

·Original Article·

## Clinical significance of the leptin and leptin receptor expressions in prostate tissues

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### Abstract

**Aim:** To evaluate the expression of leptin and leptin receptor in benign prostatic hyperplasia (BPH) and prostate cancer (PCa), and to investigate whether they are associated with the development and progression of PCa. **Methods:** Immunohistochemical staining was performed to examine the expression of leptin and leptin receptor in BPH and PCa. PCa was divided into three groups: localized PCa, locally advanced PCa and metastatic PCa. The positive staining was identified and the percentage of the positive staining was graded. We also assessed the relationship between both the Gleason score and body mass index (BMI) and PCa. **Results:** The percentage of the leptin expression in PCa was significantly higher than that in BPH ( $P < 0.01$ ). For the PCa group, the expressed levels of leptin showed a considerable correlation with localized PCa and metastatic PCa ( $P < 0.05$ ). Leptin receptor, however, did not reveal a definite difference between BPH and PCa. The expression of leptin indicated a significant difference between well-differentiated PCa (Gleason score  $\leq 6$ ) and poorly differentiated PCa (Gleason score 8–10) ( $P < 0.05$ ). The relation between the leptin expression level in PCa and the BMI was not remarkable ( $P = 0.447$ ). **Conclusion:** Our results suggest that leptin might have a promoting effect on the carcinogenesis and progression of PCa. (*Asian J Androl* 2008 Nov; 10: 923–928)

**Keywords:** leptin; leptin receptor; prostatic neoplasms

### 1 Introduction

As people's diets and lifestyles have changed in the process of development, obesity has become a significant issue, in Asia as well as in the rest of the world. Obesity is related to health care as well as to social

problems. It causes many pathophysiological problems in the human body, and it is closely linked to coronary arterial diseases, diabetes, hypertension, malignancy, as well as various other diseases [1, 2]. Adipose tissue produces diverse cytokines and hormones; therefore, it influences the metabolism of lipids and carbohydrates. Cytokines secreted from adipose tissue, such as leptin, adiponectin or resistin, are called adipokines [3]. It is well known that obesity and insulin resistance cause metabolic syndrome. Moreover, several studies concerned with the relation between other diseases and the secretion of cytokines and hormones are now underway.

Leptin is a hormone secreted from adipose tissue

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that affects the ingestion of food and body weight [4]. Furthermore, it plays a role in keeping the energy balance in the body by stimulating the generation of heat through activation of the sympathetic nerve system [5]. It also has a role in reproduction, hematogenesis, neovascularization, wound healing and secretion of insulin, and it increases the blood concentration of male hormones, such as testosterone [6, 7]. According to a recent study, the higher the blood leptin concentration, the greater the negative effect on cellular differentiation and cancer progression in prostate cancer (PCa) [8]. It is also known that leptin stimulates the cellular proliferation of benign prostatic hyperplasia (BPH).

It has been reported that the blood concentration of leptin, as well as the amount of leptin in the tissue, is related to the progression of several cancers [9, 10]. It was revealed in a study regarding leptin and leptin receptor expression in breast cancer and stomach cancer tissues that leptin is connected with the progression and metastasis of cancer. However, there is no study considering how leptin and leptin receptor in BPH tissue and PCa tissue are expressed, and what functions leptin and leptin receptor perform on the progression of cancer. Therefore, in the present study, we investigate the expression of leptin and leptin receptor using BPH tissue and PCa tissue that were acquired from operations or biopsies.

## **2 Materials and methods**

### *2.1 Patients*

The present study was conducted on patients who visited the Chung-Ang University Hospital in Korea from November 2005 to October 2006. A total of 26 patients were diagnosed with BPH and 30 patients were diagnosed with PCa. The prostate-specific antigen (PSA) blood concentration of the patients with BPH was 4 ng/mL and below. Tissues of BPH and PCa were obtained by performing transurethral prostatectomy or suprapubic prostatectomy. The PCa patients were divided into three groups. Those patients with PCa without capsular invasion and no lymphatic or bone metastasis were categorized as the localized PCa group. The second group was defined as those patients with locally advanced PCa: this group did not show lymphatic or other organ metastasis; however, their cancer extended beyond the prostate capsule and it had invaded the tissues around it. The last group included those patients for whom their PCa was found to metastasize to other organs, such as lymphatic

glands, bones and lungs, regardless of the capsule invasion of cancer: this was the metastatic PCa group. The number of patients with local PCa was 12. Of these, six had locally advanced PCa. The tissue acquired from radical prostatectomy or prostate checkup was used for the experiments. Twelve patients with metastatic PCa were not operable for operation, so their tissue samples were acquired from prostate biopsies, and they were diagnosed at that time.

### *2.2 Immunohistochemistry*

Immunohistochemistry for leptin and leptin receptor was conducted on the tissue samples of the study patients. Sections that were 4–5  $\mu\text{m}$  thick were cut from each paraffin-embedded tissue block and then the sections were deparaffined. In turn, they were dipped in a series of graded anhydrous alcohol solutions (90%, 75% and 50%) and then washed with distilled water for 5 min. To suppress the endogenous peroxidase activity, the sections were dipped in hydrogen peroxide and washed in distilled water for 10 min. For serotype retrieval, the sections were dipped in a buffer solution of 10 mmol/L citrate (pH 6.0) and irradiated in microwaves 2 times for 5 min, cooled at room temperature and washed in 50 mmol/L of Tris buffer solution (TBS, pH 7.5). To stop nonspecific immunohistochemistry reactions, the sections were treated with chlorine serum for 30 min and then washed to remove the rest of the solution. The sections were treated with antibody to leptin (A-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:150 and leptin receptor Ob-R (M18; Santa Cruz Biotechnology) at a dilution of 1:30 at room temperature for 1 night and the sections were then washed out with TBS three times for 5 min each time. After that, the sections were reacted with the second antibody (Zymed, San Francisco, CA USA), attached to biotin for 20 min, and peroxide enzyme complex combined with streptavidin was added for 20 min. Finally, coloring was done with AEC chromogen (3-amino-9-ethylcarbazole) solution and the results were observed with an optical microscope after contrast dyeing with Mayer hematoxylin stain.

### *2.3 Reaction and analysis of results*

The leptin staining was deemed negative when there were no cells dyed a red-brown color among all the cells. The other staining was defined as + if < 25% of the cells were stained positive, as ++ if 25%–50% of the cells were

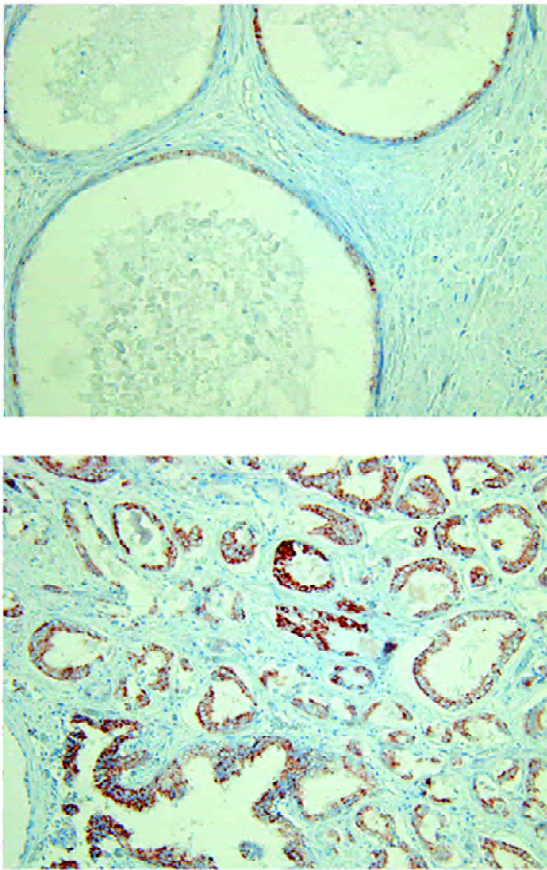


Figure 1. Immunohistochemical stainings for leptin located in epithelial cells ( $\times 100$ ). Benign prostatic hyperplasia (BPH, upper), graded (+); prostate cancer (PCa, lower), graded (++++).

stained positive, as +++ if 50%–75% of the cells were stained positive and ++++ if  $> 75\%$  of the cells were stained positive (Figure 1). The same method was applied to the results of the leptin receptor staining (Figure 2).

We conducted the immunohistochemistry using the above method and we determined the degree of the leptin and leptin receptor expression in the BPH and PCa tissues. After that, we analyzed the differences of expression in each patient group, and we tried to verify the relation between the degree of leptin expression and the body mass index (BMI), which is strongly associated with the amount of adipose tissue that secretes leptin. Following the Korean BMI standards, the patients were divided into groups: those who had a normal weight that was less  $25 \text{ kg/m}^2$ , those who were overweight at  $25\text{--}30 \text{ kg/m}^2$  and those who were obese at over  $30 \text{ kg/m}^2$ . We then compared the difference of the expressions of

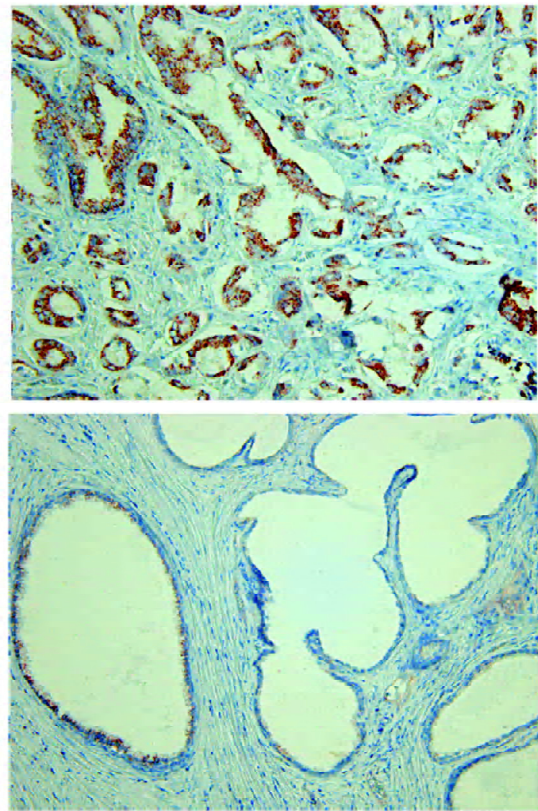


Figure 2. Sections of benign prostatic hyperplasia (BPH) and prostate cancer (PCa) immunostained for leptin receptors ( $\times 100$ ). BPH (lower), graded (+); PCa (upper), graded (++++).

leptin and leptin receptor in each group.

We also analyzed and compared the degree of malignancy of cancer cells with the expression of leptin by classifying the PCa patients according to their Gleason score. We categorized the patients into three groups: the first had a Gleason score of 6 or below, the second had a Gleason score of 7, which indicates moderate differentiation, and the third group had a Gleason score of 8, which indicates a high degree of differentiation. In addition, we compared the degree of expression of leptin and leptin receptor.

#### 2.4 Statistical analysis

The SPSS program version 12.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The difference between the leptin and leptin receptor expressions in BPH and PCa tissues was statistically analyzed using unpaired *t*-test and analysis of variance.  $P < 0.05$  was regarded as statistically significant.

### 3 Results

#### 3.1 Characteristics of the patients

The average age of the BPH patients was  $67.92 \pm 8.27$  years and that for the PCa patients was  $68.23 \pm 7.24$  years, which showed no statistical difference. The average volume of the prostate of the BPH patients, as measured by transrectal ultrasonography, was  $40.28 \pm 20.09$  mL and that of the PCa patients was  $42.30 \pm 19.37$  mL, which showed no significant difference. In terms of the BMI of the two patients groups, the BPH patients' BMI was  $24.05 \pm 2.62$  kg/m<sup>2</sup> and PCa patients' BMI was  $23.92 \pm 2.48$  kg/m<sup>2</sup>: there was no distinctive difference (Table 1).

#### 3.2 BPH and PCa

When comparing the expressions of leptin and leptin receptor in the BPH and PCa patients, in terms of leptin, there were five cases who reacted as negative, 10 as +, eight as ++ and three as +++ among the 26 BPH patients. In contrast, for the PCa patients, one reacted as negative, four as +, 10 as ++ and +++ and five as +++++, which means the expression of leptin was much stronger in the PCa patients ( $P < 0.01$ ). In regard to the expression of leptin receptor in the BPH patients, 13 patients were negative, 10 were +, three were ++ and none were +++

or +++++. In the case of the PCa patients, 11 cases were negative, 13 were +, five were ++ and one was +++; therefore, there were no statistically significant differences between the groups ( $P = 0.226$ ) (Table 2).

#### 3.3 PCa

The tissue staining conducted by dividing the PCa patients into three groups showed that the patients with metastatic PCa had a higher degree of leptin expression in their tissue compared with that of the localized PCa patients ( $P < 0.05$ ). The expression of leptin receptor did indicate the differentiation according to the degree of the progression of cancer ( $P = 0.816$ ) (Table 3).

#### 3.4 Classification of PCa by the Gleason score

When the tissue was classified according to the Gleason score regardless of the progress of cancer, the group with a high Gleason score (a Gleason score 8–10) had higher leptin expression than the low Gleason score group (Gleason score  $\leq 6$ ) ( $P < 0.05$ ) (Table 4). In this case, there was no difference between the two groups, as was the case with expression of leptin receptor.

#### 3.5 Classification of PCa using the BMI

In regard to the BMI, there were 19 cases who had normal weight ( $< 25$  kg/m<sup>2</sup>), 11 were overweight (25–30 kg/m<sup>2</sup>), and nobody was obese ( $\geq 30$  kg/m<sup>2</sup>) among the PCa patients. There was also no statistically significant difference in leptin expression between the normal weight and overweight patients ( $P = 0.447$ ) (Table 5).

Table 1. Baseline characteristics for the patients with benign prostatic hyperplasia or prostate cancer. Values are means  $\pm$  SD. BPH, benign prostatic hyperplasia; PCa, prostate cancer; BMI, body mass index.

	BPH (n = 26)	PCa (n = 30)
Age (year)	67.92 $\pm$ 8.29	68.23 $\pm$ 7.24
Prostate volume (mL)	40.28 $\pm$ 20.00	42.30 $\pm$ 19.37
BMI (kg/m <sup>2</sup> )	24.05 $\pm$ 2.62	23.92 $\pm$ 2.48

### 4 Discussion

Many recent studies have focused on the role of leptin in the body, and, in particular, have examined the relation between leptin and cancer. In one previous study

Table 2. Expression of leptin and leptin receptor in benign prostatic hyperplasia (BPH) and prostate cancer (PCa).

	Leptin			Leptin receptor		
	BPH (%)	PCa (%)	P-value	BPH (%)	PCa (%)	P-value
-	5 (19.2)	1 (3.3)		13 (50.0)	11 (36.7)	
+	10 (38.5)	4 (13.3)		10 (38.5)	13 (43.3)	
++	8 (30.8)	10 (33.3)		3 (11.5)	5 (16.7)	
+++	3 (11.5)	10 (33.3)		0 (0)	1 (3.3)	
++++	0 (0)	5 (16.7)		0 (0)	0 (0)	
Total	26	30	< 0.01	26	30	0.226

Table 3. Expression of leptin and leptin receptor in localized prostate cancer (PCa), locally advanced PCa and metastatic PCa.

	Leptin			P-value	Leptin receptor			P-value
	Localized PCa (%)	Locally advanced PCa (%)	Metastatic PCa (%)		Localized PCa (%)	Locally advanced PCa (%)	Metastatic PCa (%)	
-	1 (8.3)	0 (0)	0 (0)		4 (33.3)	2 (33.3)	5 (41.7)	
+	3 (25.0)	0 (0)	1 (8.3)		5 (41.7)	3 (50.0)	5 (41.7)	
++	4 (33.3)	4 (66.7)	2 (16.7)		3 (25.0)	1 (16.7)	1 (8.3)	
+++	4 (33.3)	1 (16.7)	5 (41.7)		0 (0)	0 (0)	1 (8.3)	
++++	0 (0)	1 (16.7)	4 (33.3)		0 (0)	0 (0)	0 (0)	
Total	12	6	12	< 0.05	12	6	12	0.816

Table 4. The expression of leptin, according to the Gleason score, in prostate cancer. P < 0.05 for the comparison between the Gleason scores ≤ 6 and the Gleason scores 8-10.

Gleason score	-	+	++	+++	++++	Total
≤ 6	0	2	2	5	0	9
7	1	2	6	1	1	11
8-10	0	0	2	4	4	10
Total	1	4	10	10	5	30

Table 5. The expression of leptin, according to the body mass index (BMI), in prostate cancer. P = 0.447 for the comparison between the normal weight group and the overweight group. Normal (BMI < 25 kg/m<sup>2</sup>); overweight (25 kg/m<sup>2</sup> ≤ BMI < 30 kg/m<sup>2</sup>); obese (BMI ≥ 30 kg/m<sup>2</sup>).

BMI	-	+	++	+++	++++	Total
Normal	0	3	6	6	4	19
Overweight	1	1	4	4	1	11
Obese	0	0	0	0	0	0
Total	1	4	10	10	5	30

regarding breast cancer, leptin and leptin receptor were more expressed in breast cancer than in normal breast tissue [9]. That study indicated that the degree of the expressions of leptin and leptin receptor was significantly related to distant metastasis of breast cancer. In the case of stomach cancer, it has been reported that as leptin and leptin receptor are highly expressed, the occurrence of cancer and the malignant degree of it are also greatly affected [10]. In a study of renal cell carcinoma, it is revealed that the increase of the leptin concentration and the expressed degree of leptin receptor in blood is extraordinarily increased with tissue invasion of renal cell carcinoma [11].

There have been studies focusing on the relation between prostate disease and leptin. Lagiou *et al.* [12] reported that there is no special relation between the leptin concentration in blood and the occurrence of BPH and the degree of PCa. In contrast, there is another study that finds that leptin concentration in blood is associated with the testosterone and PSA levels of PCa patients. Yet another report determines that the increase in leptin is related to the occurrence and progress of PCa [13-15]. Stattin *et al.* [16] measured the concentration of leptin and leptin receptor in blood and the expression of

leptin and leptin receptor in PCa tissue, and proved that they are all related to the progression of PCa. The present study shows that the expressed degree of leptin is higher in PCa tissue than in BPH tissue. This leads us to believe that leptin possibly has an influence on the occurrence of PCa. However, in the case of leptin receptor, there was no difference of the expressed degree of leptin receptor between the BPH tissue and the PCa tissue, which is a different result from the previous study [16]. In the present study, the number of patients was small and immunohistochemical staining of normal prostate tissue was not performed. Hence, future studies are needed that include a greater number of patients. In addition, normal prostate tissue should be included in the research.

Although subject to debate, according to a previous epidemiologic study, an increase in BMI affects the increased occurrence of PCa [17]. It is already known that leptin is associated with adipocytes, which are the cells in which leptin is directly formed, and the blood concentration of leptin is different according to the range of obesity. In the present study, although the result was restricted because there were no obese patients included,

and there was not much statistical difference of the leptin expression between the normal weight patients and the overweight patients, as measured by BMI. This indicates that the expression of leptin in PCa tissue is not influenced by the range of obesity, which is unlike the leptin concentration in blood.

The Gleason score divides PCa into five grades according to the range of tissue differentiation, and this is shown in the present study as the sum of the two grades that were most prevalent. The Gleason score is a valuable prognosis factor that can predict capsular invasion of PCa, metastasis to lymph nodes and so on. The tissue with a higher Gleason score is more likely to have a higher expressed level of leptin among the samples of PCa tissue. In contrast, when measuring the degree of leptin expression as categorized into three groups according to the progress of PCa, the metastatic PCa tissue expressed leptin more than that in the localized PCa tissue. These two results suggest that the degree of leptin expression is closely linked with the progression and the degree of malignancy of PCa.

Therefore, the expression of leptin is higher in PCa tissue than in BPH tissue, and for PCa tissue, the lower the degree of differentiation and progression of PCa, the higher the expression of leptin.

The results of this study suggest that an increase in leptin expression in prostate tissue is related to the progression and the degree of malignancy of PCa. These findings support the idea that further studies are needed that focus not only on leptin, but also on other adipokines in regard to the occurrence and progression of BPH and PCa.

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