Increased Soluble Leptin Receptor Levels Are Associated with Advanced Tumor Stage in Colorectal Cancer Patients

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Abstract. *Background: The leptin receptor is involved in modulating leptin activity, acting as a carrier protein. A link between leptin or leptin receptor and cancer development has been proposed and here, the hypothesis that leptin and its receptor might be implicated in colorectal cancer (CRC) progression and invasion was investigated. Patients and Methods: A total of 71 consecutive patients with CRC were enrolled in the study. Serum leptin and leptin receptor levels were evaluated by commercial ELISA kits. Results: The multinomial logistic regression model showed a positive association of leptin and leptin receptor with advanced tumor stages, which was significant for the leptin receptor in stage IV of disease. Conclusion: High circulating levels of leptin receptor occur in patients with advanced stage of colon cancer, suggesting a role for leptin in cancer progression and aggressiveness.*

Leptin is a 16-kDa polypeptide involved in the regulation of food intake and body composition (1) and it is an important signal of fat stores (1, 2). Circulating leptin levels are down-regulated by fasting and increased by refeeding and inflammatory mediators (3, 4). Leptin exerts its physiological action through the leptin receptor.

Leptin receptors are members of the cytokine receptor class I superfamily (5) and a soluble isoform is involved in modulating leptin activity, in fact it can regulate serum leptin concentration acting as a carrier protein (6).

Leptin targets colonocytes and promotes cell proliferation (7), and it has been suggested that this could be a mechanism by which nutrition uptake contributes to colon tumor growth (8). Leptin has been shown to regulate the proliferation and invasiveness of colonic and renal epithelial cells (9), of gastric cancer, as well as of breast cancer (10). Leptin expression has shown a positive correlation with the invasiveness of tumors in pituitary adenomas (11), and the enhanced expression of leptin and its tissue receptor were also correlated with hematogeneous metastasis or recurrence in distant organs (10), and other studies have also indicated that leptin may potentiate cancer cell growth (12,13). However, the precise role of leptin and its receptor in the development and promotion of colorectal cancer (CRC) remains unknown.

In view of the critical role of leptin in cell proliferation, the hypothesis that leptin and its receptor might be implicated in the modulation of CRC progression and invasion was proposed. The serum concentration of leptin and its soluble receptor were evaluated in patients with CRC in order to investigate their involvement in tumor stage and aggressiveness.

Patients and Methods

Patients. A total of 71 consecutive patients (29 females and 42 males) with CRC and without concomitant disease, such as hyperlipidemia, diabetes mellitus, thyroid disease or metabolic syndrome, were enrolled in the study. No patient had subjective complaints of appetite loss, reduced food intake, weight loss or abnormal liver function tests. The body mass index (BMI), expressed as body weight in kg/height in meters squared, was measured for each patient.

Serum levels of leptin and leptin receptor were examined in early morning samples taken before breakfast on days 2-4 before surgery and serum was immediately frozen at –80˚C.

All the participants provided written informed consent.

Leptin assay. Serum leptin levels were evaluated by a commercially available ELISA kit (Leptin ELISA, DBC-Diagnostics Biochem Canada Inc., London, Ontario) following the manufacturer's recommendations. Briefly, calibrators, controls and serum samples were pipetted into a 96-well capture plate in duplicate. Monoclonal anti-leptin-biotin conjugate was added and the plate was incubated for one hour at room temperature on a plate shaker. The plate was then washed three times with wash buffer. Streptavidin-Horseradish peroxidase conjugate was pipetted into each well and the plate was incubated for 30 min at room temperature. Serum leptin levels were visualized by color change upon addition of tetramethyl-benzidine substrate followed by the addition of stopping solution. Absorbance

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Table I. *Demographic characteristics, BMI, tumor location and serum variables in relation to the tumor stage.*

	Tumor stage			
	I+II (n=33)	III (n=30)	IV (n=8)	*P*-value[#]
Gender (Males) (%)	20 (60.6)	18 (60.0)	4 (50.0)	0.86
Age (year) (mean±SD)	69.7±12.6	66.5±11.0	63.4±12.4	0.25
Body Mass Index (kg/m^2) (mean±SD)	26.2±3.4	28.6±7.0	25.6±2.5	0.85
Tumor site* (left side) (%)	23 (69.7)	26 (86.7)	6 (75.0)	0.34
Leptin (ng/mL) (mean±SD)	7.2±8.1	10.8±13.6	10.2±10.6	0.56
Leptin receptor (ng/mL) (mean±SD)	33.3±11.8	33.1±9.7	42.4±18.8	0.40

[#]Chi-square test for categorical variables, Kruskal-Wallis rank test for continuous variables. *Left side: descending colon, sigmoid and rectum; Right side: hepatic flexure, cecum and ascending colon.

Table II. *Most parsimonious multinomial multiple logistic regression model of tumor stage (I+II versus III and I+II versus IV) on leptin and leptin receptor and other potential confounders (backward method).*

	O.R.	S.E.(OR)	*p*-value	95 % C.I.
Tumor Stage: III				
Gender (Males=1)	1.60	1.13	0.51	0.40-3.40
Age (year)	0.97	0.02	0.28	0.93-1.02
Tumor site (Left side=1)	2.24	1.45	0.21	0.63-7.93
Leptin (ng/mL)	1.06	0.04	0.15	0.98-1.14
Leptin receptor (ng/mL)	1.01	0.03	0.58	0.96-1.07
Tumor Stage: IV				
Gender (Males=1)	1.13	1.29	0.91	0.12-10.53
Age (year)	0.95	0.04	0.20	0.87-1.03
Tumor site (Left side=1)	1.68	2.05	0.67	0.15-18.32
Leptin (ng/mL)	1.09	0.06	0.10	0.98-1.21
Leptin receptor (ng/mL)	1.11	0.05	0.02	1.01-1.20

O.R.: Odds ratios; S.E.: standard error, C.I.: confidence interval.

values were read at 450 nm and leptin concentrations were determined by interpolation from the standard curve. Reported leptin levels were the mean of two determinations of the sample. Sensitivity of the test was 0.50 ng/mL, the intra and inter-assay coefficient of variation was 4.62% and 6.07%, respectively.

Leptin receptor assay. The serum concentration of the leptin receptor was evaluated by an ELISA kit (BioVendor Laboratory, Modrice, Czech Republic) following the manufacturer's recommendations.

Briefly, 100 μL of diluted standard, quality controls, dilution buffer (blank) and serum samples were pipetted into a 96-well capture plate in duplicate. The plate was incubated for one hour at room temperature and then washed five times with wash solution. One hundred μL of monoclonal anti-human leptin receptor antibody conjugated with horseradish peroxidase were added into each well. The plate was incubated for one hour at room temperature and the wash was repeated. Substrate solution was pipetted into each well and the color development was stopped by adding stop solution. The absorbance value was read at 450 nm using a spectrophotometer. Human leptin receptor concentrations were determined by interpolation from the standard curve and the expressed data were the mean of two determinations of the sample. The intra and inter-assay coefficient was 6.95% and 6.35%, respectively.

Statistical analysis. To evaluate the differences among the tumor stages on the individual variables examined, sex, age, BMI, tumor site, leptin, and leptin receptor, the Chi-square test and Kruskal-Wallis rank test were used where appropriate.

A multinomial multiple logistic regression model was used to determine if the serum levels of leptin and leptin receptor were associated with tumor stage, after correction for sex, age and tumor site. The threshold for statistical significance was set at $p \leq 0.05$.

Statistical analysis was performed with StataCorp. 2007. Stata Statistical Software: release 10. (College Station, TX, USA).

Results

Table I shows the descriptive statistics of the demographic characteristics, BMI, tumor location, and serum variables in the categories of tumor stage of the enrolled patients. Clinical staging was performed using the staging system of the International Union Against Cancer.

The patients with tumor stage IV had higher serum levels of leptin receptor than the patients with tumor stage I+II or III, although the difference was not statistically significant.

The multinomial logistic regression model (Table II) showed a positive association of leptin and leptin receptor with advanced tumor stages (Stage III and Stage IV), after adjustment for sex, age, and location of the tumor. A significant association of the leptin receptor in stage IV of disease was detected.

Discussion

The serum leptin receptor levels increased in association with tumor aggressiveness and were significantly higher in the patients with more advanced tumor stage compared with stage I and II, suggesting that circulating leptin receptor expression could be associated with the presence of metastasis in CRC patients.

Controversial data have been reported for serum leptin concentrations and CRC. Although it has been suggested that circulating levels of leptin were pronounced in the late stages of colon carcinoma (14), other studies (1, 15) found decreased leptin serum levels associated with tumor aggressiveness or did not observe any significant differences in agreement with the present results.

The expression of leptin and its receptor have been demonstrated to correlate with grade of tumor differentiation, depth of bowel wall invasion, Dukes' stage and distant metastasis in CRC patients, suggesting that the binding of leptin to its cellular receptor promotes the proliferation of CRC (13).

Although the physiological function of the soluble leptin receptor has not been fully elucidated, the present study provided evidence of high circulating levels of this receptor in patients with advanced stage of disease. The overexpression of the leptin receptor in serum could cause an increase of leptin bioactivity or a reduction of leptin clearance due to association with the high molecular binding protein. Thus, a new role for leptin in cancer susceptibility may involve stimulating the invasive capacity of colonic epithelial cells. The present results have potential clinical implications for colon cancer progression and the management of CRC patients.

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