



# Orexin expression in different prostate histopathologic examinations: Can it be a marker for prostate cancer? A preliminary result

## *Farklı prostat histopatolojilerinde oreksin varlığı: Prostat kanserinde bir belirteç olabilir mi? Ön çalışma*

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### ABSTRACT

**Objective:** The aim of this study was to evaluate the expression of the orexin receptor in different prostate pathologies, including prostate adenocarcinoma, benign prostate hyperplasia and chronic prostatitis.

**Material and methods:** A total of 90 patients (mean age 64.01±7.2 years) were enrolled in the study. The patients were divided into three groups of equal numbers based on their histopathologic findings: prostate cancer (Group 1), benign prostate hyperplasia (Group 2) and chronic prostatitis (Group 3). All the tissues were incubated with a primary antibody recognizing the Orexin receptor. The specific cytoplasmic immunoreactivity of the Orexin receptor was semiquantitatively scored for intensity and distribution based on a grading scale. The staining intensity and orexin expression were evaluated using Pearson  $\chi^2$  test.

**Results:** A heterogeneous staining pattern of the Orexin receptor was observed between the groups. The expression rates were 90% (27/30) in Group 1, 53.3% (16/30) in Group 2 and 26.7% (8/30) in Group 3. While 5 patients (9.3%) in Group 1 showed strong staining, all samples from the other 2 groups showed only weak staining. There were significant differences in staining intensity between the three groups. The expression and distribution of the Orexin receptor was more widespread in Group 1 than in the other groups and was higher in patients with poorly differentiated malignancy. However, there was no significant difference based on Gleason score.

**Conclusion:** Orexin receptors are found in human prostate tissues and their expression is widespread in prostate cancer and in patients with a higher Gleason score. Therefore, we believe that Orexin immunoreactivity can be considered to be an indicator of poor prognosis and of poorly differentiated prostate cancer cases.

**Key words:** Orexin A; Orexin B; prostate; prostate cancer.

### ÖZET

**Amaç:** Bu çalışmanın amacı Prostat Adenokarsinomu, Benign Prostat Hiperplazisi ve kronik prostatit dahil olmak üzere farklı prostat patolojilerinde oreksin reseptörlerinin varlığını değerlendirmektir.

**Gereç ve yöntemler:** Toplam 90 hasta (yaş ortalaması 64.01±7.2 yaş) çalışmaya alındı ve histopatolojik bulgularına göre eşit sayıda hasta içermek üzere, Prostat kanseri (Grup 1), benign prostat büyümesi (Grup 2) ve Kronik prostatit (Grup 3) olmak üzere üç gruba ayrıldı. Tüm dokular Oreksin reseptör primer antikoruna ile birlikte inkübe edildi. Oreksin reseptörü spesifik sitoplazmik immünoreaktivitesi yoğunluk ve dağılım için derecelendirme skalasında semikantitatif olarak skorlandı. Boyanma yoğunluğu ve oreksin varlığı Pearson  $\chi^2$  testi ile değerlendirildi.

**Bulgular:** Oreksin reseptör ekspresyonu gruplarda homojen bir boyama örneği göstermedi. Ekspresyon oranı Grup 1'de %90 (27/30), Grup 2'de %53.3 (16/30) ve Grup 3'de %26.7 (8/30) idi. Grup 1'de 5 hasta (%9.3) kuvvetli boyanma gösterirken, diğer iki gruptaki tüm örneklerde sadece zayıf boyanma vardı. Boyanma yoğunluğuna göre üç grup arasında istatistiksel bir fark vardı. Oreksin reseptör varlığı ve dağılımı Grup 1'de diğerlerinden daha yaygın ve kötü differansiyasyon grubunda daha fazla idi. Ancak, Gleason skoruna dayalı istatistiksel fark yoktu.

**Sonuç:** Oreksin reseptörleri insan prostat dokularında bulunur ve prostat kanseri ve yüksek Gleason skorunda yaygındır. Bu nedenle, Oreksin immünoreaktivitesinin kötü differansiye prostat kanserinde kötü prognostik kriter olarak kabul edilebileceğini düşünmekteyiz.

**Anahtar sözcükler:** Oreksin A; Oreksin B; prostat; prostat kanseri.

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## Introduction

Orexin A and Orexin B are two peptides that derive from a common 130 amino acid precursor peptide, prepro-orexin, by proteolytic cleavage. Orexin A is a 33 amino acid peptide with N-terminal pyroglutamyl residues and two intrachain disulfide bonds, while Orexin B is a linear peptide composed of 28 amino acids. Orexin A and Orexin B exert their actions by binding to and activating two different G protein-coupled receptors, termed Orexin 1 (Ox1R) and Orexin 2 (Ox2R) receptors, respectively.<sup>[1,2]</sup>

Although orexins are primarily expressed in the lateral hypothalamus, recent studies have demonstrated that both the orexins and their receptors are also expressed in the peripheral organs of the gastrointestinal and genital tracts.<sup>[3,4]</sup> Orexin A is expressed in the rat testis, where the peptide appears to play a role in steroidogenesis. In addition, the expression of mRNAs encoding the prepro-orexin and orexin receptors has been reported in the testis, penis, epididymis and seminal vesicles of several mammals.<sup>[4,5]</sup>

A prostate biopsy is the most important diagnostic technique in the differentiation of prostate pathologies and the diagnosis of prostate cancer. Additionally, some staining methods and biomarkers, such as cytokeratin, p63, prostate-derived Ets transcription factor (PDEF) and Erk5, have been described, enabling the histopathologic differentiation of prostate pathologies.<sup>[6-8]</sup> Russo et al.<sup>[9]</sup> first showed the presence of Orexin receptors in the bovine prostate and postulated that it might be related to pathologic conditions. Recently, Malendowicz et al.<sup>[10]</sup> showed ORX2 expression in the human prostate epithelial cells and smooth muscle of benign prostate hyperplasia (BPH) cases. They concluded that orexin might play a role in the pathogenesis and maintenance of BPH. However, little is known about orexin expression in different prostate pathologies.

The aim of this study was to evaluate the expression of orexin receptors in prostate tissue with different prostate pathologies, including prostate adenocarcinoma (PCa), BPH and chronic prostatitis (CP).

## Material and methods

**Patients:** The Human Studies Research Committee approved all procedures (University of Kırıkkale, Faculty of Medicine Local Ethic Committee 2006/053), and all subjects provided written informed consent prior to their inclusion in the study.

All patients who underwent transvesical or retropubic radical prostatectomies were evaluated with a detailed medical history, biochemical analysis, Prostat Specific Antigene (PSA) level,

complete blood count, urine samples and urine culture in the urology polyclinic. Patients with acute urinary infection, cardiac valve prosthesis, diabetes mellitus, rectal stenosis or any systemic disease, obesity (29.9 kg/m<sup>2</sup>) or any medication due to chronic disease were excluded from the study.

After exclusion based on the abovementioned criteria, a total of 90 patients (mean age 64±7.2, range 47-76) were enrolled in the study. The patients were divided into three groups of equal numbers based on their histopathologic findings as follows: Group 1= PCa; Group 2=BPH and Group 3=CP.

## Pathological Examination

**Immunohistochemical staining:** All the tissues, which were formalin fixed and paraffin embedded, were cut into 3-4 μm sections, deparaffinized, rehydrated and subjected to microwave pretreatment using 0.001 mol/lit sodium citrate buffer. All the slides were incubated with 3% hydrogen peroxide in PBS for 20 min at room temperature to block endogenous peroxidase activity. Immunoperoxidase staining was performed using the streptavidin-biotin peroxidase method. The tissue sections were incubated with Orexin R-1/2 primary antibody (Santa Cruz Biotechnology Inc., USA (H-300), sc-28936, 1/100) at room temperature for one hour. DAB was used as the chromogen, and Mayer hematoxylin was used as the counterstain. The positive control was normal testis tissue, which shows cytoplasmic staining in Leydig cells. For the negative control, PBS was used in place of primary antibody.

**Evaluation of expression of Orexin R1/2:** The specific cytoplasmic immunoreactivity of Orexin R1/2 was semiquantitativeley for intensity and distribution on a grading scale as follows: *Intensity of staining:* no staining as 0; weak staining as 1 and strong staining as 2; *Distribution of staining:* no cells, less than 5% positive cells, 5-50% of cells and more than 50% of cells as 0, 1, 2 and 3, respectively.

## Statistical Analysis

Statistical analysis was performed using SPSS 8.0 (Statistical Package for Social Sciences for Windows; Chicago, IL, USA). Age and serum PSA levels were compared by using one-way ANOVA post Hoc Bonferroni tests. The staining intensity and orexin expression were evaluated by using Pearson χ<sup>2</sup> test. Differences were considered to be statistically significant if the p value was less than 0.05.

## Results

The patients were divided into three groups of equal numbers (n=30) based on their histopathological results. Group 1 was defined as PCa. The mean age and prostate specific antigen (PSA) levels were 67.9±4.4 years (60-75) and 30.3±1.7 ng/

dL (10-64), respectively. Group 2 was considered as BPH. The mean age was  $61.6 \pm 8.1$  years (47-75) and the mean prostate specific antigen (PSA) value was  $5.3 \pm 3.8$  ng/dL (1-18). Group 3 included the patients diagnosed with CP by histopathologic examination. Their mean age was  $62.5 \pm 7.1$  years (48-76) and mean PSA value was  $5.5 \pm 2.9$  ng/dL (2-12). There were statistically significant differences between mean age ( $p=0.001$ ,  $F=7.581$ ) and PSA value ( $p=0.000$ ,  $F=50.216$ ) among the three groups. These differences were due to higher age and PSA values in Group 1 than in the other two groups (Mean age  $p_{\text{Group-1vs2}}=0.002$  and  $p_{\text{Group-1vs3}}=0.009$ ; PSA  $p_{\text{Group-1vs2}}=0.001$  and  $p_{\text{Group-1vs3}}=0.001$ ).

### Pathological Findings

A heterogeneous staining pattern of Orexin R1/2 was observed between the groups (Figure 1-3). Hot spots, representing areas of strong staining, were evaluated in the slides. The expression of Orexin R1/2 was detected in 90% (27/30) of Group 1, in 53.3% (16/30) of Group 2 and in 26.7% (8/30) of Group 3 patients. Five patients (9.3%) showed strong staining in Group 1 (Figure 4), whereas all samples from the other 2 groups showed only weak staining. The staining intensity was significantly different between the three groups (Pearson  $\chi^2=30.435$ ,  $p=0.001$ ). Additionally, the distribution of Orexin R1/2 was more widespread in Group 1 than in the other groups (Pearson  $\chi^2=35.671$ ,  $p=0.001$ ). The patients with PCa were divided into 3 groups, showing well, moderate and poor differentiation, based on their Gleason score; staining intensity and distribution were compared among these subgroups. While the expression and distribution of Orexin R1/2 were higher in the poorly differentiated group, there was no significant difference based on Gleason

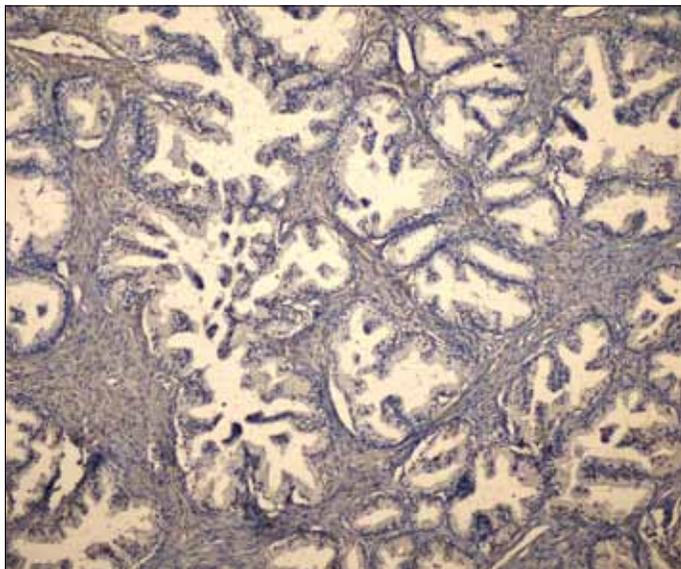


Figure 1. No expression of R1/2 in a case of BPH (Orexin, IHC x100)

score (Pearson  $\chi^2_{\text{intensity}}=6,141$   $p=0.189$ ; Pearson  $\chi^2_{\text{distribution}}=6.423$ ,  $p=0.378$ ). The staining intensity and the distribution of staining based on the pathologic findings of the prostate biopsies and the subgroups of PCa are given in Table 1.

### Discussion

In the present study, we showed that orexin receptors are found in prostate tissue and that their increased expression is associated with PCa and a high Gleason score. As such, we propose that orexin

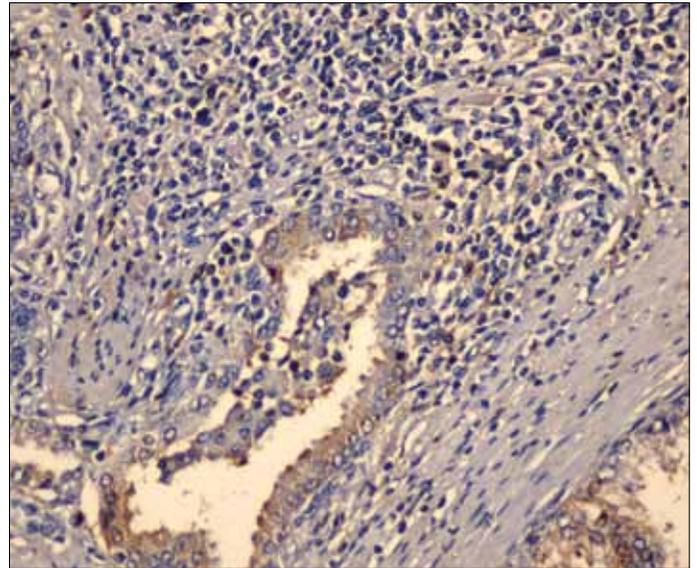


Figure 2. Heterogeneous cytoplasmic expression of R1/2 in a chronic prostatitis case (Orexin, IHC x200)

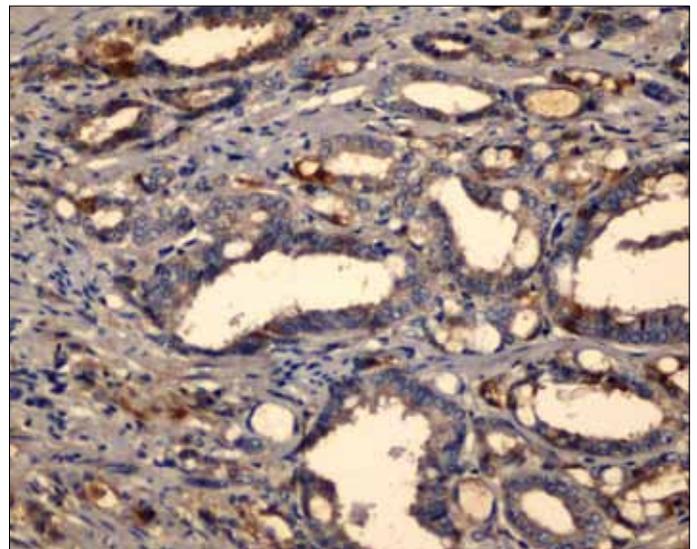


Figure 3. Weak and extensive expression of R1/2 in a moderately differentiated prostatic adenocarcinoma (Orexin, IHC x400)

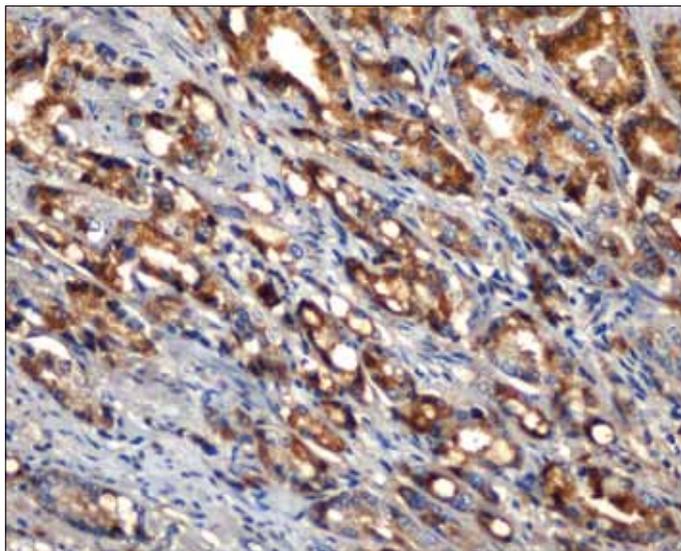


Figure 4. Strong and extensive expression of R1/2 in a poorly differentiated prostatic adenocarcinoma (Orexin, IHC x400)

**Table 1. The staining intensity and the distribution of staining in groups and subgroups of PCa (Group 1)**

	Intensity of staining (n)			Distribution of staining (n)			
	0	1	2	0	1	2	3
Group 1	3	23	4	3	14	9	4
Well-differentiated	1	11	-	1	8	3	-
Moderate	2	10	1	2	6	3	2
Poorly	-	2	3	-	-	3	2
Group 2	14	16	-	14	15	1	-
Group 3	22	8	-	22	6	2	-

immunoreactivity should be used as a histopathologic evaluation tool for the estimation of PCa grade. Additionally, like PSA, serum orexin levels should be used as a marker in the diagnosis of PCa.

Orexin receptors were originally discovered in the lateral and posterior hypothalamus of rats and were thought to regulate food intake and energy balance. The neurons expressing orexins were spread throughout the hypothalamus, and their fibers extended to the brain stem and thalamus, suggesting some central physiological effects of orexins.<sup>[1,2,11]</sup>

Since their discovery, orexins have been the focus of intense research, and there are substantial data implicating the functional effects of orexins in other systems. While orexins and their receptors have been described in different tissues, including the pancreas, kidney, adrenals, adipose and reproductive tract, their effects in many of these tissues are still unclear.<sup>[3,12-14]</sup>

Orexins have been shown to be present in the cells of the gastrointestinal mucosa that are involved in amine precursor uptake and decarboxylation (APUD), which are scattered throughout the exocrine epithelia. These cells synthesize biogenic amines and contain an acidic protein which is considered to be their own marker.<sup>[15]</sup> Orexins are also expressed in the neuroendocrine cells scattered in the glandular and lining epithelia of the gastrointestinal system. These cells are considered to be receptosecretory cells that are able to sense mechanical or chemical stimuli on their apical surfaces and respond by producing and releasing biologically active substances. Orexin containing neuroendocrine cells have been described in the gastrointestinal tract and pancreas in some animals and humans.<sup>[3,16]</sup> The functions of the orexins produced by these cells include the activation of intrinsic and extrinsic primary afferent neurons, the stimulation of intestinal secretions and the modulation of the effects of insulin and glucose metabolism.

The effects of orexin on the hypothalamo-pituitary axis and the reproductive tract have also been reported. Animal studies have shown that orexin inhibited pulsatile LH secretion from the pituitary gland and that the effects of orexin on pituitary gland secretions differs between male and female rats, thus revealing an important role for orexin in the hypothalamo-pituitary-gonadal axis.<sup>[14,17-19]</sup> Several studies have also described the expression of orexin receptors in the male genital tract, including the epididymis, penis, seminal vesicle and testis.<sup>[5,12]</sup> The studies showed that orexins had different effects based on their localization; for example, orexin receptors might have a regulatory role in Sertoli cells but be involved in spermatogonial DNA synthesis in the testis.

A study on orexin in the glandular parenchyma indicated that the peptide stimulated the synthesis of catecholamine from the adrenal glands.<sup>[20]</sup> In an animal study, it was shown that orexin 1R and orexin 2R had proliferative and antiproliferative effects in adrenocortical cells, respectively.<sup>[21]</sup> In contrast, Rouet-Benzined reported that orexin 1R suppressed cell growth by inducing apoptosis in colon cancer and neuroblastoma cells.<sup>[22]</sup> The neuroendocrine cells scattered in the urethral epithelium have been widely accepted to be a source of hormones in the genital tract.<sup>[23,24]</sup> In animal studies, it has been shown that in the urethroprostatic region, these cells contain chr-A, serotonin, somatostatin and enkephalin.<sup>[23,25]</sup> It is known that neuroendocrine cells are found in human prostate and that these cells may interact in a paracrine fashion with the stroma and with the secretory epithelial cells of prostate.<sup>[26]</sup> Prostatic neuroendocrine cells have cytoplasmic granules with dense cores in which peptide hormones and pro-hormones, such as chromogranin A, neuron-specific enolase, and bombesin are stored.<sup>[27]</sup> Additionally, in the prostate, it is thought that these neuroendocrine cells can be geared to modulate regulation, differentiation and secretion.<sup>[28]</sup> It has been reported by several groups that neuroendocrine cells are more common in higher grades and

stages of prostate cancer.<sup>[27-29]</sup> As such, neuroendocrine cell differentiation, as detected by immunohistochemical staining, can predict the prognosis of PCa.<sup>[30]</sup>

Russo et al.<sup>[9]</sup> reported the expression of OxR1/2 in the urethroprostatic complex in mammals and postulated that orexin release might have a paracrine and endocrine role on neuroendocrine secretion. Additionally, they concluded that orexins in the urethroprostatic junction might play a critical role in regulating physiological and pathological processes in the genital tract and in the prostate. In another study, Malendowicz reported the expression of OxR1/2 receptors in human prostate tissue and concluded that it might be related to BPH.<sup>[10]</sup> However, our study is the first to explore the expression of orexins in different prostate pathologies. We found the expression of orexin receptors in prostatic tissue, and their expression increased in PCa. Additionally, we found that the expression of orexins was more widespread in cases of poorly differentiated malignancy. The major limitation of our study was that we were not able to measure the serum orexin levels in our cases, and hence, we could not evaluate the relationship between serum orexin and PSA levels. Additionally, we were not able to perform molecular studies.

In conclusion, orexin receptors are found in human prostate tissues and are widespread in PCa, especially in cases with higher Gleason scores. We conclude that orexin immunoreactivity might be considered as a marker for poor prognosis in PCa and is associated with poorly differentiated malignancy. Further studies are needed to measure the serum orexin levels in PCa and BPH patients.

#### Conflict of Interest / Çıkar Çatışması

No conflict of interest was declared by the authors.

*Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.*

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#### Author Contributions / Yazar Katkıları

Concept / Fikir - M.M.B.; Design / Tasarım - M.M.B.; Ü.H.; Supervision / Denetleme - M.M.B., H.B.; Funding / Kaynaklar - M.M.B., Ü.H.; Materials / Malzemeler - M.M.B., Ü.H.; Data Collection and/or Processing / Veri toplanması ve/veya işlemesi - M.M.B., Ü.H., M.Ç., S.A., H.B.; Analysis and/or Interpretation / Analiz ve/veya yorum - M.M.B., Ü.H., M.Ç., H.B.; Literature Review / Literatür taraması - M.M.B., Ü.H., M.Ç., H.B.; Writer / Yazıyı yazan - M.M.B., Ü.H.; Critical Review / Eleştirel İnceleme - M.Ç., H.B.

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