Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2011;28:1287-1294

Accepted: December 06, 2011

Expression of the Non-gastric H^+/K^+ ATPase ATP12A in Normal and Pathological Human Prostate Tissue

Doris Streif[1], Eva Iglseder[1], Cornelia Hauser-Kronberger[2], Klaus G. Fink[3], Martin Jakab[1] and Markus Ritter[1]

[1]Institute of Physiology and Pathophysiology, Paracelsus Medical University Salzburg, Laboratory of Functional and Molecular Membrane Physiology, Salzburg, [2]Department of Pathology, University Hospital Salzburg, Paracelsus Medical University, Salzburg, [3]Department of Urology and Andrology, University Hospital Salzburg, Paracelsus Medical University, Salzburg

Key Words

ATP12A • H^+/K^+ ATPase • Prostate • Benign hyperplasia • Cancer

Abstract

Altered cellular proton handling and cell volume regulation are hallmarks of tumorigenesis. To investigate a possible involvement of the non-gastric H^+/K^+ ATPase ATP12A (ATP1AL1) in prostate cancer, we performed immunohistochemistry in formalin-fixed, paraffin-embedded histological sections from benign and malignant human prostate lesions. Normal prostate tissue displayed a membrane-bound ATP12A staining with focal accumulated pattern, whereas in the benign prostate hyperplasia (BPH) and cancerous prostate tissue (tumor grade I–III) the protein appears to be displaced in the luminal cells of the glandular epithelium. Hence, the expression pattern of ATP12A is markedly altered in BPH and prostate cancer. To test for altered gene expression of ATP12A we performed quantitative reverse transcriptase PCR (QRT-PCR) in normal (tumor-free) prostate tissue, BPH and tumor stages I-III using a prostate cancer cDNA array. However, no significantly different expression levels could be detected in the various disease states compared to normal tissue, which contrasts the findings from immunohistochemistry and points to the possibility of altered post-translational processing and/or sorting of the protein. We further show that ATP12A mRNA is expressed at different levels in PC-3 and LNCaP prostate cancer cells, with a significant ~26-fold higher expression in the latter cell type. Protein expression in these tumor cell lines was verified by Western blot.

Introduction

Accurate cellular proton handling is critical for controlling cell volume, cell proliferation, apoptosis, cell migration and senescence. Alterations in cell volume regulation and the regulation of the intra- and extracellular pH are hallmarks of tumorigenesis [1, 2]. Tumor proliferation results in areas with reduced glucose availability, hypoxia and extracellular acidification, which

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/11/0286-1287$38.00/0

Accessible online at:
www.karger.com/cpb

Markus Ritter or Martin Jakab
Institute of Physiology and Pathophysiology
Paracelsus Medical University Salzburg, Strubergasse 21, 5020 Salzburg (Austria)
Tel. +43-662-442002-1250, FAX +43-662-442002-1259
E-Mail markus.ritter@pmu.ac.at, or E-Mail martin.jakab@pmu.ac.at

requires an adaptive up-regulation of glycolysis and cellular proton extrusion pathways including Na^+/H^+ exchangers (NHEs), H^+/K^+ ATPases and H^+ pumps on a functional and/or genetic level. This promotes the evolution of glycolytic, acid-resistant and highly infiltrative cancer cell phenotypes [1]. Low interstitial pH favors matrix protein degradation by membrane-bound or secreted proteinases, which increases cancer cell invasiveness, as shown for PC-3, and DU-145 prostate cancer cells, colorectal carcinoma cell lines and immortalized breast epithelial cells [3-5]. Proton export simultaneously causes intracellular alkalinization, which is critical for the oncogenic transformation of cells and the development/maintenance of transformed phenotypes. In NIH3T3 fibroblasts infected with the E7 oncogene of HPV16, or expressing the RAS oncogene, and in human keratinozytes, intracellular alkalinization was found to be specific for transformed cells and to be mediated by NHE1; in these cell types NHE1 inhibition prevented transformation and tumor cell proliferation. Similarly, in breast cancer cells a tumor-specific activation of Na^+/H^+ exchanger, cell motility and invasion was observed [6, 7]. In microinvasive foci of breast cancer and adjacent intraductal cells NHE and glucose transporter 1 expression is up-regulated by hypoxia [1]. All these data suggest a prominent role of proton extrusion for preparing the 'soil' for tumor invasion, namely matrix degeneration on the one hand and the maintenance of transformed phenotypes by intracellular alkalinization on the other hand.

While the role of Na^+/H^+ exchangers is well established, the role of H^+/K^+ ATPases in cancerogenesis is unknown. However, evidence comes from work showing inhibition of cell migration and pro-apoptotic effects of H^+/K^+ ATPase inhibition by the proton pump inhibitors omeprazole, pantoprazole or SCH-28080 in gastric cancer cells [8], neutrophils [9-11], MDCK cells [12], pro-myelocytic HL-60 cells and INS-1E rat insulinoma cells ([13, 14]; own results; manuscripts in preparation) [15]. Cells that undergo apoptosis show characteristic signs such as intracellular acidification, K^+ loss and cell shrinkage [16, 17]. H^+/K^+ ATPase activation, besides antagonizing intracellular acidification, might concomitantly attenuate tumor cell apoptosis by counteracting K^+ loss and apoptotic cell shrinking. In fact, ion transport mechanisms that increase the tumor cells' ability to maintain cell volume constancy and intracellular pH have been identified as enhancers of apoptotic resistance in various tumors including prostate, breast and colon cancer cells [2, 18].

The catalytic alpha-subunits of H^+/K^+ ATPases are members of the P2-type family of ion pumps, which couple ATP hydrolysis to the exchange of H^+ or Na^+ against K^+ ions across the plasma membrane [19]. They comprise the gastric (HKalpha1) and the non-gastric (HKalpha2) isoform, the latter being encoded by gene ATP12A (ATP1AL1). Expression of ATP12A has been detected in the prostate, kidney, uterus, placenta, skin, brain and colon [20]. Specific functions are known in kidney and colon. Of note, ATP12A null mice display loss of luminal acidification and K^+ absorption in the prostate and kidney [21-23], indicating that the pump acts as a functional H^+/K^+ ATPase *in vivo*. Organism-wide profiling has shown differential expression of ATP12A mRNA in 88 human organism parts, 37 cell types, 39 cell lines and 78 disease states, with significant up-regulation in prostate-, and breast adenocarcinoma (EMBL-EBI arrayexpress archive, experiments E-MTAB-62, E-GEOD-24283, E-GEOD-6919; http://www.ebi.ac.uk). In human colorectal carcinoma ATP12A is significantly over-expressed, which eventually contributes to the development of the pathological condition [24]. In pro-myelocytic HL-60 cells and INS-1E rat insulinoma cells induction of apoptosis by butyrate is paralleled by ATP12A up-regulation ([13, 25]; abstracts in this issue, manuscripts in preparation). Taken together, there is strong evidence for a role of ATP12A in the development of prostate cancer and possibly other disease states.

Given its functional role in the acidification of the prostate fluid in the normal prostate [23] and its altered expression during apoptosis in cancer cells, it is feasible to assume that ATP12A expression is also deranged in pathological prostate tissue. The aim of this study was to investigate ATP12A expression on the protein level and to test for differential ATP12A expression on the mRNA level in normal (tumor-free, TF) prostate tissue, benign prostate hyperplasia (BPH) and tumor stages I-III. To this end we have analyzed 26 formalin-fixed and paraffin-embedded histological prostate sections of cancer patients by immunohistochemistry (IHC) and performed real-time quantitative PCR using a TissueScan™ prostate cancer disease array containing sets of tumor stages I-III, BPH and TF tissues. We demonstrate altered protein expression patterns in BPH as well as in tumor stages I-III. In contrast, no apparent differences in mRNA/cDNA amounts could be detected by RT-PCR in the samples investigated. We further show that ATP12A mRNA and protein is expressed in PC-3 and LNCaP prostate cancer cells, with a significantly higher mRNA expression level in LNCaP cells.

Materials and Methods

Immunohistochemistry

Experimental material and data handling: archived formalin-fixed and paraffin-embedded (FFPE) tissue blocks (histoblocks) provided by the Department of Pathology, General Hospital, Paracelsus Medical University Salzburg, were used for analyses. Experiments were performed in accordance with the Helsinki declaration of 1975 (revised 1983). All patients have signed an informed consent concerning the surgical removal and therapy of the tumors. The study did not extend to examination of individual case records. The anonymity of the patients was ensured.

1.5-µm sections were cut from FFPE tissue blocks with a Leica RM 2245 microtome, transferred onto microscope slides and deparaffinised using graded alcohol. Antigen retrieval was achieved by heat-induced epitope retrieval under pH 6.0 in antigen retrieval buffer (Dako) at 98°C for 30 minutes. Endogenous peroxidase blocking was carried out for 10 minutes with peroxidase-blocking reagent (Dako). Sections were then incubated for 30 minutes at room temperature with mouse primary antibodies directed against human ATP12A (Abnova). Primary antibodies were detected using the EnVision Detection System (Dako) employing biotinylated goat anti-mouse antibodies (1:100) and EnVision Dual Link System Peroxidase (Dako). Immunoreactivity was visualized using diamino-benzidine (DAB; Dako) as chromogenic substrate according to the manufacturer's instructions. Sections were counterstained with hematoxylin. The stained sections were digitalized using a Leica CTR 6000 Microscope. The majority of evaluated tissue sections contained more than one type of tissue/pathological state (lesion); normal (tumor-free) tissue, benign prostate hyperplasia, adenocarcinoma and atrophic tissue was present in 20, 18, 21 and 19 out of 26 sections, respectively. This allows for a direct, paired comparison of tumor free- and pathological tissues. The discrimination was performed microscopically and cancer staging was done according to the guidelines of the American Joint Committee on Cancer [26]. Images were analyzed using a modified quickscore method [27]. Briefly, this method takes into account the staining intensity (rated 0, 1, 2, or 3) and the relative amount of stained cells (based on 100 counted cells; 0=0–10%, 1=10–25%, 2=25–50%, 3=50–75%, 4=75–100%). Multiplication of staining intensity and extent yields scores ranging from 0–12, where scores from 0–3 are classified as negative, from 4–6 as weakly positive, from 7–9 as moderately positive, and from 10–12 as strongly positive (Fig. 2).

Cell culture

LNCaP and PC-3 cells were cultured at 37°C, 5% CO_2 and 95% air in RPMI 1640 medium with L-glutamine (PAA Laboratories), and Ham F-12 medium (Sigma–Aldrich), respectively, supplemented with 10% fetal bovine serum (FBS; PAA Laboratories). Media contained 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 ng/ml amphotericin B. Subcultures were established once a week by trypsin/EDTA treatment.

Western blot

The alpha2 subunit of the non-gastric H^+/K^+ ATPase ATP12A was detected in LNCaP and PC-3 cell lysates by immunoblotting after growing cells for 2–3 days under standard conditions in 9-cm cell culture dishes. Cells were scraped off with lysis buffer composed of 150 mM NaCl, 50 mM TRIS-HCl (pH 7.5), 1 mM EGTA, 1% (v/v) Triton X-100, 0.1% (w/v) SDS and proteinase inhibitor cocktail containing AEBSF, pepstatinA, E-64, bestatin, leupeptin, aprotinin (Sigma–Aldrich). After brief sonication the cell lysates were centrifuged at 14,000×g for 10 minutes. The protein concentration in the supernatants was measured with a BCA (bicinchoninic acid) protein assay kit (Pierce–Thermo Scientific). 20 µl of supernatants (200 µg protein) mixed with 2× sample buffer containing 125 mM TRIS-HCl, 4% (w/v) SDS, 0.7% (v/v) β-mercaptoethanol, 20% (v/v) glycerol and 0.004% (w/v) bromphenol blue were separated by SDS-PAGE on an 8% Tris-glycin gels (peqlab). A prestained protein ladder (PageRuler, Fermentas) served as protein size marker. Proteins were transferred onto 1.2-µm nitrocellulose membranes (BIO-RAD). Immunodetection was performed with a polyclonal rabbit anti-ATP1AL1 primary antibody (Santa-Cruz Biotechnology) at a dilution of 1:200, a secondary biotinylated goat anti-rabbit antibody (1:300; Abcam), and Avidin-HRP (1:250; eBioscience). Blots were developed using SuperSignal® West Pico Chemiluminescent Substrate (Pierce). Images were acquired using a BioSpectrum AC Imaging System and VisionWorks LS Software (UVP).

Quantitative real-time RT-PCR

SYBR-green QRT-PCR was performed on a TissueScan™ prostate cancer disease array containing sets of tumor stages I-III, BPH and TF tissues (HPRT101, OriGene Technologies) and two prostate cancer cell lines (androgen-responsive LNCaP [28] and androgene-insensitive PC-3 cells [29]). LNCaP and PC-3 cells were lysed by addition of Sure Prep™ True Total™ RNA Purification Kit (Fisher BioReagents, Fisher Scientific) and total RNA was isolated according to the manufacturer's instructions. 1 µg total RNA was subjected to DNase treatment and reverse transcription using Revert Aid™ M-MuLV Reverse Transcriptase and oligo (dT) primers (both Fermentas), according to manufacturer´s instructions. Primers for human ATP12A (GenBank acc. no. NM_001676.4) were designed using the online tool Primer3 (v.0.4.0) and synthesized by Microsynth AG (sense primer: 5'–GCTTTCTGCCCCGCACTCTCAT–3'; antisense primer: 5'–AAGCCGTGTAGCCCGTCCATTC–3'; fragment size 135 bp). Beta-actin (ACTB; GenBank acc. no. NM_001101.3) served as housekeeping gene (sense primer: 5'–CAGCCATGTACGTTGCTATCCAGG–3'; antisense primer: 5'–AGGTCCAGACGCAGGATGGCATG–3'; fragment size 140 bp). Real-time PCR was run on an Mx3000P QPCR System (Agilent Technologies). The specificity of the PCR reactions was tested by melting curve analysis and by loading the amplification products on 2% agarose gels. Threshold cycles (Ct) were determined in the exponential amplification phase of the PCR reaction. Delta Ct (dCt) values were calculated by subtraction of the Ct values of beta-actin from the individual sample's Ct values [30].

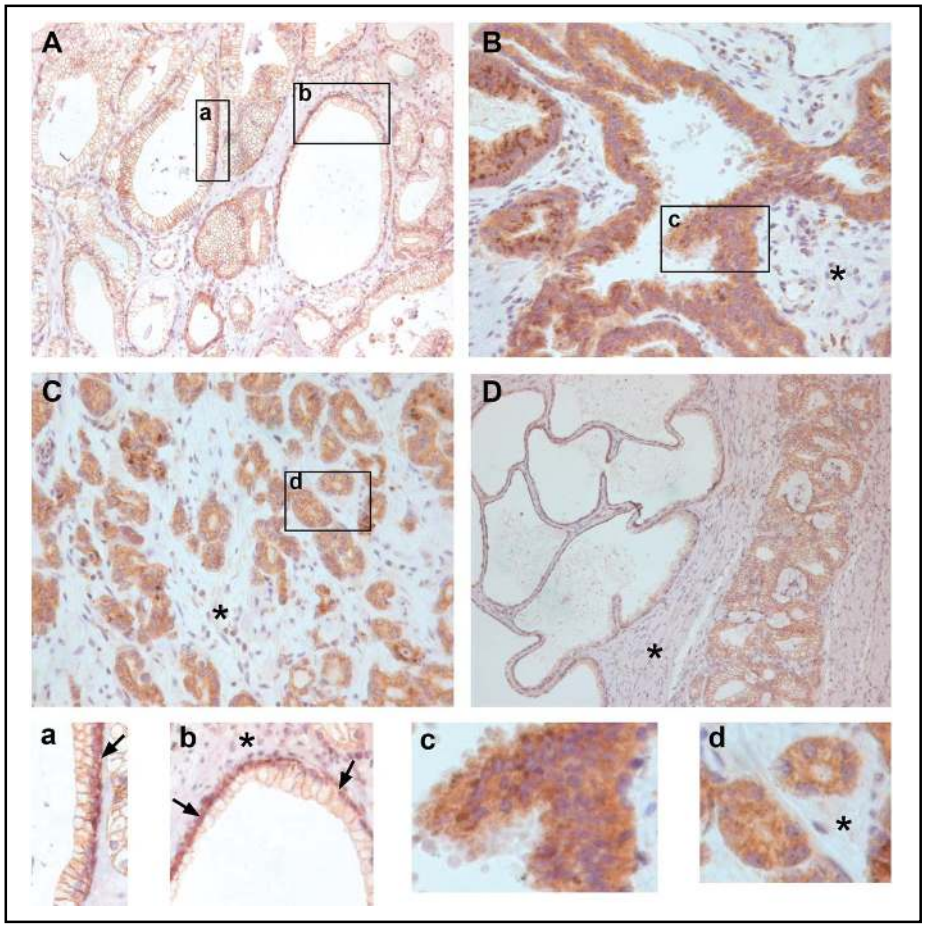

Fig. 1. Representative IHC images of ATP12A of FFPE histological prostate sections, counterstained with hematoxylin. (A) Tumor free prostate section (400× magnification). (B) Benign prostate hyperplasia (200×). (C) T3b cancerous tissue (200×). (D) Atrophic prostate tissue (400×). a–d are close-ups of the corresponding regions indicated by rectangles. In TF tissue ATP12A is frequently observed at the plasma membrane and the staining is dominant in basal cells (indicated by arrows). In all types of tissue the stroma is negative for ATP12A (asterisks).

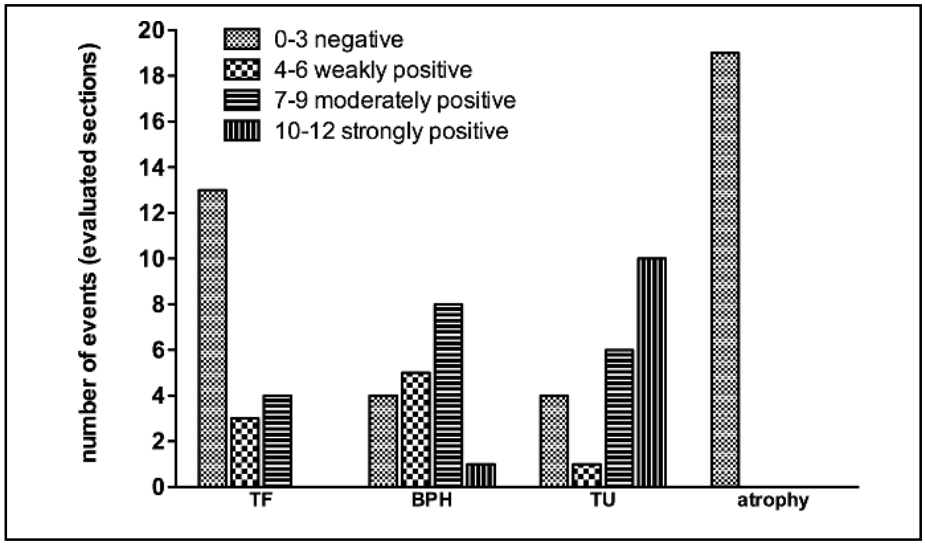

Fig. 2. Semi-quantitative IHC analysis of 26 histological prostate sections using a modified quickscore method [27] as described in the Methods. The number of analyzed sections in which the staining lies within an indicated range of scores is plotted on the y-axis. TF, tumor-free tissue; BPH, benign prostate hyperplasia; TU, tumor tissue.

Statistics

Data are presented as arithmetic mean ± standard error of the mean (SEM). Data were tested for Gaussian distribution using the Kolmogorov-Smirnov normality test and for outliers by the Grubb's test. Data were compared using 1-way ANOVA and Tukey's multiple comparison post-test, or unpaired t-test with Welsh's correction, as applicable. The significance level was 0.05 (95% confidence intervals). Analysis was done with GraphPad Prism 5 software.

Results

Immunohistochemistry

In normal (tumor-free) prostate tissue the alpha-subunit of the non-gastric H^+/K^+ ATPase ATP12A is mainly observed at the plasma membrane and staining is dominant in basal cells (arrows) with punctual

expression in clusters in some areas (Fig. 1A). This clearly differs from the distribution pattern found under benign prostate hyperplasia (BPH; Fig. 1B) and tumor tissues (Fig. 1C), where immunoreactivity is increased, indicating increased ATP12A protein abundance with homogeneous expression in the whole epithelium. In tissues with BPH cell layers extend further into the acinus lumen and the cell mass is increased. In all types of tissue the stroma is negative for ATP12A (indicated by asterisks). Atrophic cells, probably undergoing apoptosis, appear ATP12A-negative (Fig. 1D). Semi-quantitative analysis of the immunostained histological sections (n=26; Fig. 2) shows that in the majority of analyzed sections (13) tumor-free (TF) tissue is negative for ATP12A staining. In 7 sections TF tissue was weakly or moderately positive, whereas strongly positive staining was lacking. In both BPH and TU tissues immunoreactivity is increased, with a notable shift from negative and weakly positive towards strongly positive staining in the tumor tissue. In all analyzed sections containing atrophic tissue (19 out of 26) these areas were negative for ATP12A staining.

Quantitative real-time RT-PCR

QRT-PCR on a prostate cancer disease cDNA array shows expression of ATP12A in normal (tumor-free) human prostate, as well as in prostate cancer stage I-III and benign prostate hyperplasia. Individual delta Ct values (calculated as described in the Methods section) are plotted in Figure 3. Mean dCt values for tumor-free samples (TF), prostate cancer stages I and II (TUI/II; pooled data), cancer stage III (TUIII), and benign hyperplasia (BPH) were 14.5 ± 0.8 (n=7), 14.1 ± 0.5 (n=19), 13.2 ± 1.2 (n=7), and 15.5 ± 0.3 (n=11), respectively. There were no statistically significant differences between the four groups.

Gene expression studies on LNCaP and PC-3 prostate cancer cells yielded dCt values of 4.87 ± 0.13 (n=12) and 7.42 ± 1.09 (n=12), respectively (p=0.041) (Fig. 4A). This amounts to a ~26-fold (25.6 ± 7.9-fold; n=12) higher expression in LNCaP cells as calculated from the ddCt values (according to $2^{(dCt_{PC-3} - dCt_{LNCa})}$ [30]).

Western blot

By using a primary antibody against the non-gastric H^+/K^+ ATPase alpha-subunit (HKalpha2) we were able to detect specific bands at ~110 kDa in both LNCaP and PC-3 cells (Fig. 4B).

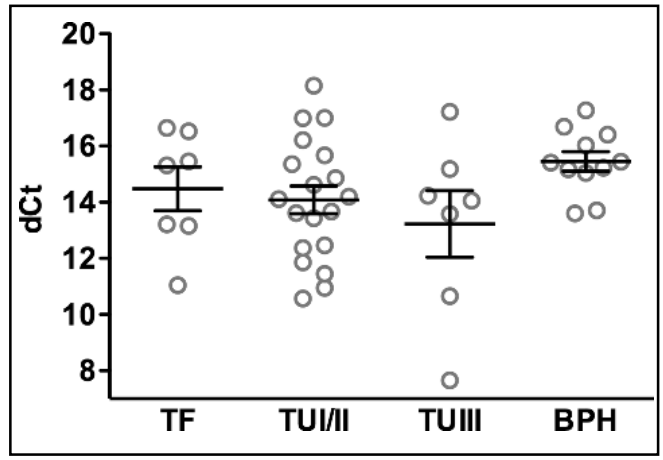

Fig. 3. ATP12A mRNA expression analysis by QRT-PCR. Scatter blot of individual dCt values calculated for tumor-free samples (TF; n=7), prostate cancer stage I/II (TU I/II; pooled data; n=19), cancer stage III (TUIII; n=7), and benign prostate hyperplasia specimen (BPH; n=11). For each group of samples the mean ± SEM is further shown in the graph (1-way ANOVA, Tukey's multiple comparison post-test).

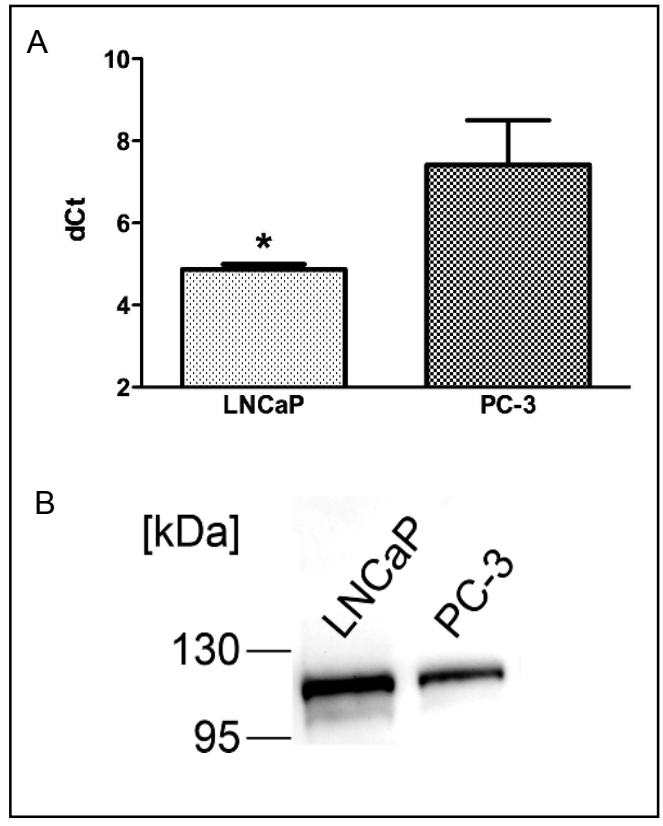

Fig. 4. Gene and protein expression of the alpha-subunit of the non-gastric H^+/K^+ ATPase ATP12A (ATP1AL1) in the prostate cancer cell lines LNCaP and PC-3 detected by (A) SYBR-green QRT-PCR (n=3; *, p<0.05; unpaired t-test with Welsh's correction), and (B) Western blot.

Discussion

As shown in this study in normal prostate tissue ATP12A is mainly restricted to basal cells and exhibits the highest immunoreactivity at or close to the cell membrane. Such localization is expected for a trans-membrane protein and indicates that ATP12A may indeed act as functional ion transporting H^+/K^+ ATPase in intact prostate glands. Lobe-specific expression and apical localization for ATP12A has been demonstrated in rodent prostate where the function of the protein is necessary for the acidification of the semen [23, 31]. In contrast, ATP12A protein abundance is increased and the expression pattern of ATP12A is markedly altered in both in BPH and TU tissues compared to normal human prostate tissue. As evident, in these tissues total ATP12A immunoreactivity is increased and the protein is no longer restricted to the basal cells but can be found more homogenously throughout the epithelium. In addition ATP12A appears to be dispersed in the cytosol of the cells and does not exhibit preferential membrane localization (Fig. 1 and 2).

The deranged expression pattern might be due to altered transcription of the ATP12A gene. Of note, significant up-regulation of ATP12A is predicted for prostate adenocarcinoma from gene array studies (EMBL-EBI arrayexpress archive, experiments E-MTAB-62, E-GEOD-24283, E-GEOD-6919; http://www.ebi.ac.uk). However, the results from our RT-PCR measurements using a cDNA library derived from normal, or defined pathological prostate tissue indicate that ATP12A mRNA expression is apparently unchanged in tumor tissue samples and BPH compared to normal tissue (Fig. 3). Therefore it seems unlikely that the observed changes on the protein level arise from altered gene expression. However, even though the provided array samples are derived from pathologically clearly defined disease states, uncertainty arises concerning the absolute amount of lesion-specific mRNA/cDNA since the tissues from which the samples were derived from may contain more than one specific lesion at a time. For example, a tissue sample of stage III grading may also contain normal cells, atrophic cells or necrotic cells at varying degrees. So the total amount of mRNA/cDNA in such an array may not necessarily reflect the transcription of lesion-specific mRNA/cDNA.

The observed ATP12A expression pattern in pathological tissue could also arise from altered post-translational processing and/or sorting of the protein. In MDCK and LLC-PK1 cells ATP12A is predominantly sorted to the apical and lateral membrane, respectively. This differential localization and sorting depends on the interaction of ATP12A with the beta-subunit of the Na^+/K^+ ATPase [32]. Recently it has been shown that in the rat anterior prostate the Na^+/K^+ ATPase beta1-subunit functions also as the authentic beta-subunit of the non-gastric H^+/K^+ ATPase and putatively accounts for the intracellular sorting of the X-K-ATPase isoforms [33]. Interestingly, the Na^+/K^+ ATPase beta1-subunit was found to be down-regulated by androgens in human LNCaP-FGC prostate cancer cells and human prostate cancer xenografts [34]. Similarly it has been shown that sorting of APT12A in stably transfected MDCK cells is regulated by protein kinase (PKC) and activation of PKC by phorbol esters causes internalization and intracellular accumulation of ATP12A [35]. Of note PKC regulation is markedly altered in prostate cancer [36, 37]. Therefore it is tempting to speculate that the alterations of ATP12A expression observed in this study might be due to deranged sorting of the protein as a consequence of tumor-driven altered expression of the Na^+/K^+ ATPase beta1-subunit and/or PKC. In order to investigate gene transcription, post-translational processing, protein sorting and the cellular functions of ATP12A in detail, experiments need to be performed in functionally, morphologically and genetically defined cancer cell lines. As shown here, we find ATP12A gene and protein expression in both androgen-insensitive PC-3 and androgen-sensitive LNCaP cells, two well-established prostate cancer cell models. Interestingly ATP12A mRNA is more abundantly expressed in LNCaP cells (Fig. 4). On-going studies are performed to investigate the expressional and functional regulation of the non-gastric H^+/K^+ ATPase in these cells, with special emphasis on a possible androgen-dependence of ATP12A processing and function.

In summary we show for the first time deranged protein expression of the non-gastric H^+/K^+ ATPase ATP12A (ATP1AL1) in prostate tissue of benign prostate hyperplasia (BPH) and tumor stages I-III compared to normal tissue without detectable differences on the transcriptional level. In addition we show that ATP12A is expressed in PC3 and LNCaP prostate cancer cells, with higher expression levels in the latter ones. Our findings might be of both diagnostic and therapeutic importance in prostate cancer.

Abbreviations

BPH (benign prostate hyperplasia); Ct (threshold cycle); FFPE (formalin-fixed paraffin-embedded); IHC (immunohistochemistry); NHE (Na^+/H^+ exchanger); QRT-PCR (quantitative real-time PCR).

Acknowledgements

The assistance of L. Emin is gratefully acknowledged. This work was supported by the PMU-Forschungsförderungsfonds, grant no. R-09/04/009-JAK and R-11/02/024-JAK to MJ, and the PMU-Forschungsförderungsfonds, grant no. 06/03/020 to MR.

References

1 Gatenby RA, Smallbone K, Maini PK, Rose F, Averill J, Nagle RB, Worrall L, Gillies RJ: Cellular adaptations to hypoxia and acidosis during somatic evolution of breast cancer. Br J Cancer 2007;97:646-653.

2 Kunzelmann K: Ion channels and cancer. J Memb Biol 2005;205:159-173.

3 Rozhin J, Sameni M, Ziegler G, Sloane BF: Pericellular pH affects distribution and secretion of cathepsin B in malignant cells. Cancer Res 1994;54:6517-6525.

4 Ackerstaff E, Artemov D, Gillies RJ, Bhujwalla ZM: Hypoxia and the presence of human vascular endothelial cells affect prostate cancer cell invasion and metabolism. Neoplasia 2007;9:1138-1151.

5 Steffan JJ, Snider JL, Skalli O, Welbourne T, Cardelli JA: Na^+/H^+ exchangers and RhoA regulate acidic extracellular pH-induced lysosome trafficking in prostate cancer cells. Traffic 2009;10:737-753.

6 Woll E, Ritter M, Offner F, Lang HJ, Scholkens B, Hussinger D, Lang F: Effects of HOE 694 - a novel inhibitor of Na^+/H^+ exchange - on NIH 3T3 fibroblasts expressing the RAS oncogene. Eur J Pharmacol 1993;246:269-273.

7 Reshkin SJ, Bellizzi A, Caldeira S, Albarani V, Malanchi I, Poignee M, Alunni-Fabbroni M, Casavola V, Tommasino M: Na^+/H^+ exchanger-dependent intracellular alkalinization is an early event in malignant transformation and plays an essential role in the development of subsequent transformation-associated phenotypes. FASEB J 2000;14:2185-2197.

8 Yeo M, Kim DK, Kim YB, Oh TY, Lee JE, Cho SW, Kim HC, Hahm KB: Selective induction of apoptosis with proton pump inhibitor in gastric cancer cells. Clin Cancer Res 2004;10:8687-8696.

9 Ritter M, Schratzberger P, Rossmann H, Woll E, Seiler K, Seidler U, Reinisch N, Kahler CM, Zwierzina H, Lang HJ, Lang F, Paulmichl M, Wiedermann CJ: Effect of inhibitors of Na^+/H^+-exchange and gastric H^+/K^+ ATPase on cell volume, intracellular pH and migration of human polymorphonuclear leucocytes. Br J Pharmacol 1998;124:627-638.

10 Wandall JH: Effects of omeprazole on neutrophil chemotaxis, super oxide production, degranulation, and translocation of cytochrome b_{-245}. Gut 1992;33:617-621.

11 Martins de Oliveira R, Antunes E, Pedrazzoli J Jr, Gambero A: The inhibitory effects of H^+ K^+ ATPase inhibitors on human neutrophils in vitro: restoration by a K^+ ionophore. Inflamm Res 2007;56:105-111.

12 Klein M, Seeger P, Schuricht B, Alper S, Schwab A: Polarization of Na^+/H^+ and Cl^-/HCO_3^- exchangers in migrating renal epithelial cells. J Gen Physiol 2000;115:599-608.

13 Schmidt S, Mercorillo D, Hufnagl C, Bulatova S, Jakab M, Ritter M: The non-gastric H^+/K^+-ATPase ATP12A exerts an anti-apoptotic effect on butyrate-treated myelomonocytic HL60 cells. Acta Physiol 2009;195:102-103.

14 Ketterl N, Jakab M, Ritter M: Expression and function of the non-gastric H^+/K^+ ATPase in pancreatic beta-cells. Cell Physiol Biochem 2011:28:1308.

15 Jakab M, Ritter M: Cell volume regulatory ion transport in the regulation of cell migration. Contrib Nephrol 2006;152:161-180.

16 Bortner C, Sifre M, Cidlowski J: Cationic gradient reversal and cytoskeleton-independent volume regulatory pathways define an early stage of apoptosis. J Biol Chem 2008;283:7219-7229.

17 Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, Blagosklonny MV, El-Deiry WS, Golstein P, Green DR, Hengartner M, Knight RA, Kumar S, Lipton SA, Malorni W, Nunez G, Peter ME, Tschopp J, Yuan J, Piacentini M, Zhivotovsky B, Melino G: Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 2009;16:3-11.

18 Prevarskaya N, Skryma R, Bidaux G, Flourakis M, Shuba Y: Ion channels in death and differentiation of prostate cancer cells. Cell death and differentiation 2007;14:1295-1304.

19 Axelsen KB, Palmgren MG: Evolution of substrate specificities in the P-type ATPase superfamily. J Mol Evol 1998;46:84-101.

20 Pestov NB, Romanova LG, Korneenko TV, Egorov MV, Kostina MB, Sverdlov VE, Askari A, Shakhparonov MI, Modyanov NN: Ouabain-sensitive H,K-ATPase: tissue-specific expression of the mammalian genes encoding the catalytic alpha subunit. FEBS Lett 1998;440:320-324.
21 Kone BC, Higham SC: A novel N-terminal splice variant of the rat H^+-K^+-ATPase alpha2 subunit. Cloning, functional expression, and renal adaptive response to chronic hypokalemia. J Biol Chem 1998;273:2543-2552.
22 Greenlee MM, Lynch IJ, Gumz ML, Cain BD, Wingo CS: The renal H,K-ATPases. Curr Opin Nephrol Hypertens 2010;19:478-482.
23 Pestov N, Korneenko T, Shakhparonov M, Shull G, Modyanov N: Loss of acidification of anterior prostate fluids in Atp12a-null mutant mice indicates that nongastric H-K-ATPase functions as proton pump in vivo. Am J Physiol Cell Physiol 2006;291:C366-374.
24 Takahashi Y, Sakai H, Kuragari M, Suzuki T, Tauchi K, Minamimura T, Tsukada K, Asano S, Takeguchi N: Expression of ATP1AL1, a non-gastric proton pump, in human colorectum. Jpn J Physiol 2002;52:317-321.
25 Jakab M, Schmidt S, Ketterl N, Streif D, Hauser-Kronberger C, Ritter M: The Non-Gastric H^+/K^+-ATPase ATP12A (ATP1AL1) Acts as Anti-Apoptotic Ion Transporter. Cell Physiol Biochem 2011;28:1305.
26 Edge SB, American Joint Committee on Cancer: AJCC cancer staging manual, ed 7th. New York, Springer, 2010.
27 Detre S, Saclani Jotti G, Dowsett M: A "quickscore" method for immunohistochemical semiquantitation: validation for oestrogen receptor in breast carcinomas. J Clin Pathol 1995;48:876-878.
28 Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP: LNCaP model of human prostatic carcinoma. Cancer Res 1983;43:1809-1818.
29 Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW: Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol 1979;17:16-23.
30 Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:e45.
31 Pestov NB, Korneenko TV, Adams G, Tillekeratne M, Shakhparonov MI, Modyanov NN: Nongastric H-K-ATPase in rodent prostate: lobe-specific expression and apical localization. Am J Physiol Cell Physiol 2002;282:C907-916.
32 Reinhardt J, Grishin AV, Oberleithner H, Caplan MJ: Differential localization of human nongastric H^+-K^+-ATPase ATP1AL1 in polarized renal epithelial cells. Am J Physiol Renal Physiol 2000;279:F417-425.
33 Pestov NB, Korneenko TV, Radkov R, Zhao H, Shakhparonov MI, Modyanov NN: Identification of the beta-subunit for nongastric H-K-ATPase in rat anterior prostate. Am J Physiol Cell Physiol 2004;286:C1229-1237.
34 Blok LJ, Chang GT, Steenbeek-Slotboom M, van Weerden WM, Swarts HG, De Pont JJ, van Steenbrugge GJ, Brinkmann AO: Regulation of expression of Na^+,K^+-ATPase in androgen-dependent and androgen-independent prostate cancer. Br J Cancer 1999;81:28-36.
35 Reinhardt J, Kosch M, Lerner M, Bertram H, Lemke D, Oberleithner H: Stimulation of protein kinase C pathway mediates endocytosis of human nongastric H^+-K^+-ATPase, ATP1AL1. Am J Physiol Renal Physiol 2002;283:F335-343.
36 Ali AS, Ali S, El-Rayes BF, Philip PA, Sarkar FH: Exploitation of protein kinase C: a useful target for cancer therapy. Cancer Treat Rev 2009;35:1-8.
37 Cornford P, Evans J, Dodson A, Parsons K, Woolfenden A, Neoptolemos J, Foster CS: Protein kinase C isoenzyme patterns characteristically modulated in early prostate cancer. Am J Pathol 1999;154:137-144.