Immunoreactivity of Ubiqitin in human prostate gland

Ziad M. Bataineh & Omar Habbal

Department of Human and Clinical Anatomy, Faculty of Medicine and Health Sciences, Sultan Qaboos University, Oman.

Correspondence to: Ziad M.Bataineh, MD., Ph.D.
Jordan University of Science and technology
Faculty of Medicine, Department of Anatomy
Irbid 22110, P.O.Box 3030, JORDAN
TEL: +962 2 7201000 ext 23838
FAX: +962 2 709 5010
EMAIL: ziad@just.edu.jo

Submitted: April 24, 2006 *Accepted: July 27, 2006*

Key words: **prostatic adenocarcinoma; benign prostatic hyperplasia; immunohistochemistry; proteasome; protein degradation**

Neuroendocrinol Lett 2006; **27**(4):517–522 **PMID:** 16892003 NEL270406A12

Abstract

BACKGROUND: Ubiquitin is a low molecular weight protein which has been detected in a variety of normal and cancerous tissues. It is involved in many regulatory processes including protein proteolysis. It has been implicated in tumor pathogenesis. The role of ubiquitin in human prostate gland is investigated in this study.

METHODS: In this study, we utilized immunohistochemistry technique to localize ubiqitin in human prostate gland and correlate it with different pathological conditions of the prostate.

RESULTS: Ubiqitin was localized in normal, benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma with variations in the distribution and intensity. In BPH, ubiquitin immunoreactivity was localized mainly in the nuclei while in the adenocarcinoma was localized mainly in the cytoplasm.

CONCLUSION: The presence of ubiquitin mainly in the nuclei and in the cytoplasm of BPH and prostatic adenocarcinoma, respectively, may suggest a role of ubiquitin in the development of the above mentioned conditions. Ubiquitin could be used as a potential marker for the diagnosis and prognosis of pathological conditions of the prostate.

ORIGINAL ARTICLE

Introduction

Ubiquitin (Ub) is a low molecular weight protein of 8.6 KD consisting of 76 amino acids and it was found in all eukarytotic cells tested [27]. It exists in cells either freely or joined covalently to a variety of cytoplasmic, nuclear and integral membrane proteins [14,17].

Ubiqitin has been proved to act as a cofactor in ATP dependent proteolysis [11]. Since then, many studies have shown that Ub is a multifunctional regulatory protein implicated in cell cycle regulation [32], DNA repair, membrane transport [4], and signal transduction [10,37], as well as in non-lysosomal protein degradation [27].

Ubiquitin has also been implicated in the pathogenesis of several disease states through either its involvement in protein degradation or its role as stress protein in the cell [21,26].

The Ub proteolytic system may be involved in cell cycle progression, since it selectively degrades nuclear proteins such as P53, N-myc and C-myc [3]. Normally, P53 appears to function as a tumor suppressor and its degradation by Ub may result in neoplastic transformation [7,15].

The role of Ub in the genital tract has been suggested. Ubiquitin was detected in human seminal plasma [19] and in the epididymal epithelium of both rat [22] and human testes [9]. Development of mature spermatozoa involves mitochondrial degradation by ubiquitylation and destroyed by proteasome machinery of the fertilized egg [34].

Immunocytochemistry utilizing anti-ubiquitin antibodies has been used to demonstrate the presence of Ub in various normal tissues such as: lung bronchial epithelium, bile duct and collecting tubules of the kidney. In addition, Ub has been detected in malignant tumors such as: lung, liver, pancreas and stomach tumors [12]. More recently, Lombardo et al have demonstrated the presence of Ub in human benign prostatic hypertrophy (BPH) utilizing SDS-PAGE technique [20].

In addition, previous studies have demonstrated the presence of Ub in normal and BPH but did not show the possibility of its expression in pathological prostate or its role in the pathogenesis of different pathological conditions of the prostate [22].

We utilize Ub-antibody for localization of Ub in abnormal specimens from human prostate gland and correlate the findings with different pathological conditions of the prostate.

Materials and Methods

Prostatic tissues were taken from patients (aged 64–80 years) with benign prostatic hyperplasia (21 cases) and prostatic adenocarcinoma (7 cases) through transurethral or saprapubic resection. Normal prostatic tissues were taken from corps by autopsy (12 cases). Specimens were clinically and histopathologically diagnosed. The procedures followed for obtaining all specimens were in accordance with the guidelines of the ethical committee of the Faculty of Medicine at Jordan University of Science and Technology. All tissues were fixed in SUSA fluid consisting of formalin, mercuric chloride and glacial acetic acid. Tissues then were routinely processed and embedded in paraffin.

Five μm thick sections were immunostained by avidin-biotin-peroxidase method. In order to abolish endogenous peroxidase activity, sections were incubated for 30 min. in 0.3% hydrogen peroxide in PBS. The sections were incubated overnight at 4° C with the rabbit polyclonal Ub-antibody (Segma MO, USA) at 1:400 dilution, and then incubated for 30 min with biotinylated goat anti-rabbit immunoglobulin as a secondary antibody (Dako) diluted 1:200. Subsequently, they were incubated for 30 min. with avidin-biotinylated peroxidase complex (Dako) diluted 1:100 in PBS. Visualization of the reaction was performed using 3.3' diamino benzidine tetrahydrochloridehydroden peroxidase as a chromogen in Tris buffer at pH 7.6 for 5 min. Between each step, slides were washed 3 times (5 min. each) with PBS. The sections were counter stained, dehydrated and mounted.

Specificity of the reaction was tested by incubating sections with either preabsorbed primary antibody with excess antigen for 48 hours or with buffer, from which the primary antibody has been deleted, followed by incubation otherwise as usual. Slides were photographed using Olympus photomicroscope (BH2-RFCA).

Results

All prostatic tissues from patients with benign prostatic hyperplasia and adenocarcinoma and from the corps showed a positive reaction for Ub, but with variation in the distribution and intensity of the reaction product.

Figure 1 showed a positive reaction for Ub in normal prostatic tissues. The reaction was confined to the epithelium but not the lumen of the acini or the connective tissue. The reaction was very mild in the nuclei and the cytoplasm as well. Both the cytoplasm and the nuclei of epithelial cells lacked uniformity in reaction.

Figure 2 showed a positive reaction for Ub in prostatic tissues from patients with benign prostatic hyperplasia. The reaction was confined to the epithelium and connective tissue as well. The reaction was most intense in the nuclei, while the cytoplasm showed a mild reaction. In contrast, the connective tissue showed heterogeneity in reaction product. Both cytoplasm and nuclei of epithelial cells lacked uniformity in reaction. The secretory products in the acinar lumen showed a mild reaction for ubiquitin.

Figure 3 showed a positive reaction for Ub in prostatic tissues from patients with prostatic adenocarcinoma. Both the epithelial cells and the connective tissue were stained. The cytoplasm showed the most intense reaction, whereas, the nuclei showed a mild

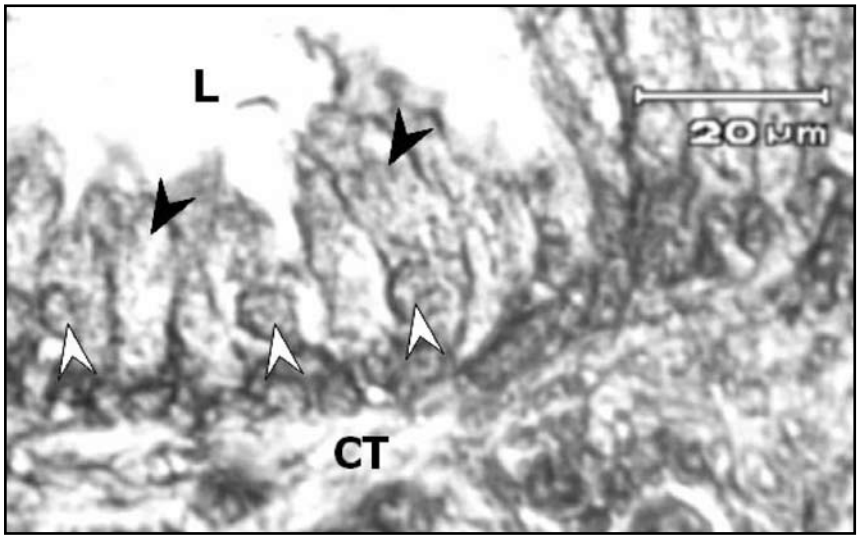

Figure 1. Photograph of normal human prostate gland after treatment with anti-ubiquitin. Portion of prostatic acinus is shown. Very mild immunoreactivity is shown in the nuclei of lining epithelium (white arrow heads) while the cytoplasm shows mild reaction for ubiquitin (black arrow heads). The lumen of the acinus (L) shows no reaction. Fibromuscular comportment of the connective tissue (CT) also shows a positive reaction for ubiquitin.

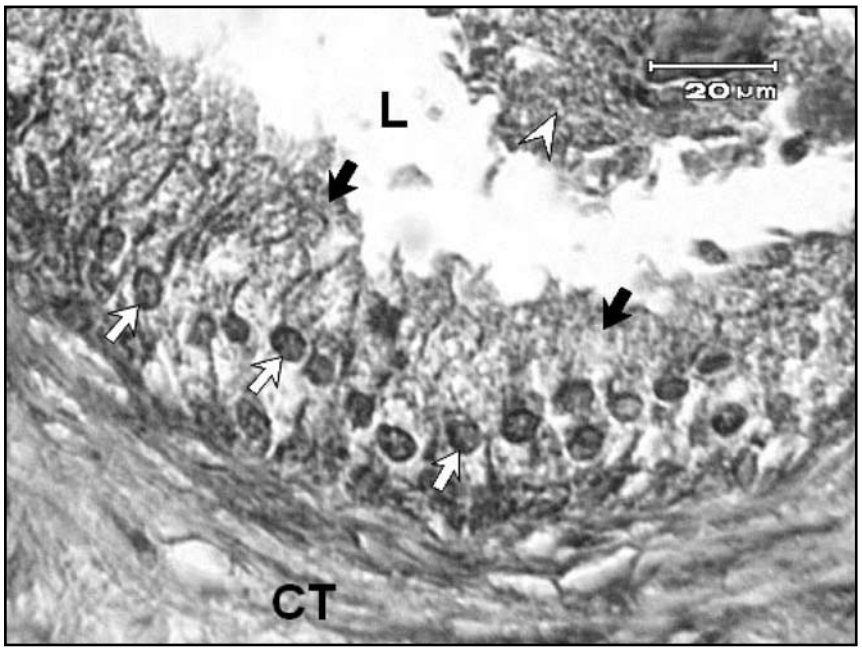

Figure 2. Photograph of benign prostatic hyperplasia of human prostate gland after treatment with anti- ubiquitin. Portion of prostatic acinus is shown. Intense immunoreactivity is shown in the nuclei of lining epithelium (white arrows) while the cytoplasm shows mild reaction for ubiquitin (black arrows). Secretory products (arrow head) in the lumen of the acinus (L) show mild reaction. Fibromuscular comportment of the connective tissue (CT) shows positive reaction for ubiquitin.

reaction for Ub in most of the epithelial cells. Some nuclei showed a faint reaction. On the other hand, the secretory products in the acinar lumina and the connective tissue showed a mild reaction for Ub.

Fig. 4 showed hyperplastic prostatic tissue which has been used as a control to validate the technique. No reaction product for Ub, neither in the epithelium nor in the connective tissue, has been detected.

Discussion

All prostatic tissues from normal, BPH, and adenocarcinoma showed a positive immunoreactivity for Ub with a variation in the distribution and intensity of the immunoreactivities. The variation in Ub distribution and intensity may reflect differences in specific functional response with different pathological conditions. In this study, BPH immunoreactivity was confined to the epithelium and connective tissue as well. This is supported by a study of Lombardo et al., where Ub was identified in BPH homogenate using SDS-PAGE [20]. Ubiquitin in the prostate gland could be the source of Ub in human seminal plasma [19]. On other study, Ub immunostaining was confined to the nuclei and cytoplasm of BPH [22]. Degradation, by Ub, of regulatory proteins in the prostate could trigger the development of BPH. Moreover, it was postulated that the predominance of Ub system component (protein gene product 9.5), in the transition zone of the prostate may be attributed to the pathogenesis of BPH [29]. This could explain the presence of Ub in the fibromuscular connective tissue as an implicated factor in hypertrophy of smooth muscle fibers in the fibromuscular connective tissue. In this regard, Ub was implicated in the degeneration of insect flight muscle [5] and inclusion body myositis [26] which may lend

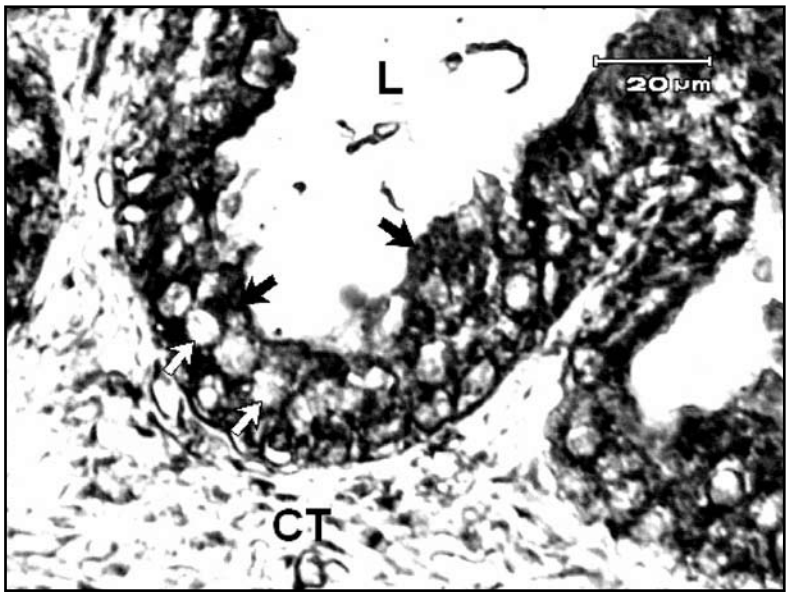

Figure 3. Photograph of prostatic adenocarcinoma of human prostate gland. Portions of prostatic acini are shown. Intense reaction for ubiquitin was shown in the cytoplasm of the lining epithelium (black arrows) while the nuclei showed a mild reaction for ubiquitin (white arrows). Connective tissue (CT) and the acinar lumen (L) show mild reaction for ubiquitin.

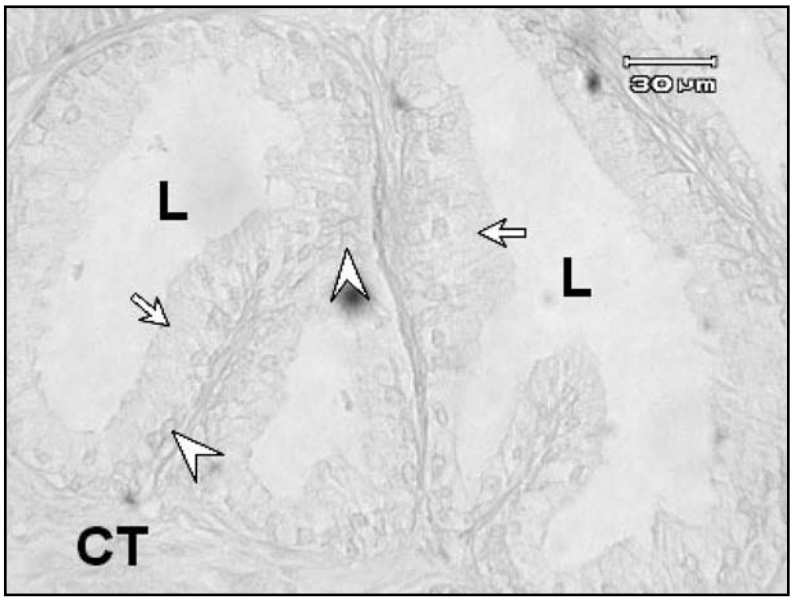

Figure 4. Photograph of the benign prostatic hyperplasia of human prostate gland. Portions of prostatic acini are shown. Control section that has been treated with buffer from which the anti-ubiquitin has been deleted. The cytoplasm (arrows) and nuclei (arrow heads) of the lining epithelium as well as the connective tissue (CT) show a negative reaction for ubiquitin.

support for the presence of Ub in the fibromuscular tissue of the prostate.

We demonstrated intense Ub immunoreactivity in the cytoplasm but not the nuclei of epithelial cells of prostatic adenocarcinoma. Many studies have shown the localization of Ub in malignant tissues .In breast neoplasm, Ub was confined to the cytoplasm but not the nuclei of acinar and ductal cells [13].In addition, ubiquitin was localized in lung, liver, stomach and pancreatic tumors. The Ub immunoreactivity was distributed uniformly in nuclei and cytoplasm of malignant cells regardless the degree of differentiation or origin of the tumor [12] .The absence of Ub immunoreactivity in many normal tissues and the intense reaction for Ub in their malignant forms [12] raises the question about the role of Ub in malignant transformation. The cytoplasm of the epithelial cells of prostatic adenocarcinoma demonstrated the presence of high amount of Ub which reflects a high rate of translational activity. Since the polyubiquitin gene expression is a cytoprotective phenomenon [8], the enhancement of Ub immunoreactivity in tumor cells may be a kind of stress responses induced by various host defense mechanisms in order to protect the cell. Apart from the stress responses, the enhanced Ub immunoreactivity in tumor cells might be due to the high metabolic/catabolic ration of the tumor vs. normal tissues [12]. The cell cycle and cell proliferation are regulated by a complex pathway composed of cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors [28]. P53, one of the cyclin-dependent kinases inhibitors, induces cell cycle arrest and apoptosis and simultaneously stimulates P21 (cyclin dependent kinase inhibitor) which results in G_1 and G_2 arrest. [35]. The role of Ub in P53 proteolytic degradation has been documented [24] and oncoproteins promote malignant transformation

in HPV-transformed cervical cell line by stimulating Ub-dependent degradation of P53 [30].

On the other hand, Ub degradation of P27, a cell cycle inhibitor, was implicated in the development of prostatic adenocarcinoma. This was associated with a concomitant high amount of Ub-ligase Skp2 levels [2]. A recent study has suggested the involvement of Ub in the pathogenesis of prostatic adenocarcinoma by degradation of proteins involved in growth inhibition or apoptosis of cancer cells [16].

Thus, accumulation of neoplastic cell specific ubiquinated proteins might be one of the explanations for the enhanced Ub immunoreactivity in prostatic adenocarcinoma.

Bortezomib, formerly known as (PS-341, LDP-341 and MLM341), is a potent anti-cancer agent against a variety of tumors including prostate cancer is a selective proteasome inhibitor which might supports the implication of Ub in prostate cancer pathogenesis [1]. On the other hand, the induction of S-phase arrest and apoptosis in LNCaP and PC-3 prostate carcinoma cells by a novel retinoid CD437 [18] was regulated by ubiqitin-mediated pathway [6].

Although it is too early to conclude the relationship between the level of Ub and some physiological states of cancerous or hypertrophied prostate, the differences found in Ub distribution could be used as a cytological parameter in the diagnosis and prognosis of disease states of the prostate.

In this regard, the intensity of prostatic acid phosphatase was used to differentiate between normal, BPH and adenocarcinoma of the prostate [33]. Other study has used the prostatic specific antigen in localization, staging and prognosis of prostatic adenocarcinoma [25]. On the other hand, overexpression of Skp2 (Ub ligase) and loss of P27 (cyclin dependent kinase) by Ub system degradation are strongly associated with poor prognosis and may thus be used as a prognostic marker for colorectal carcinoma [31] and prostatic adenocarcinoma [36], respectively. Although this study did not determine the Ub expression as an independent predictor of the pathological states of the prostate, Ub expression is of significant interest and warrants further investigation as a potential marker for cell proliferation in the prostate.

These preliminary data may set the bases for further studies which correlate Ub distribution with different stages and differentiation of prostatic tumors.

REFERENCES

1 Adams J, Kauffman M. Development of the proteasome inhibitor Velcade (Bortezomib). Cancer Invest 2004; **22**: 304–311.

2 Ben-Izhak O, Lahar-Baratz S, Meretyk S, Ben-Eliezer S, Sabo E, Dirnfeld M. et al. Inverse relationship between ubiquitin ligase and the cyclin dependent kinase inhibitor P27 Kip1 in prostate cancer. J Urol 2003; **170**: 241–245.

3 Ciechanover A, Di Giuseppe JA, Bercovich B, Orian A, Richter JD, Schwartz AL. et al. Degradution of nuclear oncoproteins by the ubiquitin system in vitro. Proe Natl Acad Sci USA 1991; **88**: 139 – 143.

4 d'Aazzo A, Bongiovanni A, Nastasi T. E_3 ubiquitin ligase as regulators of membrane protein trafficking and degradation. Traffic 2005; **6**: 429–441.

5 Davis WL, Jacoby BH, Goodman BP. Immunolocalization of ubiquitin in degenerating insect flight muscle. Histochem J 1994; **26**: 298 – 305.

6 Farhana L, Dawson M, Rishi AK, Zhang Y, Van Buren E, Trivedi C, Reichert U. et al. Cyclin B and E2F-1 expression in prostate carcinoma cells treated with the novel retinoid CD437 are regulated by the ubiquitin-mediated pathway. Cancer Res 2002; **62**:3842–3849.

7 Finlay CA, Hinds PW, Levine AJ. The P53 proto-oncogene can act as a suppressor of transformation. Cell 1989; **57**: 1083 – 1093.

8 Finley D, Özkaynak E, Vashavsky A. The yeast polyubiquitin gene is essential for resistance to light temperatures, starvation and other stresses. Cell 198; **48**: 1035–1046.

9 Fraile B, Martin R, De Miguel MP, Arenas MI, Bethencourt FR, Peinado F. et al. Light and electron microscopic immunocytochemical localization of protein gene produce 9.5 and ubiquitin immunoreactivities in the human epididymis and vas deference. Biol Reprod 1996; **55**: 291–297.

10 Gao M, Karin M. Regulating and regulators: control of protein ubiqitination and ubiquitin-like modification by extra cellular stimuli. Mol Cell 2005; **19**: 581–593.

11 Hershko A, Ciechanover A, Heller H, Haas AL, Rose IA. Proposed role of ATP in protein breakdown: conjugation of proteins with multiple chains of the polypeptide of ATP-dependent proteolysis. Proc Natl Acad Sci USA 1980; **77**: 1783–1786.

12 Ishibashi Y, Takada K, Joh K, Ohkawa K, Aoki T, Matsuda M. Ubiquitin immunoreactivity in human malignant tumors. Br J Cancer 1991; **63**: 320–322.

13 Iwaya K, Nishibori H, Osada T, Matsuno Y, Tsuda H, Sato S. et al. Immunoreaction at 43 KDa with anti-ubiquitin antibody in breast neoplasm. Jpn J Cancer Res 1997; **88**: 273–280.

14 Jentsch S, McGrath JP, Varshavsky A. The yeast DNA repair gene RAD 6 encodes a ubiquitin conjugating enzyme. Nature 1987; **329**: 131–134.

15 Kiaris H, Chatzistamou I, Trimis G, Frangou-Plemmenou M, Pafiti-Kondi A, Kalofoutis A . Evidence for non atonomous effect of P53 tumor suppressor in carcinogenesis. Cancer Res 2005; **65**: 1627–1630.

16 Ko y, Hahn T, Lu H, Ma ZL, Chen J, Rothe M. et al. A novel component of the ubiquitin pathway, ubiquitin carboxyl extension protein 1 is overexpressed in prostate cancer. Int. J Mol Med 2005; **15**: 183–196.

17 Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ. et al. Growth hormone receptor and serum binding protein: punfication, cloning and expression. Nature 1987; **330**: 537–543.

18 Liang JY, Fontana JA, Rao JN, Ordonez JV, Dawson MI, Shroot B. et al. Synthetic retinoid CD437 induces S-phase arrest and apoptosis in human prostate cancer cells LNCaP and PC-3. Prostate 1999; **38**:228–236.

19 Lippert TH, Seeger H, Schieferstein G, Voelter W. Immunoreactive Ubiquitin in human seminal plasma. J Androl 1993; **14**: 130–131.

20 Lombardo ME, Meyer-Siegler K, Hakky SI, Hudson PB. Preliminary studies on isolation and characterization of predominant prostatic proteins.Prostate 1996; **29**: 381–385.

21 Mani A, Gelmann EP. The ubiquitin-proteasome pathway and its role in cancer. J Clin Oncol 2005; **23**: 477–489.

22 Martin R, Fraile B, Peinado F, Arenas MI, Elices M, Alonso L. et al. Immunohistochemical localization of protein gene product 9.5, ubiquitin, and neuropeptide Y immunoreactivities in epithelial and neuroendocrine cells from normal and hyperplastic human prostate. J Histochem Cytochem 2000; **48**: 1121–1130.

23 Martin R, Santamaria L, Fraile B, Paniagua R, Polak JM. Ultrastructural localization of PGP 9.5 and ubiquitin immunoreactivities in rat ductus epididymis epithelium. Histochem J 1995; **27**: 431–439.

24 Pallares-Trujillo J, Agell N, Garcia-Martinez C, Lopez-Soriano FJ, Argiles JM. The ubiquitin system: a role in disease? Medicinal Res Rev 1997; **17**: 139–161.

25 Partin AW, Carter HB, Chan DW, Epstein JI, Oesterling JE, Rock RC. et al. Prostatic specific antigen in the staging of localized prostate cancer: Influence of tumor differentiation, tumor volume and benign hyperplasia. J Urolo 1990; **143**: 747–752.
26 Prayson RA, Cohen ML. Ubiquitin immunostaing and inclusion body myositis. Study of 30 patients with inclusion body myositis. Human Pathology 1997; **28**: 887–892.
27 Rechsteiner AM. Ubiquitin-mediated pathways for intracellular proteolysis. Annu Rev Cell Biol 1987; **3**: 1–30.
28 Sanchez-Beato M, Sanchez-Aguillera A, Piris MA. Cell cycle deregulation in B-cell lymphamas. Blood 2003; **101**: 1220–1235.
29 Santamaria L, Martin R, Martin JJ, Alonso L. Stereologic estimation of the number of neuroendocrine cells in normal human prostate detected by immunohistochemistry. Appl Immunohistochem Mol Morphol 2002; **10**: 275–281.
30 Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 1990; **63**: 1129–1136.
31 Shapira M, Ben-Izhak O, Linn S, Futerman B, Minkov I, Hershko DD. The prognostic impact of the ubiquitin ligase subunits Skp2 and Cks1 in colorectal carcinoma. Cancer 2005; **103**:1336–1346.
32 Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression.Gene Dev 1990; **13**: 1501–1512.
33 Sinha AA, Gleason DF, Wilson MJ, Wick MR, Reddy PK, Blackard CE. Relationship of prostatic acid phosphatose localization in human prostate by a monoclonal antibody with the Gleason grading system. Prostate 1988; **13**: 1–15.
34 Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C, Schatten G. Ubiquitin tag for sperm mitochondria. Nature 1999; **402**: 371–372.
35 Takimoto R, El-Deiry WS. DNA replication blockade impairs P53 – transactivation. Proc. Natl Acad Sci. USA 2001; **98**: 781–783.
36 Tsihlias J, Kapusta LR, DeBoer G, Morava-Protzner I, Zbieranowski I, Bhattacharya N. et al. Loss of cyclin-dependent kinase inhibitor p27Kip1 is a novel prognostic factor in localized human prostate adenocarcinoma. Cancer Res 1998; **58**: 542–548.
37 Welchman RL, Gordon C, Mayer RJ. Ubiquitin and ubiquitin-like proteins as amultifunctional signals. Natl Rev Mol Cell Biol 2005; **6**: 599–609.