.44	0.001

Table 6. — *Differences in expression of leptin receptors in neoplastic endometrial and ovarian tissue*.

Receptor	Neoplastic ovarian tissue No.	25^{th}-50^{th}-75^{th}	Neoplastic endometrial tissue No.	25^{th}-50^{th}-75^{th}	*p*
Rb	9	0.005 - 0.02 - 0.05	10	0.008 - 0.07 - 0.35	n.s.
Ra	22	0.66 - 1.06 - 1.45	12	0.29 - 0.53 - 1.45	n.s.

n.s. = non significant.

The majority of neoplasms were adenocarcinomas and serous neoplasms. All adenocarcinomas came from the endometrium, while serous carcinomas were mainly in the ovaries (Table 2). Most neoplasms were of low differentiation (grade 3). Uterine neoplasms were statistically significantly correlated with higher BMI values, compared with ovarian ones (Table 3). A statistically significant difference was traced in normal tissue Rb concentrations depending on BMI. A BMI > 30 was correlated with increased expression of leptin receptors (Table 4). A statistically significant difference in leptin receptor expression was detected between normal and neoplastic tissue, with expression being around 3-5 fold higher in neoplatic tissue (Table 5). In the neoplastic endometrial tissue, as the 75^{th} percentile was approached, the expression of Rb acquired the same size order as Ra and the difference was minimized (Table 6).

Discussion

According to the results of the present study, there was a statistically significant difference in the expression of leptin receptors between normal and neoplastic tissue of the endometrium and ovaries, while endometrial neoplasms were associated with a higher BMI. The knowledge that obesity is combined with increased danger of carcinogenesis has been well established, and obesity treatment has been proposed as a cancer prevention measure [7]. Although obesity is known to be related with endometrial cancer, data on ovarian cancer are not explicit. Recent studies lead to the conclusion that also ovarian neoplasms are associated with obesity. Nevertheless, compared with the endometrium, the relative risk is smaller (1.14 for the ovary compared with 2.89 for endometrial neoplasms) [8, 9].

Gynecological cancers have been associated with leptin blood levels and the density of leptin tissue receptors. Both in normal and neoplastic cells, leptin promotes development, metastasis and infiltration while it enhances angiogenesis. However the role of leptin and its receptors in the development of reproductive system carcinomas remains unclear [6].

In the present study the concentrations of short type, as well long type receptors were calculated, both in normal and neoplastic tissues. There is some indication of receptor over-expression in endometrial cancers. Koda *et al*. did not detect any receptors in normal endometrial tissue with the immunochemistry method, while they traced ObR-b presence in 30% of neoplastic preparations [4]. Yuan *et al*. found no difference in long isoform concentrations between normal and neoplastic tissues, while there was a reduction in short isoform concentrations observed. The concentration of ObR-a was found higher than ObR-b. Whereas no statistical difference was observed between obese and non-obese women as regards ObR-a, the ObR-b concentrations were significantly elevated in obese individuals [10]. Rb expression exhibits variation according to menses and it is the prominent receptor in the endometrium [11]. Sharma *et al*. found that leptin induces enzyme phosphorylation, leading to increased infiltration capacity. Inhibiting the relevant pathways resulted in inhibition of cell infiltration induced by leptin [5]. Carino *et al*. also found that the effects of leptin on proangiogenic molecules involved in endometrial cancer were more evident in malignant versus benign cells [12]. According to the above findings, it could be speculated that some difference in receptor expression in favor of Rb could be responsible for endometrial carcinogenesis. The increase in expression of Rb appears to be

more rapid than the increase in Ra expression. This event might be of clinical importance because of the different roles the two receptor types play in cellular proliferation. Even though a protective role is preserved for ObR-a, this effect seems to be neutralized, probably due to the ObR-b influence as a result of their higher concentration. As a consequence, the rapid increase of ObR-b together with some lowering in ObR-a concentration might account for carcinogenesis.

Conclusions

Endometrial neoplasms were associated with increased BMI and obesity was associated with increased expression of long leptin isoform receptors in normal tissue. In neoplastic tissue an increase in ObR-b was observed, approaching the same size order of ObR-a. These results support an important role of long isoforms in endometrial carcinogenesis.

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