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LEPTIN INFLUENCES CELLULAR DIFFERENTIATION AND PROGRESSION IN PROSTATE CANCER

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ABSTRACT

Purpose: Several studies have shown a positive association of dietary fat with prostate cancer. Leptin, a peptide hormone that has a role in the regulation of body weight, currently serves as a more accurate biomarker for total body fat. We designed a study to determine whether leptin influences cellular differentiation and the progression of prostate cancer.

Materials and Methods: In this study we investigated serum leptin in 21 patients with prostate cancer, 50 with benign prostatic obstruction and 50 healthy individuals matched for sex, body mass index and age. Patients with cancer were stratified into 2 groups by the disease spread, including groups 1—organ confined and 2—advanced disease, and into 3 groups by the differentiation degree, including groups 3—Gleason sum 2 to 4 or well differentiated, 4—Gleason sum 5 to 7 or moderately differentiated and 5—Gleason sum 8 to 10 or poorly differentiated.

Results: We noted significant differences in serum leptin in the cancer versus control and cancer versus benign prostatic obstruction groups. In addition, in the prostate cancer group serum leptin correlated with prostate specific antigen and biopsy Gleason score. We also observed significant differences in serum leptin in groups 1 versus 2, 3 versus 5 and 4 versus 5.

Conclusion: Leptin may have roles in the development of prostate cancer through testosterone and factors related to obesity. It influences cellular differentiation and the progression of prostate cancer.

KEY WORDS: prostate, prostatic neoplasms, leptin, obesity, testosterone

Prostate cancer is becoming an increasingly important public health problem, particularly in countries with trends toward an aging population in the era of prostate specific antigen (PSA).[1] Although the incidence of latent or microfocal prostate carcinoma is higher compared with a much lower clinical incidence, about the same throughout the world, unrelated to race and increases with age, there is wide variation in the incidence of clinically significant prostate cancer and disease specific mortality rates around the world with lowest rates in Asian countries, while North America and western Europe have the highest rates.[2,3] Furthermore, Chinese and Japanese men who immigrate to the United States subsequently have an increased incidence of and mortality from prostate cancer compared with men in their native countries with the rates increasing steadily as Western diets and lifestyles are adopted in those countries.[4–6]

These epidemiological studies suggest that environmental factors associated with the Western life-style, encompassing a high caloric intake from an energy dense diet, particularly dietary fat and a sedentary life-style, often resulting in obesity, may promote the progression of latent or microscopic prostate cancer to clinically significant and metastatic prostate cancer.[7,8] Moreover, data from case control and cohort studies show a positive association of dietary fat with prostate cancer.[3,9] In addition, several ecological studies show a high correlation of average per capita fat consumption with prostate cancer mortality in a number of countries worldwide.[7–9] Furthermore, numerous animal studies have provided direct evidence that changing the fat composition of diet could modulate the growth rate of already established prostatic tumors.[3,9]

To date a few tools, such as body mass index, have been used to quantify obesity.[6,8,10,11] However, leptin currently serves as a more accurate biomarker for total body fat.[10] Leptin is a circulating hormone controlled by an obese gene that is expressed abundantly, especially in adipose tissue.[7,8,10,11] It is involved in the regulation of food intake, energy expenditure, and the neuroendocrine/reproductive (pituitary, thyroid, adrenal cortex and gonads) and hematopoietic systems.[6–8,11] Like other growth factors, cytokines and neuroendocrine peptides the leptin system consists of receptors, binding proteins and ligands in human serum.[12] Plasma leptin correlates with body fat content in that it is elevated in obesity and decreased in anorexia nervosa.[11] Leptin has a significant role in the development and maintenance of reproductive tissues, including the prostate, it induces angiogenesis in vivo and in vitro, and its receptors are present in the human prostate and vasculature.[7,8] Moreover, neuroendocrine peptides and growth factors are modulators of prostate epithelial cell proliferation, differentiation and invasiveness.[13]

Given the observed association of obesity and dietary fat intake with prostate cancer risk, and the known features and effects of leptin on the prostate we designed a cross-sectional study to determine whether leptin influences cellular differentiation and progression of prostate cancer. We also determined its relationship with totaltestosterone, PSA, free testosterone, estradiol and sex hormone binding globulin. We evaluated plasma leptin and other parameters in a study of patients with prostate cancer diagnosed at our institution.

MATERIALS AND METHODS

Patients. From September 1999 to April 2000, 21 men with a median age of 66 years (range 49 to 84) with prostate cancer who were consecutively diagnosed by transrectal ultrasonography-guided biopsy, 50 with a median age of 63.5 years (range 43 to 84) with clinically benign prostatic obstruction and pathologically nodular hyperplasia diagnosed after transurethral resection of the prostate and 50 healthy male volunteers with a median age of 66 years (range 48 to

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TABLE 1. *Parameters assessed in patients with prostate cancer and benign prostatic obstruction, and controls*

Parameters	Prostate Ca	Benign Prostatic Obstruction	Controls	p Value		
				Ca Vs. Control	Ca Vs. Obstruction	Obstruction Vs. Control
No. pts.	21	50	50			
Mean body mass index ± SE (kg./m.^{2})	25.45 ± 3.08	26.11 ± 3.98	25.91 ± 4.69	0.960	0.528	0.622
Mean leptin (ng./ml.)	27.33 ± 12.50	16.96 ± 6.27	17.55 ± 7.20	<0.001	<0.001	0.775
Mean PSA (ng./ml.)	18.90 ± 14.15	3.17 ± 2.30	3.36 ± 1.96	<0.001	<0.001	0.208
Mean estradiol ± SE (pg./ml.)	29.07 ± 10.48	42.15 ± 16.88	38.27 ± 17.99	0.035	0.002	0.359
Mean totaltestosterone ± SE (ng./dl.)	624.57 ± 138.87	491.70 ± 192.46	498.46 ± 170.53	0.003	0.003	0.738
Mean free testosterone ± SD (pg./ml.)	24.41 ± 9.95	15.17 ± 6.56	17.33 ± 8.26	0.001	<0.001	0.593
Mean sex hormone binding globulin ± SE (nmol./ml.)	43.14 ± 10.96	50.35 ± 16.56	50.70 ± 10.81	0.100	0.118	0.898

78) were enrolled in our study. The size of the study population was based on a power calculation. One of us obtained patient written informed consent form and a blood sample, recorded age, and measured the height and weight of all participants at study enrollment. We calculated the body mass index as weight in kg./height in m.^{2}. None of these patients were on any treatment when blood samples were obtained.

Prostate cancer was staged according to the TNM system by digital rectal examination, PSA at diagnosis, transrectal ultrasonography, biopsy Gleason sum, ultrasound guided biopsy, pelvic computerized tomography, magnetic resonance imaging and radionuclide bone scan. Patients were stratified into 2 groups by disease spread, including groups 1—organ confined and 2—advanced disease, including locally advanced, stage T3N0M0 disease, and into 3 groups by the differentiation degree, including groups 3—Gleason sum 2 to 4 or well differentiated, 4—Gleason sum 5 to 7 or moderately differentiated and 5—Gleason sum 8 to 10 or poorly differentiated. All patients with prostate cancer were treated properly. For study standardization we did not examine the correlation of biopsy with radical prostatectomy specimen grade.

Serum leptin measurement. Serum leptin was measured by radioimmunoassay using a Human Leptin RIA Kit (Linco Research, Inc., St. Louis, Missouri). Test sensitivity was 0.5 ng./ml., and the variation interassay coefficient was 8.3% in 4.9 ng./ml. and 3.4% in 25.6 ng./ml. Blood samples were obtained within 1 week before surgery. After 12 hours of fasting peripheral venous blood was drawn at 8 a.m. Blood samples were placed into tubes with ethylenediaminetetraacetic acid, centrifuged for 20 minutes at 2,000 rpm and the serum was separated. They were stored at −70C until analysis. No patients were on any other drugs that could affect serum leptin. PSA, total and free testosterone, estradiol and sex hormone binding globulin levels were measured by chemiluminescent using a DPC Kit (Diagnostic Products Corp., Los Angeles, California).

Statistical analysis. A commercially available software program was used for statistical analysis. Age, body mass index and baseline leptin in patients and controls were compared by the nonparametric Wilcoxon rank sum W test. Comparison of leptin in patients and controls was done with the Mann-Whitney U test. Leptin in patients with prostate cancer, those with benign prostatic obstruction and controls was compared with the Mann-Whitney U test. In the study of the correlation of leptin with PSA and Gleason score the bivariate Spearman correlation test was used with $p \leq 0.05$ considered statistically significant. Results are shown as the mean ± SE.

RESULTS

There were no statistical significant differences in age, the body mass index or sex hormone binding globulin in any groups. We noted significant differences in serum leptin, PSA, estradiol, total and free testosterone in the cancer versus control and cancer versus benign prostatic obstruction groups ($p < 0.005$, table 1). In addition, in the prostate cancer group serum leptin correlated with PSA and biopsy Gleason score ($r = 0.728$, $p < 0.0001$ and $r = 0.701$, $p < 0.001$, respectively). Also, PSA also correlated with biopsy Gleason sum in the same group ($r = 0.957$, $p < 0.001$).

We observed significant differences in serum leptin, PSA and sex hormone binding globulin in groups 1 and 2 (table 2). There were significant differences in serum leptin, PSA, estradiol and sex hormone binding globulin in groups 3 versus 5 but in only leptin and PSA in groups 4 versus 5 (table 3).

DISCUSSION

Overweight and diet induced obesity, representing an excess of body fat, is highly prevalent due to the diet consumed in Western countries and it has been associated with several common forms of cancer, including hormone dependent tumors such as prostate cancer and breast cancer.[7,9] Although it has been suggested that the impact of obesity on prostate cancer development and progression may involve the androgen action axis since androgen is the most potent mitogen for prostatic cells in vivo, the underlying mechanism and specific dietary risk factors remain unclear.[3,7,9,10,13,14]

Leptin as a marker for obesity. The relationships among body fat, body mass index, testosterone and leptin are complex, and the role of each factor in prostate carcinogenesis has not yet been clearly elucidated.[10] International comparisons and epidemiological studies have repeatedly shown a strong association of prostate cancer with high caloric intake, often resulting in obesity.[3,7,9,13,14] However, determining that obesity is a risk factor for prostate cancer has many difficulties. Most studies have quantified obesity as a body mass index of greater than 30 kg./m.^{2} and there are inherent limitations to using body mass index to characterize body fat composition. Men with the same body mass index usually have different heights and weights and, thus, body mass index provides no information on body fat distribution, which has metabolic implication for steroid hormone balance.[6,10] More interestingly epidemiological studies show that overt obesity, as calculated by body mass index, is not a risk factor but mild overweight may be a risk factor for prostate cancer.[8] In contrast to the disadvantages of using body mass index, an

TABLE 2. *Comparisons of groups 1 and 2*

Parameters	Group 1	Group 2	p Value
No. pts.	11	10	
Mean body mass index ± SE (kg./m.^{2})	25.36 ± 2.60	25.55 ± 3.69	0.863
Mean leptin ± SE (ng./ml.)	19.01 ± 2.72	36.47 ± 12.73	<0.001
Mean PSA ± SE (ng./ml.)	10.49 ± 1.57	28.16 ± 16.15	<0.001
Mean estradiol ± SE (pg./ml.)	26.96 ± 9.53	31.39 ± 11.48	0.387
Mean totaltestosterone ± SE (ng./dl.)	605.36 ± 153.71	645.70 ± 125.13	0.468
Mean free testosterone ± SE (pg./ml.)	23.69 ± 10.36	25.21 ± 23.69	0.705
Mean sex hormone binding globulin ± SE (nmol./ml.)	48.59 ± 11.76	37.16 ± 6.10	0.006

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TABLE 3. *Comparisons of groups 3 to 5*

Parameters	Group 3	Group 4	Group 5	p Value		
				3 Vs. 4	3 Vs. 5	4 Vs. 5
No. pts.	5	9	7			
Mean body mass index ± SE (kg./m.^{2})	25.10 ± 3.28	25.38 ± 1.91	25.78 ± 4.34	0.898	0.935	0.758
Mean leptin ± SE (ng./ml.)	19.52 ± 2.02	27.14 ± 17.05	33.15 ± 6.36	0.699	0.003	0.05
Mean PSA ± SE (ng./ml.)	9.14 ± 1.15	13.61 ± 3.25	32.68 ± 17.60	0.001	0.003	0.001
Mean estradiol ± SE (pg./ml.)	25.48 ± 5.36	25.88 ± 10.67	35.72 ± 10.85	0.797	0.042	0.125
Mean totaltestosterone ± SE (ng./dl.)	532.20 ± 88.84	659.55 ± 155.19	645.57 ± 132.11	0.162	0.123	0.837
Mean free testosterone ± SE (pg./ml.)	22.80 ± 12.40	23.00 ± 7.99	27.38 ± 11.28	0.947	0.432	0.368
Mean sex hormone binding globulin ± SE (nmol./ml.)	46.62 ± 6.17	45.21 ± 14.86	38.01 ± 5.79	0.898	0.03	0.210

advantage of measuring plasma leptin is the ability to evaluate, independent of other aspects of body composition.[10] Because plasma leptin represents the body fat mass and the 2 factors correlate highly, leptin as a biomarker for total body fat may be a more precise measure of fat mass than body mass index.[7]

Leptin and prostate cancer. We noted that increased leptin is significantly associated with testosterone and serum PSA in men with prostate cancer compared with men who have benign prostatic hyperplasia and controls. To date only a few studies have been published on a possible relationship of leptin to prostate cancer.[6,8,10,11] However, the results of these studies are inconsistent.

Stattin et al measured leptin expression in the prostate by immunohistochemistry.[8] They reported that increased leptin is associated with prostate cancer, consistent with our findings, and leptin may be a possible key link between the Western life-style and the transition from a premalignant lesion to overt prostate cancer. Chang et al also reported that a higher concentration of leptin is associated with a greater risk of larger volume tumor, consistent with our findings in the advanced prostate cancer subgroup.[10] Thus, leptin may affect the risk of clinically relevant prostate cancer through testosterone, and factors related to stature and obesity.[10] Hsing et al reported that the association of leptin with prostate cancer risk was not statistically significant.[6] However, they also stated that the association of leptin with prostate cancer risk was confined largely to Chinese men with a waist-to-hip ratio than 0.87, suggesting that leptin may interact with markers related to abdominal obesity, such as sex hormones or insulin-like growth factor I, to increase the risk of prostate cancer.

Lagiou et al detected a weak, inverse but nonsignificant association of elevated leptin with prostate cancer in a heterogeneous patient group.[11] However, if leptin does not exert the same effect at all stages, as suggested by our findings, the design of studies including men diagnosed at different disease stages in a single but heterogeneous prostate cancer group may decrease the detection of an association of leptin with prostate cancer. Therefore, we used 2 study groups, namely localized and advanced prostate cancer stratified by clinical tumor stage to determine the role of leptin in prostate cancer progression. We observed that elevated leptin is significantly associated with advanced disease compared with organ confined disease.

Leptin and the cellular differentiation/progression of prostate cancer. This preliminary study, which to our knowledge is the first investigation of the relationship of leptin to cellular differentiation and progression in prostatic carcinogenesis, shows that elevated leptin is significantly associated with poorly differentiated cancer and extraprostatic cancer compared with well/moderately differentiated cancer and localized disease, respectively. West et al noted that tumor aggressiveness in the older age group increases with increasing fat intake.[15] Similarly, Kolonel et al reported a significant relationship of dietary fat and prostate cancer mortality in men 70 years and older.[16]

Because all prostate cancer foci do not become clinically apparent cancer,[3,7] the hypothesis that elevated leptin influences prostate tumor development, particularly during the later stages of carcinogenesis, is biologically plausible.[10] In vitro and in vivo studies show that leptin, as a proinflammatory cytokine, can promote angiogenesis, an important determinant in the growth and spread of many human cancers, including prostate cancer.[7,8] In addition, leptin may enhance other cytokines and growth factors, particularly vascular endothelial growth factor, which is involved in neoplastic transformation.[17] These data suggest that leptin may be a tumor growth promoter and thus, may stimulate tumor progression by inducing angiogenesis. On the other hand, others have noted that leptin modulates cell proliferation in hematopoietic and embryonic stem cells, and CD4+ human T lymphocytes, and findings suggest that leptin has a role in the neoplastic process of several cancer forms.[17–19] Attoub et al reported that leptin promotes the invasiveness of kidney and colonic epithelial cells through several mechanisms at various stages of neoplastic transformation.[19]

Leptin, testosterone and PSA. We noted that high leptin and testosterone were associated in the case group. However, higher circulating testosterone in prostate cancer cases has not been observed consistently.[10,13] It is now clear that PSA is the most powerful biomarker for detecting prostate cancer. We observed that serum PSA and leptin were significantly parallel in case and in the 2 subgroup analyses (tables 1 to 3). To our knowledge this association has not been reported previously.

The natural history of prostate cancer is influenced by several prognostic factors. These factors were classified into 3 categories according to prognostic importance and usefulness in clinical management with or without support in the literature at the College of American Pathologists Consensus Conference.[20] The high association of elevated serum leptin with advanced and poorly differentiated prostate cancer can be proposed as a component of category III and future research may confirm our study. The possible limitations of our series were the potential confounding of variables, cross-sectional design and small sample size. Confounding was possible but it should be emphasized that our study purpose was to determine possible the relationship of leptin to cellular differentiation and prostate cancer progression.

CONCLUSIONS

The results of our cross-sectional study suggest that leptin has roles in the development of prostate cancer through testosterone and factors related to obesity, and it influences cellular differentiation and prostate cancer progression. These findings indicate a need for further examination of the role of leptin in prostate cancer.

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