Serum resistin levels in benign prostate hyperplasia and non-metastatic prostate cancer: Possible role in cancer progression

D. HOUSA*, Z. VERNEROVA, J. HERACEK[1], P. CECHAK[2], B. ROSOVA, J. KUNCOVA[1], M. HALUZIK[3]

Department of Pathology, Third Faculty of Medicine, Charles University in Prague, Czech Republic; [1]Department of Urology, Third Faculty of Medicine, Charles University in Prague; [2]Department of biochemistry and pathobiochemistry, Third Faculty of Medicine, Charles University in Prague; [3]3rd Department of Internal Medicine, 1st Faculty of Medicine, Charles University in Prague, e-mail: dhous@lf1.cuni.cz

Received: January 15, 2008

Resistin is a member of adipokine family involved in the regulation of inflammatory reactions and insulin sensitivity. In presented study its possible role in the development of benign prostate hyperplasia and prostate cancer was evaluated.

Blood samples and prostate specimens were collected from 26 patients with benign prostate hyperplasia (BPH) and from 42 patients with prostate cancer (PCa) stage pT2 (n=18) and pT3 (n=24). Selected metabolic and biochemical parameters and serum resistin levels were measured and anthropometric measurements were performed as well as tissue immunohistochemistry for resistin.

Serum resistin levels did not differ significantly between benign hyperplasia and prostate cancer but in cancer patients there was a trend towards decrease with higher cancer stage. Moreover, serum resistin levels were significantly lower in patients with seminal vesicle invasion in comparison to those without invasion. While in BPH serum resistin levels correlated with insulin resistance, inflammatory status and cortisol, in PCa positive correlation with F/T PSA ratio and cortisol was observed. Tissue immunohistochemistry did not show any differences in staining pattern between benign and neoplastic prostate tissue.

We conclude that serum resistin levels do not significantly differ between patients with benign prostate hyperplasia and prostate cancer, but there is a trend towards decrease in resistin serum levels in advanced cancer cases.

Key words: Immunohistochemistry, inflammation, insulin resistance, prostate cancer, resistin

Introduction

Obesity and diet rich in saturated fat is associated with several malignancies, including prostate cancer. As prevalence of both entities increases in Western countries, it is not surprising that the rate of prostate cancer has been continuously increasing [1–3].

Adipose tissue produces a vast array of biologically active molecules acting by both paracrine and endocrine fashion [4]. Adipocyte-derived factors commonly referred to as adipokines may represent a possible link between obesity and cancer development as is known from studies in breast, endometrial and colon cancer [5]. It has been mainly leptin and adiponectin that were demonstrated to participate in the development and progression of different forms of malignancies, including prostate cancer [6–10] while the role of resistin in cancerogenesis remains less clear.

Resistin is a member of cystein-rich proteins that has been the subject of much controversy concerning its role in the pathogenesis of obesity-induced insulin resistance and type 2 diabetes mellitus. Experimental data suggested that recombinant resistin administration induced insulin resistence and that hyperresistinemia contributed to impaired insulin sensitivity in obese rodents [11]. In contrast to rodents, the physiological role of resistin in humans is unclear. This fact is not surprising as there is not a direct homology between resistin in rodents and humans and additionally humans lack one of three murine isoforms [12]. In humans, resistin de-

* Corresponding author

BPH benign prostate hyperplasia, BMI body mass index, CRP C-reactive protein, cT2 clinical stage T2, F/T PSA free/total PSA ratio, FAI free androgen index, HDL high-density lipoproteins, LDL low-density lipoproteins, PCa prostate cancer, PSA prostate specific antigen, pT2, pT3 histopathological stage T2,T3, SHBG sex-hormone binding globulin, SVI seminal vesicle invasion

rives primarily from stromavascular fraction of adipose tissue and immunocompetent cells of peripheral blood and its role probably lies in the regulation of inflammatory reactions associated with obesity rather than insulin sensitivity [13]. Several studies in humans have highlighted resistin expression in adipose tissue, particularly in abdominal depots [14], though mRNA expression in isolated mature adipocytes is low in comparison with pre-adipocytes. Concerning its role in cancerogenesis, resistin was recently reported to contribute to *in vitro* human choriocarcinoma cell invasiveness and to the control of angiogenesis [15] and increased serum resistin levels were described in breast cancer patients [16].

We hypothesized that resistin could be involved in the development of prostate cancer. To evaluate this we measured serum resistin levels and tissue expression in well-characterized group of patients that underwent simple prostatectomy for benign prostate hyperplasia (BPH); suprapubic radical prostatectomy for organ-confined (pT2) or locally advanced (pT3) prostate cancer (PCa), respectively and studied the relationship of resistin levels with tumor stage, grade and selected hormonal, metabolic and biochemical parameters.

Patients and methods

Study population. Sixty-eight men referred to undergo either simple prostatectomy for benign prostate hyperplasia or radical prostatectomy for prostate cancer of pre-operative clinical stage T2 were enrolled to the study. Of those, 26 patients had benign prostate hyperplasia, 18 patients had organ-confined (pT2) and 24 patients locally advanced disease (pT3), respectively. A complete medical history and physical examination were performed and the patients' medication was recorded. None of the patients had previously actinotherapy, chemotherapy, hormonal treatment, including androgen deprivation therapy or suffered from an acute illness. The study protocol was approved by the local ethical committee of Faculty Hospital Kralovske Vinohrady. All participating subjects were informed about the purpose of the study and provided written informed consent.

Anthropometric measurement and hormonal and biochemical analysis. BMI was calculated as the weight in kilograms divided by the height in square meters. Peripheral venous blood was collected after an overnight fasting in the morning of the day of surgery between 6:00am and 7:00am into tubes with EDTA and centrifuged for 20 minutes at 2000 rpm. The serum was separated, aliquoted and kept frozen at -80°C until further analysis. Serum resistin levels were measured using commercial ELISA kit (Biovendor, Brno, Czech Republic). Sensitivity was 1.0 ng/ml, and the intra- and interassay variability were 1.78% and 9.25%, respectively. Serum total PSA and free PSA levels, cortisol, testosterone, SHBG, FAI, DHEAS, estradiol, progesterone, LH, FSH, triglycerides, high-density lipoprotein, cholesterol, low-density lipoprotein and total cholesterol were measured in the Department of Biochemistry, Teaching Hospital Královské Vinohrady, Prague, by standard laboratory methods. Assays for each parameter were performed in one batch to reduce interassay variability. Fasting levels of serum glucose (G_0) and insulin (I_0) were measured and HOMA-IR index was calculated [HOMA-IR = fasting insulin (μU/mL) x glucose (mmol/L)/22.5]. Atherogenic index was calculated as serum total cholesterol divided by serum HDL levels.

Histopathologic characteristics of prostate specimens. Each specimen of radical or simple prostatectomy was fixed in 4% buffered formaldehyde, totally embedded and processed as complete sampling with routine sections. All specimens were examined at the Department of Pathology, Teaching Hospital Královske Vinohrady, Prague. Tumor grading according to the Gleason grading scheme and pathological staging based on UICC TNM Classification of Malignant Tumours, Sixth edition staging manual (Czech edition 2004) were performed. The patients in pT2 group were followed-up for up for up to 30 months and none of the subjects reported recurrence, biochemical relapse or metastatic disease development.

Immunohistochemistry. Five-micron-thick sections cut from formalin-fixed, paraffin-embedded tissue samples were deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was inhibited by 3% H_2O_2 in methanol for 30 minutes followed by 15 minutes rinsing in tap water. Non-specific reactivity was avoided by pre-treatement sections for 2 hrs with 1% normal goat serum (Dako Cytomation, Glostrup, Denmark) with 1% bovine fetal albumine diluted in ChemMate Antibody Diluent (Dako Cytomation, Glostrup, Denmark). The slides were incubated with resistin rabbit antiserum (Phoenix Pharmaceuticals, Inc., California, USA), diluted to 1:750 with ChemMate Antibody Diluent (Dako Cytomation, Glostrup, Denmark) for 1 hour at room temperature. The Histofine® kit (Nichirei, Tokyo, Japan) was used to visualize sections incubated with primary antibody. The chromogen 3,3-diaminobenzidine (Liquid DAB+Substrate, Dako Cytomation, Glostrup, Denmark) was applied to all sections and counterstaining was performed with Mayer's hematoxylin. Tissue sections incubated either without primary antibody or with normal rabbit immunoglobulin fraction (Dako Cytomation, Glostrup, Denmark) were used as negative controls.

All sections were analyzed using Nikon Eclipse E600 microscope in a random order by two pathologists who were unaware of clinical data.

Statistical analysis. The mean values of the various parameters studied were calculated and compared between groups using a two-tailed independent sample *t*-test or ANOVA as appropriate. The correlations among variables were analyzed using Spearman's correlation coefficients (*rho*). Two-sided Fishers exact test was used to determine the relationship between resistin immunostaining and the investigated clinicopathological factors. Statistical analyses were performed using SigmaStat (Jandel Scientific, USA) statistical package. Statistical significance was defined as a two-sided $***P \leq 0.001$, $** P \leq 0.01$ and $*P \leq 0.05$, respectively and data were reported as mean ± SD.

Results

Hormonal and biochemical characteristics of BPH and combined organ-limited and locally advanced PCa group (PCa = pT2+pT3) are shown in Tables 1 and 2. We did not find a significant difference between the latter and former group in terms of BMI, total and free PSA but there was a statistically significant increase in F/T PSA ratio (0.22 ± 0.20 vs. 0.10 ± 0.06, P < 0.001) and serum cortisol levels (378.10 ± 222.38 vs. 488.81 ± 173.15, P = 0.002) in PCa in comparison to BPH patients. Moreover, age in the BPH was biased towards advanced age when compared with PCa group. When the patients in "combined" PCa group were subdivided into pT2 and pT3 stages, the only difference encountered between both stages was increased total PSA level in pT3 (pT2 vs. pT3, 6.24 ± 2.17 vs. 11.55 ± 10.10, P = 0.04).

Patients with BPH and PCa did not differ significantly with respect to serum resistin levels (7.09 ± 2.83 vs. 7.33 ± 4.98, P = 0.80) and neither did after stratification for organ-confined and locally advanced prostate cancer (pT2 vs. pT3, 6.90 ± 2.67 vs. 6.07 ± 2.76, P = 0.34). Similarly, we did not observe any difference in PCa group with respect to serum resistin levels between low-grade and high-grade disease (pathological Gleason sum 7 or greater) (7.64 ± 5.03 vs. 6.77 ± 5.01, P = 0.59), the presence or absence of vascular invasion (6.21 ± 3.00 vs. 6.47 ± 2.70, P = 0.62) but we noticed the statistically significant difference between the cases with (n=13) and without (n=33) seminal vesicle invasion (SVI) (5.04 ± 2.03 vs. 6.91 ± 2.79, P = 0.049) (Fig.1). When comparing both groups with and without SVI in terms of CRP (3.35 ± 2.74 vs. 5.48 ± 6.54, P = 0.15) and cortisol (437.46 ± 210.31 vs. 508.70 ± 163.53, P = 0.26), there was no statistically significant difference.

Table 1. Anthropometric and biochemical parameters in patients with benign prostate hyperplasia and prostate cancer.

	Benign prostate hyperplasia (n=26)	Prostate cancer (n=42)	P
Age (years)	70.73 ± 8.62	63.50 ± 4.70	< 0.001**
BMI (kg/m²)	27.47 ± 3.31	27.36 ± 3.09	0.85
Serum resistin (ng/ml)	7.09 ± 2.83	7.33 ± 4.98	0.34
Total PSA (ng/ml)	8.02 ± 6.83	9.40 ± 8.58	0.23
Free PSA (ng/ml)	1.37 ± 2.07	0.91 ± 0.79	0.17
F/T PSA (ng/ml)	0.22 ± 0.20	0.10 ± 0.06	< 0.001 ***
Cortisol (nmol/l)	378.10 ± 223.38	488.81 ± 173.15	0.002 **
CRP (mg/l)	7.06 ± 13.63	5.42 ± 6.48	0.27
Glucose (mmol/l)	5.78 ± 2.42	5.28 ± 1.81	0.18
Insulin (mUI/l)	5.22 ± 4.52	4.94 ± 4.11	0.75
Cholesterol (mmol/l)	4.62 ± 1.07	5.08 ± 0.91	0.10
LDL (mmol/l)	2.82 ± 0.79	3.12 ± 0.67	0.16
HDL (mmol/l)	1.14 ± 0.31	1.24 ± 0.23	0.16
Triglycerides (mmol/l)	1.46 ± 0.68	1.67 ± 0.76	0.33
HOMA IR index	1.59 ± 2.37	1.36 ± 1.97	0.59
Athero index	4.16 ± 0.80	4.18 ± 0.72	0.90

Data presented as mean ± SD, *** P < 0.001, ** P < 0.01

Table 2. Anthropometric, clinico-pathological, metabolic and biochemical parameters in patients with organ-confined (pT2) and advanced prostate cancer (pT3) group.

	pT2 (n=18)	pT3 (n=24)	P
Age (years)	62.50 ± 5.14	64.42 ± 4.33	0.35
BMI (kg/m²)	27.07 ± 3.21	27.50 ± 2.10	0.57
Serum resistin (ng/ml)	6.90 ± 2.67	6.07 ± 2.76	0.32
Total PSA (ng/ml)	6.24 ± 2.17	11.55 ± 10.10	0.04*
Free PSA (ng/ml)	0.72 ± 0.45	1.03 ± 0.98	0.82
Cortisol (nmol/l)	507.35 ± 185.25	477.00 ± 174.50	0.30
CRP (mg/l)	5.18 ± 5.89	4.72 ± 5.91	0.93
Glucose (mmol/l)	5.57 ± 2.27	5.10 ± 1.39	0.37
Insulin (mUI/l)	5.25 ± 4.12	4.62 ± 4.18	0.42
Cholesterol (mmol/l)	4.82 ± 0.95	5.18 ± 0.81	0.18
LDL (mmol/l)	2.98 ± 0.76	3.14 ± 0.56	0.45
HDL (mmol/l)	1.17 ± 0.24	1.29 ± 0.22	0.10
Triglycerides (mmol/l)	1.60 ± 0.64	1.69 ± 0.87	0.95
HOMA IR index	1.58 ± 2.51	1.17 ± 1.56	0.31
Athero index	4.19 ± 0.66	4.11 ± 0.76	0.73

Data presented as mean ± SD, HDL – high-density cholesterol, LDL – low-density cholesterol, CRP – C-reactive protein * P < 0.05

When the patients with prostate cancer were divided into the two groups based on median value of plasma resistin concentration (5.8 ng/ml) a P trend with decreased levels of free PSA in a group of serum resistin concentration < 5.8 ng/ml was observed (0.67 ± 0.42 vs. 1.12 ± 1.02, P = 0.07) but not in other parameters monitored. When prostate cancer group was divided into pT2 and pT3 TNM classification substages, there was a P trend towards the decrease of serum resistin levels in advanced cancer stage (r = -0.28, P trend = 0.08). Additionally, statistically significant correlation of disease stage with PSA levels, Gleason sum and local aggressiveness factors (capsule penetration and/or SVI) was observed (data not shown).

No statistically significant differences between all studied groups were found with respect to BMI, fasting plasma glucose, serum insulin levels, HOMA IR and lipid metabolism parameters.

In the population of men with BPH, resistin levels positively correlated with HOMA IR (rho 0.49, P = 0.02), serum insulin concentration (rho 0.47, P = 0.03) and CRP (rho 0.57, P = 0.003), while no such relationships were found in prostate cancer patients. In PCa patients, positive correlation was found with F/T PSA ratio (rho 0.37, P = 0.02). In both BPH and PCa, a positive correlation with cortisol was found (rho 0.58, P = 0.01 and rho 0.37, P = 0.02, respectively). No correlation between BMI and serum resistin levels was found in our study.

Tissue immunostaining with rabbit anti-resistin antiserum showed cytoplasmatic positivity both in benign and malignant prostate glands and in stromal elements (i.e. smooth muscle cells). Comparable staining intensity in normal and tumorous samples was observed in all examined structures. There was no obvious association between staining intensity and histological grade of tumor (data not shown).

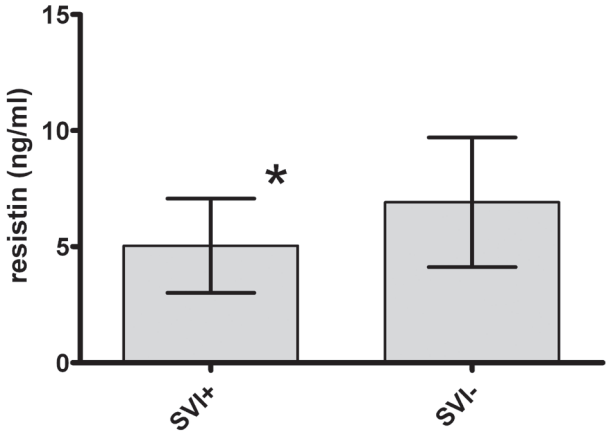

Figure 1. Serum resistin concentrations in patients with seminal vesicle invasion (SVI+) relative to patients without seminal vesicle invasion (SVI-). Results are expressed as means ± SD. * P < 0.05.

Discussion

Obesity is a well-established risk factor for the development of several types of malignancies. Adipose tissue is the source of several hormonally active substances that can participate in the process of cancerogenesis by stimulating growth, migration and invasion of tumor cells both *in vitro* and *in vivo* [5]. While adiponectin and leptin have been extensively studied so far, the role of resistin is less clear. Resistin was originally proposed to be a link between obesity and insulin resistance/diabetes based on studies in rodents. However, its role in humans seems to be different and lies probably in the regulation of inflammatory processes rather than in insulin sensitivity [17].

Here we show that circulating resistin levels did not differ significantly between patients with benign prostate hyperplasia and prostate cancer. In prostate cancer, no relation of resistin serum levels to disease grade was observed but we found a statistically significant decrease of resistin serum levels in patients with SVI. This observation is of particular interest as SVI is a negative prognostic finding that confers a high rate of prostate cancer recurrence [18]. This finding is in accordance with observed trend of decreasing serum resistin levels with advancing prostate cancer stage. As the similar trend with disease stage was not observed in CRP, cortisol or HOMA-IR we may speculate that this finding is directly attributable to cancer growth rather than to secondary general metabolic changes. Moreover, positive correlation was observed between F/T PSA ratio and serum resistin levels which further supports the previous findings. Our results are opposite to those described recently in Korean breast cancer patients [16] and it might be speculated that it reflects enhanced metabolic turnover/hypercatabolic status in advanced stage of tumor. The similar trend toward decrease of serum resistin levels was observed in hyperthyroidism [19] and anorexia nervosa patients [20]. Moreover, in our study serum resistin levels in patients with benign hyperplasia positively correlated with CRP and HOMA IR, the finding that was not observed in patients with prostate cancer. In both groups the correlation with cortisol as the marker of stress conditions was observed.

Our findings show that resistin´s role in metabolism, stress and inflammation at least partially overlaps in both cancerous and non-cancerous patients and that there is a possible role for resistin in cancer progression. Taken together, our data suggest that similarly to serum leptin and adiponectin levels, resistin might participate in prostate cancer progression.

This work was supported by the Research Project of MZO VFN2005

References

[1] HARDING MA, THEODORESCU D. Prostate tumor progression and prognosis. interplay of tumor and host factors. Urol Oncol 2000; 5: 258–264.

[2] ROTH J, QIANG X, MARBAN SL et al. The obesity pandemic: where have we been and where are we going? Obes Res 2004; 12 Suppl 2: 88S–101S.
[3] SONN GA, ARONSON W, LITWIN MS. Impact of diet on prostate cancer: a review. Prostate Cancer Prostatic Dis 2005; 8: 304–310.
[4] Matsuzawa Y The metabolic syndrome and adipocytokines. FEBS Lett 2006; 580: 2917–2921.
[5] HOUSA D, HOUSOVA J, VERNEROVA Z et al. Adipocytokines and cancer. Physiol Res 2006; 55: 233–244.
[6] FREEDLAND SJ, SOKOLL LJ, MANGOLD LA et al. Serum leptin and pathological findings at the time of radical prostatectomy. J Urol 2005; 173: 773–776.
[7] BAILLARGEON J, PLATZ EA, ROSE DP et al. Obesity, adipokines, and prostate cancer in a prospective population-based study. Cancer Epidemiol Biomarkers Prev 2006; 15: 1331–1335.
[8] GOKTAS S, YILMAZ MI, CAGLAR K et al. Prostate cancer and adiponectin. Urology 2005; 65: 1168–1172.
[9] MICHALAKIS K, WILLIAMS CJ, MITSIADES N et al. Serum adiponectin concentrations and tissue expression of adiponectin receptors are reduced in patients with prostate cancer: a case control study. Cancer Epidemiol Biomarkers Prev 2007; 16: 308–313.
[10] HOUSA D, VERNEROVA Z, HERACEK J et al. Adiponectin as a potential marker of prostate cancer progression: studies in organ-confined and locally advanced prostate cancer. Physiol Res 2008; 57:
[11] STEPPAN CM, LAZAR MA. The current biology of resistin. J Intern Med 2004; 255: 439–447.
[12] ARNER P Resistin: yet another adipokine tells us that men are not mice. Diabetologia 2005; 48: 2203–2205.
[13] KERSHAW EE, FLIER JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89: 2548–2556.
[14] McTERNAN PG, MCTERNAN CL, CHETTY R et al. Increased resistin gene and protein expression in human abdominal adipose tissue. J Clin Endocrinol Metab 2002; 87: 2407.
[15] DI SIMONE N, DI NICUOLO F, SANGUINETTI M et al. Resistin regulates human choriocarcinoma cell invasive behaviour and endothelial cell angiogenic processes. J Endocrinol 2006; 189: 691–699.
[16] KANG JH, YU BY, YOUN DS. Relationship of serum adiponectin and resistin levels with breast cancer risk. J Korean Med Sci 2007; 22: 117–121.
[17] HALUZIK M, HALUZIKOVA D. The role of resistin in obesity-induced insulin resistance. Curr Opin Investig Drugs 2006; 7: 306–311.
[18] POTTER SR, EPSTEIN JI, PARTIN AW. Seminal vesicle invasion by prostate cancer: prognostic significance and therapeutic implications. Rev Urol 2000; 2: 190–195.
[19] IGLESIAS P, ALVAREZ FIDALGO P, CODOCEO R et al. Serum concentrations of adipocytokines in patients with hyperthyroidism and hypothyroidism before and after control of thyroid function. Clin Endocrinol (Oxf) 2003; 59: 621–629.
[20] DOSTALOVA I, SMITKA K, PAPEZOVA H et al. Increased insulin sensitivity in patients with anorexia nervosa: the role of adipocytokines. Physiol Res 2007; 56: 587–94.