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ORIGINAL ARTICLE

Overexpression of Aquaporin-1 is a Prognostic Factor for Biochemical Recurrence in Prostate Adenocarcinoma

Ji Y. Park[1,2] · **GhilSuk Yoon**[2]

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Abstract Aquaporins (AQP) are transmembrane proteins that provide channels for water and solutes, and some are involved in tumor progression and invasion. We evaluated the expression of AQP-1, AQP-3, and AQP-5 and their clinicopathological significance in prostate adenocarcinomas (PCA). Prostatectomy specimens ($n = 99$) were retrieved from the surgical pathology archives and clinicopathological data were obtained from the medical database at Kyungpook National University Hospital. Immunohistochemical staining for AQP-1, AQP-3, and AQP-5 was performed on tissue microarrays comprising paired malignant and benign prostatic tissues. Seventeen PCA cases (17.2 %) showed AQP-1 overexpression, specifically 7 tumors (9.7 %) with lower Gleason scores (GS) and 10 tumors (37.0 %) with higher GS, with statistical significance ($P = 0.001$). AQP-1 overexpression was significantly associated with higher GS ($P = 0.001$), higher pathologic T (pT) stages ($P = 0.024$), and biochemical recurrence (BR) ($P = 0.002$). The difference in AQP-3 and AQP-5 expression between neoplastic and non-neoplastic tissues was not established and there were no correlations with clinicopathological parameters. AQP-1 overexpression was evident in tumors with higher GS, it was less evident in tumors with lower GS, and it was associated with BR and a higher pT stage. AQP-1 overexpression is associated with prostate cancer progression.

Keywords Aquaporin · Prostate adenocarcinoma · Biochemical recurrence · Gleason score

Introduction

Prostate adenocarcinoma (PCA) is the most common malignancy among men in westernized countries [1]. Although most PCAs have indolent clinical courses, PCA remains a dominant cause of cancer-related death in males, accounting for 10 % of all cancer deaths in men in the western world [2].

Clinical and laboratory parameters, including preoperative prostate-specific antigen (PSA) levels, the combined Gleason score (GS), and the tumor extent on biopsies are established prognostic factors, however, they do not provide all of the information necessary to achieve optimal treatment and to predict patient outcomes.

The aquaporins (AQPs) are a family of transmembrane proteins that act as selective channels for water and small solutes, including glycerol and urea [3]. Since AQPs are ubiquitously expressed in bacterial, animal, and human cells, they are considered essential for cellular function [4]. Thirteen members of the AQP family have been identified in humans, and several AQP subtypes are associated with oncogenesis, including tumor development, angiogenesis, migration, and the aggressive behavior of malignant tumors [5–12]. Several studies have demonstrated that the aberrant expression of AQP-1, AQP-3, or AQP-5 is associated with tumor invasion and progression in breast, colon, lung, and ovarian cancers, but these studies have mainly been carried out in vitro using cell lines [5–8, 10–15]. More recent studies have focused on the expression of AQPs in human cancer tissues. For example,

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✉ Ji Y. Park
pathpjy@naver.com

[1] Department of Pathology, Daegu Catholic University Medical Center, School of Medicine, Catholic University of Daegu, Duryugongwon-ro 17-gil, Nam-gu Daegu 42472, Republic of Korea

[2] Department of Pathology, Kyungpook National University Medical Center, Kyungpook National University School of Medicine, Daegu, Republic of Korea

Yoshida et al. demonstrated that AQP-1 expression in colon adenocarcinoma was associated with lymph node involvement, frequent vascular invasion, and poor survival rates [5].

A few studies have investigated the expression and biological significance of AQPs in human PCA tissue [7]. Previous studies have demonstrated that the different AQP subtypes are associated with specific locations and functions in benign prostate tissue. Hence, while AQPs might characterize human benign and malignant prostatic tissues, investigations into the clinicopathological implications of AQP distribution are limited [16–19].

Therefore, we aimed to evaluate the expression profiles of AQP-1, AQP-3, and AQP-5 in prostate cancer tissues, to compare these profiles with those in matching benign tissues, and to determine the clinicopathological significance of these aquaporins in PCA.

Materials and Methods

Case Selection

Prostatectomy specimens that had been obtained between 2008 and 2010 were retrieved from the surgical pathology archives at Kyungpook National University Medical Center, Kyungpook National University School of Medicine, Daegu, Korea. Patients who had received preoperative hormonal therapy and those who had malignancies of other organs at the time of surgery were excluded from the study. A total of 99 cases of acinar (conventional) PCA were selected and included in the study.

The patients' clinical information was acquired from the medical database at Kyungpook National University Medical Center and, it included the following: the age at PCA diagnosis; data relating to the operation; serum PSA levels before and after the operation, and during follow-up assessments; data relating to biochemical recurrence (BR); information about tumor recurrence and/or metastasis; and information about the survival outcome.

The pathologic data were obtained from reviews of the hematoxylin and eosin-stained tissue sections and comprised the following: the tumor size; the histologic tumor type; the dominant pattern and the sum of the combined GS; the presence of lymphovascular or perineural invasion; the presence of extraprostatic extension or seminal vesicle involvement; whether complete resection was achieved; and the presence of lymph node metastases.

BR was defined as follow-up PSA levels that were ≥ 0.2 ng/ml higher than the first recorded PSA level when they were assessed more than 30 days after surgery, without any evidence of recurrence in the imaging investigations [20]. The combined GS was determined using the criteria from the International Society of Urological Pathology Consensus Conference, 2005. The pathological tumor-node-metastasis stage was determined using the seventh edition of the Cancer Staging Manual prepared by the American Joint Committee on Cancer.

The Kyungpook National University Medical Center's Institutional Review Board approved this study.

Tissue Microarrays and Immunohistochemistry

Tissue microarrays (TMA) were prepared from each formalin-fixed paraffin-embedded prostatectomy specimen, with 3 cores, each 1 mm in diameter, obtained from each specimen. For each tumor, 2 cores were obtained from the areas that showed the highest GS patterns and 1 core was obtained from a corresponding benign area. Immunohistochemical (IHC) staining was performed on the tissues using a Ventana Benchmark XT autoimmunostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA). Briefly, 4-μm tissue sections were transferred onto adhesive slides and dried at 62 °C for 30 min. Following standard heat-induced epitope retrieval for 60 min in ethylenediaminetetraacetic acid (pH 8.0), the tissue samples were incubated with antibodies to AQP-1 at a dilution of 1:5,000 (Abcam, Cambridge, UK), AQP-3 at a dilution of 1: 800 (Abcam, Cambridge, UK), or AQP-5 at a dilution of 1:200 (Abcam, Cambridge, UK). The sections were subsequently incubated with the ChromoMap DAB Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). Appropriate positive controls were used for each antibody on each tissue. The slides were counterstained with Hematoxylin II (Ventana Medical Systems, Inc., Tucson, AZ, USA).

IHC staining of the TMAs was quantified by the standard Hirsch (H-)score method [21]. The IHC H-score assigned a continuous scale of 0–300, based on the staining intensity and the extent of tumor cell staining. Staining intensity was graded as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. The extent of tumor cell staining was quantified using a Nikon Eclipse 80*i* microscope (Nikon Instruments Inc., Melville, NY, USA) with a grid scale in the eyepiece and by determining the level of positively stained tumor cells as a percentage of the total tumor area. Cohorts were finally grouped as negative (H-score < 200) or positive (H-score ≥ 200) for AQP-1, AQP-3, and AQP-5 expression, respectively. Sublocalization of the immunoreactivity was also evaluated by comparing reactivity of normal prostatic tissues and PCAs: cytoplasmic staining, reactivity was involved in the cytoplasm, but there was no accentuation in the submembranous cytoplasmic region; cytoplasmic-membranous reactivity, which was mainly accompanied in the cytoplasm with submembranous accentuation; membranous staining, it was shown as prominent cellular outlines of cells, the immunoreaction was mainly conducted in the submembranous cytoplasmic region.

Two pathologists (G.S.Y. and J.Y.P.) evaluated all of the histopathological and the subsequent IHC slides.

Statistical Analysis

Associations between the expression of the AQPs and each clinicopathological parameter were determined using the chi-squared test or Fisher's exact test. Univariate analyses of the risk factors for BR were performed using log-rank tests of the Kaplan-Meier analyses and Cox regression models. Multivariate analyses were based on the Cox proportional hazard model using the backward likelihood ratio. *P* values of < 0.05 were considered statistically significant. All statistical analyses were performed using PASW Statistics for Windows, Version 18.0 (IBM Corporation, Armonk, NY, USA).

Results

Clinicopathological Characteristics of the Patients

Table 1 summarizes the patients' clinicopathological features. The mean (standard deviation [SD]) age at diagnosis was 66.5 (5.6) years. The mean (SD) size of the dominant tumor nodule (if multiple tumors) was 2.7 (0.9) cm. The mean (SD) preoperative PSA level was 15.6 (13.9) ng/ml and the median preoperative PSA level was 10.9 ng/ml (range, 3–99 ng/ml). All patients were managed by radical prostatectomy.

The combined GS were distributed as follows: 9 cases (9.1 %) had scores of 6 (3 + 3), 30 cases (30.3 %) had scores of 7 (3 + 4), 33 cases (33.3 %) had scores of 7 (4 + 3), 12 cases (12.1 %) had scores of 8 (4 + 4), 9 cases (9.1 %) had scores of 9 (4 + 5), 5 cases (5.1 %) had scores of 9 (5 + 4), and 1 case (1.0 %) had a score of 10 (5 + 5). The tumors comprised 32 (32.3 %) at the pathologic T (pT)2 stage, 43 (43.4 %) at the

Table 1 Clinicopathological characteristics of the patients with prostate adenocarcinomas and baseline analyses associated with biochemical recurrence

Clinicopathological parameter		All PCA ($n = 99$)	BR positive ($n = 16$)	BR negative ($n = 83$)	*P* value
Mean (SD) age (years)		66.3 (5.4)	66.0 (5.3)	66.3 (5.4)	*0.820*
DRE, *n* (%)	+	26 (26.3)	2 (12.5)	24 (28.9)	*0.572*
	–	73 (73.7)	14 (87.5)	79 (71.1)	
Mean (SD) tumor size (cm)		2.7 (0.9)	3.1 (0.8)	2.6 (0.9)	***0.010***
Mean (SD) preoperative serum PSA level (ng/ml)		15.0 (10.9)	21.9 (14.9)	13.5 (10.3)	*0.202*
Multiplicity, *n* (%)	Single	68 (68.7)	13 (81.3)	55 (66.3)	*0.237*
	Multiple (≥2)	31 (31.3)	3 (18.8)	28 (33.7)	
Combined Gleason score, *n* (%)	6 (3 + 3)	9 (9.1)	0 (0.0)	9 (10.8)	***0.004***
	7^{Low} (3 + 4)	30 (30.3)	2 (12.5)	28 (33.7)	
	7^{High} (4 + 3)	33 (33.3)	4 (25.0)	29 (34.9)	
	8 (4 + 4)	12 (12.1)	3 (18.7)	9 (10.8)	
	9 and 10	15 (15.2)	7 (43.8)	8 (9.6)	
Pathologic T stage, *n* (%)	pT2	32 (32.3)	3 (18.7)	29 (34.9)	***0.022***
	pT3a	43 (43.4)	5 (31.3)	38 (45.8)	
	pT3b	24 (24.2)	8 (50.0)	16 (19.3)	
Lymph node metastasis, *n* (%)	+	1 (1.0)	0 (0.0)	1 (1.2)	*0.875*
	–	98 (99.0)	16 (100.0)	82 (98.8)	
Perineural invasion, *n* (%)	+	74 (74.7)	14 (87.5)	60 (72.3)	*0.184*
	–	25 (25.3)	2 (12.5)	23 (27.7)	
Lymphovascular invasion, *n* (%)	+	17 (17.2)	4 (25.0)	13 (15.7)	*0.295*
	–	82 (82.8)	12 (75.0)	70 (84.3)	
Marginal status, *n* (%)	Involved	53 (53.5)	10 (62.5)	43 (51.8)	*0.384*
	Not involved	46 (46.5)	6 (37.5)	40 (48.2)	
Mean (SD) follow-up duration (months)		36.8 (20.4)	31.4 (18.1)	37.9 (20.8)	*0.246*

*Variables with statistically significant associations ($p < 0.05$) with biochemical recurrence are indicated in bold

§PCA prostate adenocarcinoma, *SD* standard deviation, *BR* biochemical recurrence, *DRE* digital rectal examination, *PSA* serum prostate-specific antigen

pT3a stage, and 24 (24.2 %) at the pT3b stage. The mean (SD) follow-up period was 36.8 (20.4) months.

At the time of the analysis, BR was identified in 16 patients (16.2 %) with a mean (SD) follow-up duration of 15.1 (5.9) months (range, 4–27 months). There were significant associations between BR and a larger tumor size ($P = 0.010$), a higher combined GS (≥8) ($P = 0.001$), and a higher pT stage ($P = 0.022$). There were no differences between the BR-positive and BR-negative groups in relation to age, digital rectal examination positivity, tumor multiplicity, lymphovascular or perineural invasion, and the marginal status. None of the patients had regional recurrences or distant metastases during the follow-up period.

Immunohistochemical Expression of Aquaporin-1, Aquaporin-3, and Aquaporin-5

AQP1 expression was identified in the capillaries and venules within the prostatic interstitial tissues with a mild, sometimes, moderate staining intensity. In benign prostatic tissue, most of the secretory cells were negative for AQP-1 expression (Fig. 1a), but 3 cases (3/99, 3.0 %) were positive for AQP-1, which was observed in the cytoplasm and membrane of the secretory cells, with luminal accentuation.

The overexpression of AQP-1 was observed in 17 cases (17/99, 17.2 %) of PCA (Fig. 1d), specifically in 7 tumors with lower GS (6 + 7) (7/72, 9.7 %) and in 10 tumors with higher GS (≥8) (10/27, 37.0 %), a difference that was statistically significant ($P = 0.001$). There was no significant difference between benign prostatic glands and PCAs with lower GS in relation to AQP-1 expression ($P = 0.067$).

Positive staining for AQP-3 (Fig. 1b) and AQP-5 (Fig. 1c) was apparent in 92 samples (92/99, 92.9 %) and 43 samples (43/99, 43.4 %) of non-neoplastic prostatic glands, respectively. The expression of AQP-3 in PCAs was observed in 49 cases (49/99, 49.5 %); in detail, 36 tumors with lower GS (36/72, 50.0 %) and 13 tumors in high GS (13/27, 48.1 %); there was no statistical significance ($P = 0.887$). The AQP-5 was expressed in 13 cases (13/99, 13.1 %); concretely, 7 lower GS tumors (7/72, 9.7 %) and 6 high GS tumors (6/27, 22.2 %); a statistical difference was not evident ($P = 0.323$). In both benign prostatic glands and PCAs, AQP-3 staining was predominantly cytoplasmic-membranous pattern, while AQP-5 staining was mainly cytoplasmic with a variable intensity, and was sometime concentrated at the apical surface. Unlike AQP-1 expression, AQP-3 and AQP-5 were seldom expressed in the vascular endothelial cells.

The expression of AQP-3 was significantly greater in the benign prostatic glands compared with malignant prostatic tissues ($P = 0.044$) (Fig. 1e). The expression of AQP-5 tended to be higher in benign prostatic glands compared with neoplastic glands ($P = 0.067$) (Fig. 1f). We did not identify any correlations between the clinicopathological parameters and the expression of either AQP-3 or AQP-5. Table 2 summarizes

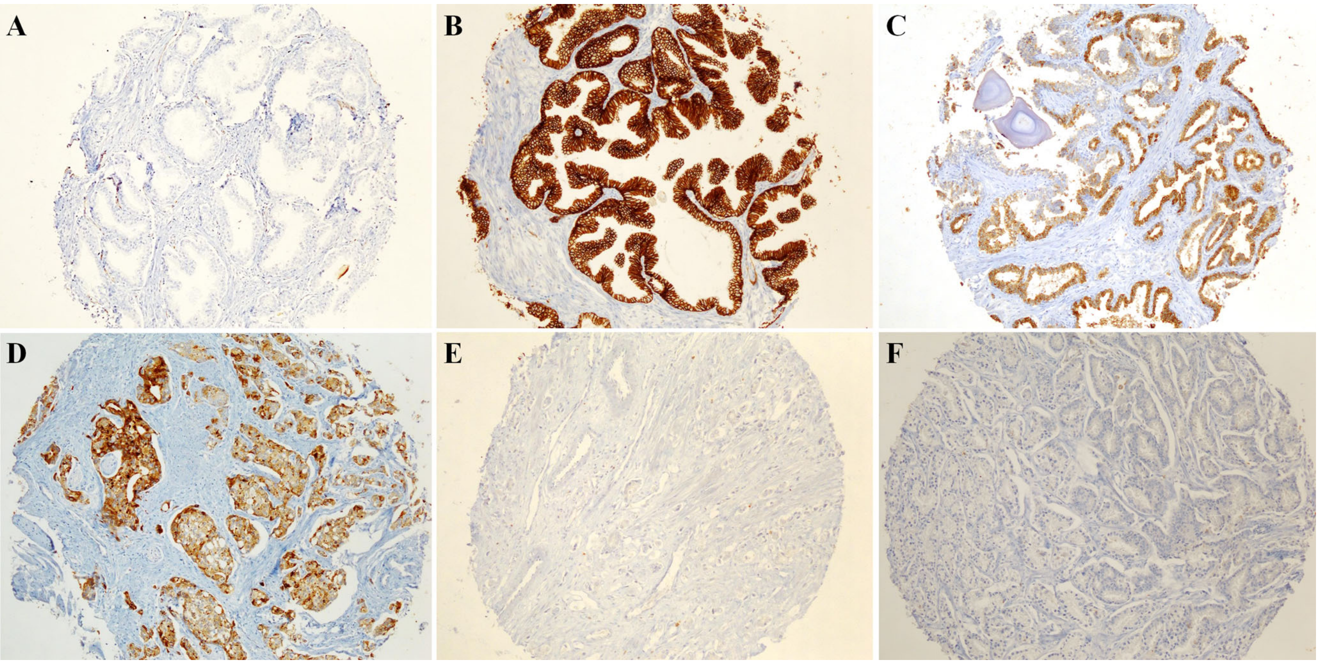

Fig. 1 Representative features of aquaporin (AQP)-1, aquaporin-3, and aquaporin-5 expression in benign and malignant prostatic tissue. In benign prostatic tissue, most of the secretory cells are negative for AQP-1 (**a**), but they are positive for AQP-3 and AQP-5 and characteristic cellular expressions are evident, namely, a predominantly cytoplasmic-membranous pattern for AQP-3 (**b**) and cytoplasmic pattern for AQP-5 (**c**). In prostate adenocarcinomas, AQP-1 overexpression is evident in tumors with higher Gleason scores (≥8) (**d**). The expression of AQP-3 (**e**) and AQP-5 (**f**) tend to be reduced in cancer tissues compared with the corresponding benign tissues. (**a**–**f**, immunohistochemical stains, magnification × 200)

Table 2 Immunohistochemical profiles of aquaporin-1, aquaporin-3, and aquaporin-5 and clinicopathological correlations in prostate adenocarcinomas

Clinicopathological features		AQP-1 (*n* = 99)		*P* value	AQP-3 (*n* = 99)		*P* value	AQP-5 (*n* = 99)		*P* value
		−	+		−	+		−	+	
Age (years)	<65	28 (75.7)	9 (24.3)	*0.173*	17 (45.9)	20 (54.1)	*0.483*	32 (86.5)	5 (13.5)	*0.931*
	≥65	54 (87.1)	8 (12.9)		33 (53.2)	29 (46.8)		54 (87.1)	8 (12.9)	
Tumor size (cm)	<3	46 (85.2)	8 (14.8)	*0.596*	31 (57.4)	23 (42.6)	*0.132*	46 (85.2)	8 (14.8)	*0.587*
	≥3	36 (80.0)	9 (20.0)		19 (42.2)	26 (57.8)		40 (88.9)	5 (11.1)	
Combined Gleason score, *n* (%)	6 + 7	65 (90.3)	7 9.7)	***0.001***	36 (50.0)	36 (50.0)	*0.870*	65 (90.3)	7 (9.7)	*0.101*
	≥8	17 (63.0)	10 (37.0)		14 (51.9)	13 (48.1)		21 (77.8)	6 (22.2)	
Pathologic T stage, *n* (%)	pT2	30 (93.8)	2 (6.2)	***0.024***	18 (56.3)	14 (43.8)	*0.185*	29 (90.6)	3 (9.4)	*0.227*
	pT3a	35 (81.4)	8 (18.6)		23 (53.5)	20 (46.5)		38 (88.4)	5 (11.6)	
	pT3b	17 (70.8)	7 (29.2)		9 (37.5)	15 (62.5)		19 (79.2)	5 (20.8)	
Lymphovascular invasion, *n* (%)	−	68 (82.9)	14 (17.1)	*0.954*	38 (46.3)	44 (53.7)	*0.069*	71 (86.6)	11 (13.4)	*0.855*
	+	14 (82.4)	3 (17.6)		12 (70.6)	5 (29.4)		15 (88.2)	2 (11.8)	
Perineural invasion, *n* (%)	−	59 (79.7)	15 (20.3)	*0.160*	36 (48.6)	38 (51.4)	*0.525*	63 (85.1)	11 (14.9)	*0.380*
	+	23 (92.0)	2 (8.0)		14 (56.0)	11 (44.0)		23 (92.0)	2 (8.0)	
Marginal status, *n* (%)	−	38 (82.6)	8 (17.4)	*0.957*	25 (54.3)	21 (45.7)	*0.476*	41 (89.1)	5 (10.9)	*0.535*
	+	44 (83.0)	9 (17.0)		25 (47.2)	28 (52.8)		45 (84.9)	8 (15.1	
Biochemical recurrence, *n* (%)	−	73 (88.0)	10 (12.0)	***0.002***	44 (53.0)	39 (47.0)	*0.256*	73 (88.0)	10 (12.0)	*0.467*
	+	9 (56.3)	7 (43.8)		6 (37.5)	10 (62.5)		13 (81.3)	3 (18.8)	

*Variables with statistically significant associations ($p < 0.05$) with the expression profiles of AQPs and clinicopathological parameters are indicated in bold

§*AQP* aquaporin

the IHC profiles of AQP-1, AQP-3, and AQP-5 in PCA and the associations of these AQPs with the clinicopathological parameters.

Associations between Clinicopathological Parameters and Biochemical Recurrence

The overexpression of AQP-1 was significantly associated with higher GS (≥8) ($P = 0.001$), higher pT stage ($P = 0.024$), and BR ($P = 0.002$).

Survival analyses revealed that higher GS ($P = 0.001$) (Fig. 2a), higher pT stage ($P = 0.023$), and AQP-1 overexpression ($P = 0.013$) (Fig. 2b) were associated with BR. Univariate analysis showed that larger tumor size (≥3 cm), higher GS (≥8), higher pT stage, and AQP-1 overexpression were prognostic factors for BR (Table 3). Age, the preoperative PSA tendency (data not shown), lymphovascular invasion, perineural invasion, and marginal status were not associated with BR. Multivariate analysis determined that a higher combined GS (≥8) was an independent factor for BR. AQP-3 and AQP-5 expression did not show any statistical associations with BR.

When parameters other than the GS were entered into the multivariate analysis model, the overexpression of AQP-1 (hazard ratio [HR], 3.095; 95 % confidence interval [CI], 1.201–8.198; $p = 0.042$) and tumors staged at pT3b (seminal vesicle invasion) (HR, 3.135; 95 % CI, 1.139–8.993; $p = 0.029$) were independent prognostic factors for BR.

Table 3 summarizes the analysis of the prognostic factors for BR in association with the clinicopathological parameters.

Discussion

Prostate cancer is the second-most common cause of death from cancer in men [22]. Despite a considerable amount of research effort, limited information has been published about biomarkers that may be able to discriminate between those PCAs that have the greatest potential to progress and those that will remain latent [23].

AQPs are a family of membrane proteins that are involved in the selective transport of water and solutes across cell membranes, and they may be involved in cell proliferation, extravasation, migration, and metastasis in a variety of tissues [3, 24–27]. Within this family of proteins, AQP-1 and AQP-5 act as water channels mainly, and AQP-3 transports water and small molecules, including glycerol and urea [3, 24].

In this study, we examined the potential of AQP-1, AQP-3, and AQP-5 as novel biomarkers and their utility in assessing individual patient outcomes in PCA.

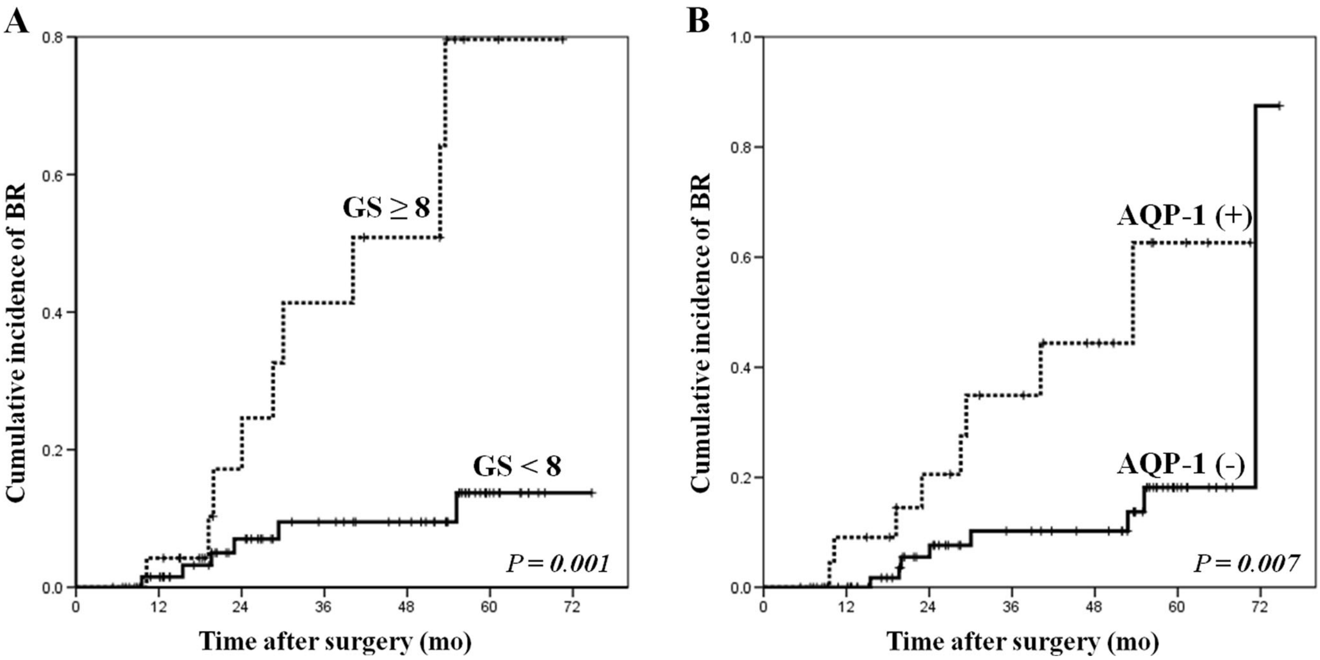

Fig. 2 Cumulative incidence of biochemical recurrence (BR). A higher Gleason score (GS) (≥8) (**a**) and the overexpression of aquaporin (AQP)-1 (**b**) are significantly correlated with BR

We demonstrated a distinct AQP-1 overexpression in tumors with higher GS (≥8) compared with those with lower GS (6 + 7) and benign prostatic tissues. In addition, the overexpression of AQP-1 was correlated with a higher pT3 stage (pT3b) and BR. This finding might represent a pathophysiological change in relation to the biological behavior of the tumors.

Previous studies have shown that AQP-1 expression affected angiogenesis, cell proliferation and migration, local invasion, tumor cell extravasation, and metastasis [8, 9, 28–30].

AQP-1 promoted endothelial cell migration and tumoral angiogenesis, and, it therefore enhanced tumor progressions [31]. Furthermore, the overexpression of AQP-1 was common in malignancies within a variety of organs and tissues [5, 8, 32, 33]. Several cancer cell lines that showed AQP-1 overexpression, exhibited an increased cell migration potential in vitro and greater metastatic potential in vivo [9]. Recent studies proposed that AQP-1 overexpression of tumor cells facilitates abnormally active metabolism that changes intra-

Table 3 Univariate and multivariate analyses of factors potentially associated with biochemical recurrence

Clinicopathological parameters		Univariate analysis			Multivariate analysis		
		HR	95 % CI	*P* value	HR	95 % CI	*P* value
Age (years)	<65 vs. ≥ 65	0.881	0.312–2.488	*0.811*	–	–	–
Tumor size (cm)	<3 vs. ≥ 3	3.364	1.070–10.574	***0.038***	1.819	0.413–8.010	*0.429*
Combined Gleason score (sum)	6 + 7 vs. ≥ 8	5.406	1.944–15.033	***0.001***	5.406	1.944–15.033	***0.001***
Pathologic T (pT) stage	pT2	1	Reference		1	Reference	
	pT3a	1.084	0.242–4.848	*0.916*	0.610	0.122–3.052	*0.473*
	pT3b	3.886	1.027–14.702	***0.046***	1.673	0.362–7.727	*0.572*
Lymphovascular invasion	– vs. +	1.870	0.591–5.916	*0.287*	–	–	–
Perineural invasion	– vs. +	2.013	0.453–8.949	*0.358*	–	–	–
Marginal status	– vs. +	1.297	0.461–3.652	*0.622*	–	–	–
AQP-1	– vs. +	3.346	1.220–9.174	***0.019***	1.931	0.620–6.107	*0.256*
AQP-3	– vs. +	1.710	0.621–4.708	*0.299*	–	–	–
AQP-5	– vs. +	1.928	0.543–6.843	*0.310*	–	–	–

*Variables with statistically significant associations ($p < 0.05$) are indicated in bold

§*AQP* aquaporin, *HR* hazard ratio, *CI* confidence interval and extracellular osmotic pressure, which might be important in the development and progression of tumors [9, 34, 35].

In addition, the treatment of AQP inhibitor or RNA interference to inhibit the overexpression of AQP1 demonstrated that progression of tumor cells was inhibited thougth suppressing of tumor cell metabolism [36]. Therefore, AQP1 is closely associated with tumor development and progression through multiple pathways.

In the present study, a significant overexpression of AQP-1 staining was observed in PCAs with higher GS compared with PCAs with lower GS. Although the multivariate analysis showed that the GS was the only significant prognostic factor for BR, when parameters other than the GS were entered into the multivariate analysis model, the overexpression of AQP-1 and pT3b stage were independent prognostic factors for BR. Therefore, our results suggest that the overexpression of AQP-1 might be associated with prostate cancer progression.

Since this study focused on the AQPs expression within tumor cells and the AQP-1 expression of endothelial cells was not evaluated, we are not in a position to discuss the implication of AQP-1 for tumor angiogenesis. However, the positive AQP-1 staining within tumor cells was predominantly cytoplasmic and it was sometimes concentrated towards the lumen, which indicated that AQP-1 participate actively in cellular function; as well, the interpretation of staining pattern might be useful in clinical practice.

Recently, it has been suggested that an AQP inhibitor could be used for cancer therapy, a proposal that was based on restraining a tumor's malignant potential by limiting increase in tumor cell migration and proliferation, and arresting angiogenesis within the tumor. Bin et al. reported that acetazolamide, a carbonic anhydrase inhibitor, inhibited AQP-1 expression in colon cancer xenograft-models; hence, AQP-1 could be a target for cancer treatment [37].

We also evaluated the expression of AQP-3 and AQP-5 in malignant and benign areas of the same tissues. AQP-3 staining showed a cytoplasmic-membranous pattern, and positivity for AQP-5 was mainly located in the cytoplasm. The expressions of AQP-3 and AQP-5 were less frequent in the malignant tissues, but these differences were not statistically significant compared with the expression of these AQPs in benign tissues, and correlations with clinicopathological parameters were not established. However, the AQP-3 and AQP-5 expressions in PCA would appear to have specific cellular function at the appointed sublocal regions. Wang et al. demonstrated AQP-3 expression in the membrane region of normal prostate cells, whereas AQP-3 expression was apparent in the cytoplasm of prostate cancer cells [29]. They explained that the distinct sublocalization of AQP-3 would serve to transport water and small solutes into the intracellular compartment and would induce functional changes in tumor cells. Another study showed that AQP-5 interacted with non-receptor cytoplasmic tyrosine kinase, an activated form of proto-oncogene tyrosine-protein kinase Src, and increased cellular migration and invasion [38]. Therefore, these proteins may play key roles within the secretory cells of the prostate gland, and they may be involved in maintaining normal cellular function and homeostasis. Although the statistical significances were not established, in this study, their reduced expressions in malignant tissues compared with benign tissues may indicate their specific roles in prostatic tumorigenesis, which are rendered to establish.

Conclusions

To the best of our knowledge, this is the first report that describes an evaluation of AQP-1 as a prognostic biomarker for prostate cancer. We have provided fundamental information about the expression of AQP-1, AQP-3, and AQP-5 in malignant and benign prostatic tissues, and we have evaluated the clinicopathological significance of these AQPs.

It appears that the overexpression of AQP-1 is associated with prostate cancer progression and that it is a prognostic factor for BR. These findings concur with those from previous studies that have suggested that AQP-1 may be involved in tumor growth, invasiveness, and progression in a variety of tumors.

Even though the IHC results did not address the significance of AQP-3 and AQP-5 expression in PCA, these proteins appear to have specific cellular locations that might imply that AQPs have different functional roles depending on their subtypes and cellular locations.

This study was limited in its ability to determine conclusive associations between the expression of aquaporins and the clinicopathological parameters because of the small number of patients. However, further studies involving larger numbers of patients are warranted to investigate the roles of the different aquaporins in PCA progression.

AQP, aquaporin; ATP, adenosine triphosphate; BR, biochemical recurrence; GS, Gleason score; IHC, immunohistochemical; PCA, prostate adenocarcinomas; PSA, prostate-specific antigen; pT stage, pathologic T stage; SD, standard deviation; TMA, tissue microarray.

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Compliance with Ethical Standards

Conflict of Interest The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

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