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ORIGINAL ARTICLE

Different Prognostic Implications of Aquaporin-1 and Aquaporin-5 Expression among Different Histological Types of Ovarian Carcinoma

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Abstract

The aquaporins (AQPs) are a family of transmembrane water channel proteins that are distributed in various human tissues. Recent studies have suggested that AQP expression correlates with various aspects of cancer biology that determine the aggressiveness of different cancers. Ovarian carcinoma is one of the most lethal gynecological cancers. Some studies have suggested that AQPs are expressed in ovarian carcinoma, and are associated with cancer cell growth and migration. In this study, we immunohistochemically evaluated the expression of AQP1, 3, 5, and 9 in a total of 300 ovarian carcinomas using tissue microarrays. In our analyses of correlations between aquaporin expression and overall survival, high AQP5 expression was significantly associated with poorer prognosis ($P = 0.029$). For AQP1, the low expression group trended towards poorer prognosis than the high expression group, but the difference was not statistically significant. When ovarian carcinomas were divided by histological types, high AQP5 expression correlated with poorer prognosis in serous carcinoma ($P = 0.015$), and low AQP1 expression correlated with poorer prognosis in clear cell carcinomas ($P = 0.0055$). By contrast, high AQP1 expression correlated with poorer prognosis in mucinous carcinoma ($P = 0.0001$) and endometrioid carcinoma ($P = 0.021$). Our studies suggest that AQPs can be useful prognostic markers in ovarian carcinoma, but their correlation with prognosis depends on the histological type of ovarian carcinoma.

Keywords Aquaporin · Water channel · Ovarian carcinoma · Immunohistochemistry · Tissue microarray

Introduction

Aquaporins (AQPs) are transmembrane water channels. Thirteen human AQP genes have been identified [1]. Aquaporins 1, 2, 4, 5 and 8 function as water-selective transporters, while AQP3, 7, 9 and 10, termed 'aquaglyceroporins', can transport water and small neutral solutes such as glycerol [2]. AQPs play important roles in physiological conditions including urinary concentration, exocrine gland fluid secretion, and fat metabolism, and pathological conditions including brain edema [1]. Recent discoveries suggest that AQPs are involved in cell proliferation and migration, and play key roles in tumor biology [3–8]. AQP-expressing cancer cells have been shown to display enhanced migration and proliferation in vitro, and greater invasion and metastasis in vivo [4–8]. Aquaporins are expressed in tumor cells of various origins, including some particularly aggressive tumor types [9–13].

Ovarian carcinoma is the most lethal gynecological cancer. The prognosis for patients with advanced-stage ovarian carcinoma remains poor despite aggressive surgery and recent advances in chemotherapy [14, 15]. Some studies have suggested that members of the AQP family are expressed in ovarian carcinoma [16–22], but the relationship of their expression to prognosis has not been elucidated. In this study, we have investigated expression of AQPs in ovarian carcinoma by immunohistochemistry in order to clarify the clinical implications of their expression. Past research suggests that expression of AQPs 1, 3, and 5 correlates most strongly with prognosis in various cancers [8]. In addition, some authors have shown clinical significance of AQP9 expression in ovarian carcinoma [18]. Therefore, we focused our study on these four AQPs.

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Materials and Methods

Patient Data

Formalin-fixed paraffin-embedded tissue blocks from 300 patients with ovarian carcinoma who received surgery at the National Defense Medical College Hospital between 1983 and 2010 were collected. A total of 300 patients who met the inclusion criteria were enrolled in this investigation: i) patients who received no prior chemotherapy before surgical therapy; ii) patients who were diagnosed to have ovarian carcinoma by pathological evaluation; iii) patients whose histological subtype was serous, endometrioid, mucinous, and clear cell type; iv) patients whose medical information, and tissue blocks were available. Patient characteristics are summarized in Table 1. The tissue blocks included 123 serous carcinomas (SC), 53 mucinous carcinomas (MC), 40 endometrioid carcinomas (EC), and 84 clear cell carcinomas (CCC). Patients' ages were between 16 and 82 years; the median was 53 years. The stage of each cancer was determined according to the International Federation of Gynecology and Obstetrics (FIGO) system. The number of cases in FIGO stage I, II, III, and IV were 118 (39.3%), 35 (11.7%), 110 (36.7%), and 37 (12.3%), respectively. Optimal surgery (residual tumor ≤1 cm) was performed in 183 cases (61.0%), and suboptimal surgery (residual tumor >1 cm) was performed in 117 cases (39.0%). For 273 patients (91.0%), postoperative chemotherapy was performed: 140 cases with cyclophosphamide, adriamycin and cisplatin (CAP), 60 cases with paclitaxel and carboplatin (TC), 33 cases with irinotecan and cisplatin (CPT-P), 15 cases with etoposide and cisplatin (EP), 9 cases with docetaxel and carboplatin (DC), and 16 cases by the other regimens. Patients who received neoadjuvant chemotherapy were excluded. The research protocol was approved by the Institutional Ethical Review Board Committee of the National Defense Medical College, Tokorozawa, Japan. Informed consent was obtained from the individuals included in this study.

Table 1 Clinicopathological characteristics of the patients

Characteristic	Number of patients (%)
Age (years)	
< 50	112 (37.3)
≥ 50	188 (62.7)
FIGO stage	
I	118 (39.3)
II	35 (11.7)
III	110 (36.7)
IV	37 (12.3)
Histological types	
Serous carcinoma	123 (41.0)
Mucinous carcinoma	53 (17.7)
Endometrioid carcinoma	40 (13.3)
Clear cell carcinoma	84 (28.0)
Lymph node metastasis	
Positive	35 (11.7)
Negative	119 (39.7)
Not evaluated	146 (48.7)
Residual tumor at primary surgery	
Optimal	183 (61.0)
Suboptimal	117 (39.0)
Postoperative chemotherapy	
Yes	273 (91.0)
No	26 (8.7)
Uncertain	1 (0.3)

Tissue Microarray Construction

From each tumor tissue block, two 1.5-mm cores were punched. These cores were arranged on a tray, and tissue microarray (TMA) blocks were constructed. All TMA blocks were cut into 4-μm-thick slices to make sections for immunohistochemical (IHC) staining.

Immunohistochemistry

For IHC staining, we used rabbit polyclonal antibodies for AQP1 (dilution 1:300; Bioss bs-1506R, Boston, MA, USA), AQP3 (dilution 1:200; Bioss bs-1253R), AQP5 (dilution 1:400; Bioss bs-1554R), and AQP9 (1:400; Bioss bs-2060R). Tissue microarray slides were deparaffinized in xylene and hydrated with alcohol. Antigen retrieval was performed by incubating slides with Dako Target Retrieval Solution, pH 9, for 60 min at 98 °C. Endogenous peroxidase activity was blocked with 0.3% H_2O_2/methanol. Slides were incubated with primary antibodies overnight at 4 °C, then exposed to the DAKO REAL EnVision system/HRP, containing Rabbit/Mouse secondary antibodies for 60 min at 18 °C. Specific antigen-antibody reactions were visualized with Dako REAL DAB+ chromogen from the kit, and counterstained with Mayer hematoxylin. We also stained positive controls including human lung, kidney and liver, simultaneously.

A semi-quantitative evaluation of the AQPs immunoreactivity was done scoring both the staining intensity (no staining =0, weak staining = 1, strong staining = 2) and the amount of positivity stained cells (0 = 0%, 1 = 1–50%, 2 ≥ 50%). The multiplied scoring resulted in an immunoreactivity score between 0 and 4. If the immunoreactivity score was 4, that tissue was defined as high expression. Tissue in which less than score 4 were defined as low expressing.

Statistical Analysis

The JMP Pro software version 12 (SAS Institution Inc., Cary, NC, USA) was used for statistical analysis. Progression-free survival (PFS) was defined as the interval between completion of upfront treatment and death/disease progression. Overall survival (OS) was defined as the interval between diagnosis or the start of treatment and death. The χ^2 test and Fisher's exact test were used to evaluate differences in correlation between expression of AQPs and clinicopathological parameters. PFS and OS curves were generated using the method of Kaplan and Meier. Comparison of survival distributions was performed with a log-rank test. Univariate and multivariate analyses were performed using Cox's proportional hazards model to evaluate risk factors for cancer-related mortality. Statistical significance was defined as $P < 0.05$.

Results

Distribution of AQPs in Ovarian Carcinomas

High AQP1, AQP3, AQP5, and AQP9 expression were detected in 29 (9.7%), 57 (19%), 204 (68%), and 5 (1.7%) cases of ovarian carcinoma, respectively. AQP1 was observed in the plasma membrane, and occasionally in the cytoplasm of highly expressing cancer cells (Fig. 1a, b). Although it was also strongly expressed in the microvascular endothelium (Fig. 1c), we evaluated only cancer cells in this study. AQP3 was found in the basolateral membrane (Fig. 1d) or cytoplasm of highly expressing cancer cells. AQP5 and AQP9 were mainly found in the cytoplasm of highly expressing cancer cells (Fig. 1e–h). In cells with low expression, AQPs were weakly observed mainly in the cytoplasm (Fig. 1c, f).

Relations of AQP Expression with FIGO Stage and Histological Type

Relationships between AQP expression and clinicopathological characteristics are summarized in Table 2. Low AQP3 expression was significantly more frequent in ovarian carcinomas at FIGO stage III or IV (127 of 146, 87.0%) than in ovarian carcinomas at FIGO stage I or II (116 of 154, 75.3%) ($P = 0.012$). Such a relationship was not observed for AQP1, AQP5, and AQP9. Our analyses of AQP expression in each histological type of ovarian carcinoma revealed that the percentage of tissues expressing high levels of AQP1 was significantly greater in CCC (19 of 84, 22.6%) than in SC (3 of 123, 2.4%), MC (4 of 53, 7.6%), and EC (3 of 40, 7.5%) ($P < 0.0001$), and the percentage of tissues expressing high levels of AQP3 was significantly greater in MC (20 of 53, 37.7%), EC (9 of 40, 22.5%) and CCC (22 of 84, 26.2%) than in SC (6 of 123, 4.9%) ($P < 0.0001$). The percentage of tissues expressing high levels of AQP5 was also significantly greater in MC (34 of 53, 64.2%), EC (31 of 40, 77.5%) and CCC (64 of 84, 76.2%) than in SC (75 of 123, 61.0%) ($P = 0.033$). The level of AQP9 expression did not correlate with the histological type of ovarian carcinoma. AQP expression did not significantly correlate with patient age or lymph node metastasis.

Relationship between AQP Expression and Prognosis

Overall survival curves were significantly different between patient groups with high and low AQP5 ovarian carcinomas ($P = 0.029$) (Fig. 2a). Overall survival rates 5 years after initial treatment were 59.7% in the high AQP5 group, and 74.4% in the low AQP5 group. The difference of PFS curves between the high and low AQP5 expression groups was not statistically significant ($P = 0.216$) (data not shown). The differences of OS and PFS curves between patient groups with high and low AQP1 expression were not statistically significant ($P = 0.325$ and 0.168, respectively) (Fig. 2b). Overall survival and progression free survival rates 5 years after initial treatment were 63.7 and 52.5% in the low AQP1 group, and 71.2 and 64.5% in the high AQP1 group, respectively. Therefore, the prognostic impacts of AQP5 and AQP1 expression in ovarian carcinomas appear to be different. Overall survival and progression free survival rates did not correlate with levels of AQP3 or AQP9 expression (Fig. 2c, d).

Prognostic Significance of AQP Expression in Different Histological Types of Ovarian Carcinoma

When ovarian carcinomas were divided by histological type, high AQP5 expression was significantly associated with poorer prognosis in SC ($P = 0.015$) (Fig. 3a). Five years after initial treatment, OS rates were 44.2% in the high AQP5 subgroup, and 66.9% in the low AQP5 subgroup. This correlation between AQP5 expression and OS was not observed in other histological types. Low AQP1 expression was significantly associated with poorer prognosis in CCC ($P = 0.0055$) (Fig. 3b). Overall survival rates 5 years after initial treatment were 58.8% in the low AQP1 subgroup, and 94.4% in the high AQP1 subgroup. By contrast, high AQP1 expression was significantly associated with worse OS in MC ($P = 0.0001$) and EC ($P = 0.021$) (Fig. 3c, d). In MC, OS rates 5 years after initial treatment were 0% in the high AQP1 subgroup, and 83.2% in the low AQP1 subgroup. Likewise, in EC, OS rates in high and low AQP1 subgroups 5 years after initial treatment were 33.3 and 81.8%, respectively. Similar

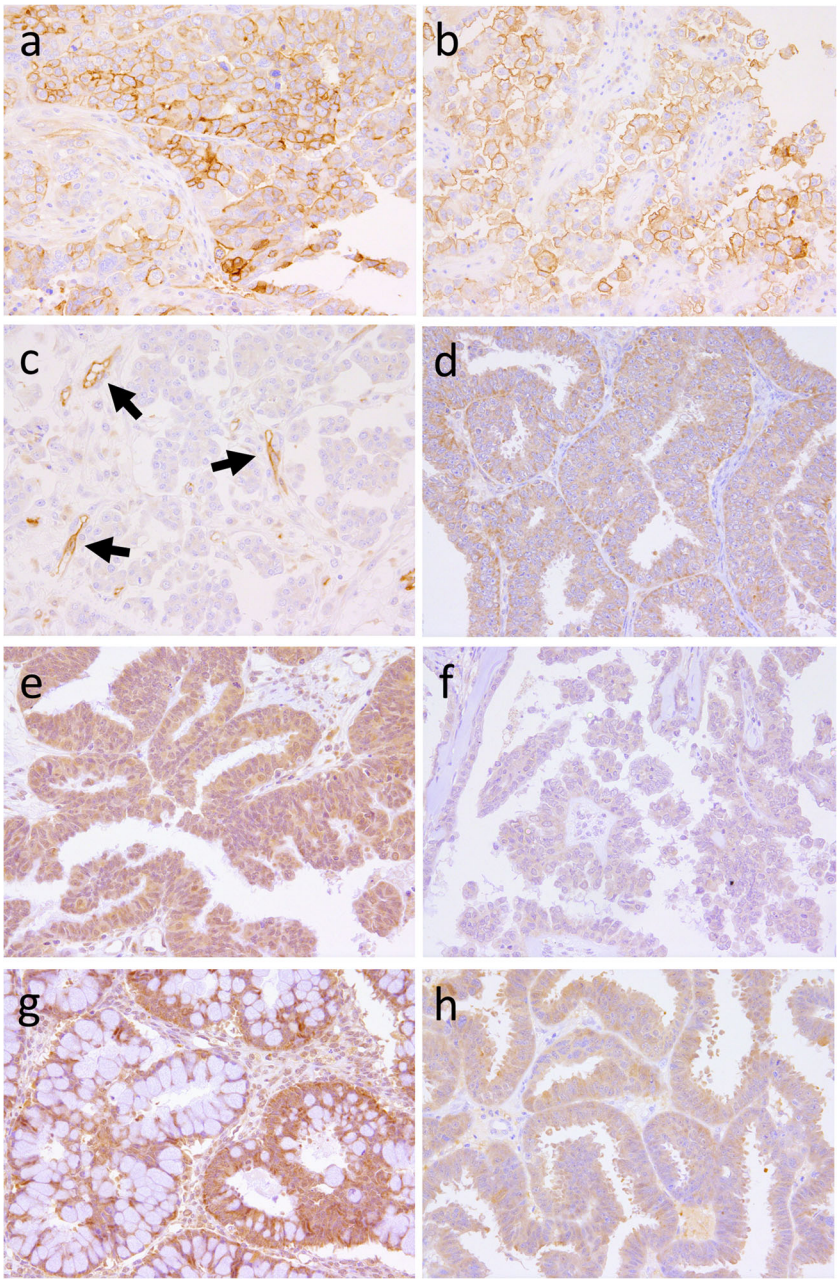

Fig. 1 Representative IHC stains of AQPs in tissue-microarray based samples of ovarian carcinoma (×400). **a** to **c** AQP1. **a** Intensity score 2 in serous carcinoma and (**b**) intensity score 2 in clear cell carcinoma. Highly expressed AQP1 was immunoreactive mainly on cell membrane, and occasionally in cytoplasm. **c** Intensity score 1 in serous carcinoma. Microvessels were also positive (*arrows*). **d** AQP3. Intensity score 2 in endometrioid carcinoma. Basolateral membranes of cancer cells were positive in this case. **e** to **g** AQP5. **e** Intensity score 2 and (**f**) intensity score 1 in serous carcinoma. Highly expressed AQP5 (**e**) is immunoreactive in cytoplasm. **g** Intensity score 2 in mucinous carcinoma. **h** AQP9. Intensity score 2 in serous carcinoma

correlations were observed between AQP1 expression and PFS in CCC, EC, and MC, and these correlations were statistically significant ($P = 0.0036$, $P = 0.019$, $P = 0.0022$, respectively) (data not shown).

Analyses using Cox's univariate proportional hazard model revealed that FIGO stage III/IV, suboptimal surgery, and high AQP5 were significant indicators of poor prognosis. Multivariate analysis including these three parameters revealed that they are independent indicators of poor prognosis (hazard ratio = 3.027, $P < 0.0001$; hazard ratio = 2.059, $P = 0.002$; hazard ratio = 1.694, $P = 0.013$, respectively) (Table 3). High AQP5 was an independent indicator of poor prognosis in SC (hazard ratio = 1.859, $P = 0.025$) (Table 4). FIGO stage III/IV (hazard ratio = 3.415, $P = 0.014$), and low AQP1 (hazard ratio = 9.124, $P = 0.002$) are independent indicators of poor prognosis (Table 5).

Table 2 Expression of AQPs and clinicopathological characteristics

Parameters	Number of patients (%)	AQP1			AQP3			AQP5			AQP9		
		High (n = 29)	Low (n = 271)	*P*-value	High (n = 57)	Low (n = 243)	*P*-value	High (n = 204)	Low (n = 96)	*P*-value	High (n = 5)	Low (n = 295)	*P*-value
Age (years)													
<50		9 (8.0)	103 (92.0)	0.547	24 (21.4)	88 (78.6)	0.448	74 (66.1)	38 (33.9)	0.610	2 (1.8)	110 (98.2)	1.000
≥50		20 (10.6)	168 (89.4)		33 (17.6)	155 (82.5)		130 (69.2)	58 (30.9)		3 (1.6)	185 (98.4)	
FIGO stage													
I -II		17 (11.0)	137 (89.0)	0.440	38 (24.7)	116 (75.3)	**0.012***	105 (68.2)	49 (31.8)	1.000	1 (0.7)	153 (99.4)	0.204
III -IV		12 (8.2)	134 (91.8)		19 (13.0)	127 (87.0)		99 (67.8)	47 (32.2)		4 (2.7)	142 (97.3)	
Histological types													
Serous carcinoma		3 (2.4)	120 (97.6)		6 (4.9)	117 (95.1)		75 (61.0)	48 (39.0)		3 (2.4)	120 (97.6)	
Mucinous carcinoma		4 (7.6)	49 (92.5)		20 (37.7)	33 (62.3)	**<0.0001***	34 (64.2)	19 (35.9)	**0.033***	0 (0)	53 (100)	
Endometrioid carcinoma		3 (7.5)	37 (92.5)	**<0.0001***	9 (22.5)	31 (77.5)		31 (77.5)	9 (22.5)		2 (5.0)	38 (95.0)	
Clear cell carcinoma		19 (22.6)	65 (77.4)		22 (26.2)	62 (73.8)		64 (76.2)	20 (23.8)		0 (0)	84 (100)	
Lymph node metastasis													
Positive		6 (17.1)	29 (82.9)	0.588	4 (11.4)	31 (88.6)	0.157	25 (71.4)	10 (28.6)	0.685	0 (0)	35 (100)	1.000
Negative		16 (13.5)	103 (86.6)		28 (23.5)	91 (76.5)		80 (67.2)	39 (32.8)		2 (1.7)	117 (98.3)	

*Statistically significant

AQP, aquaporin; FIGO, The International Federation of Gynecology and Obstetrics

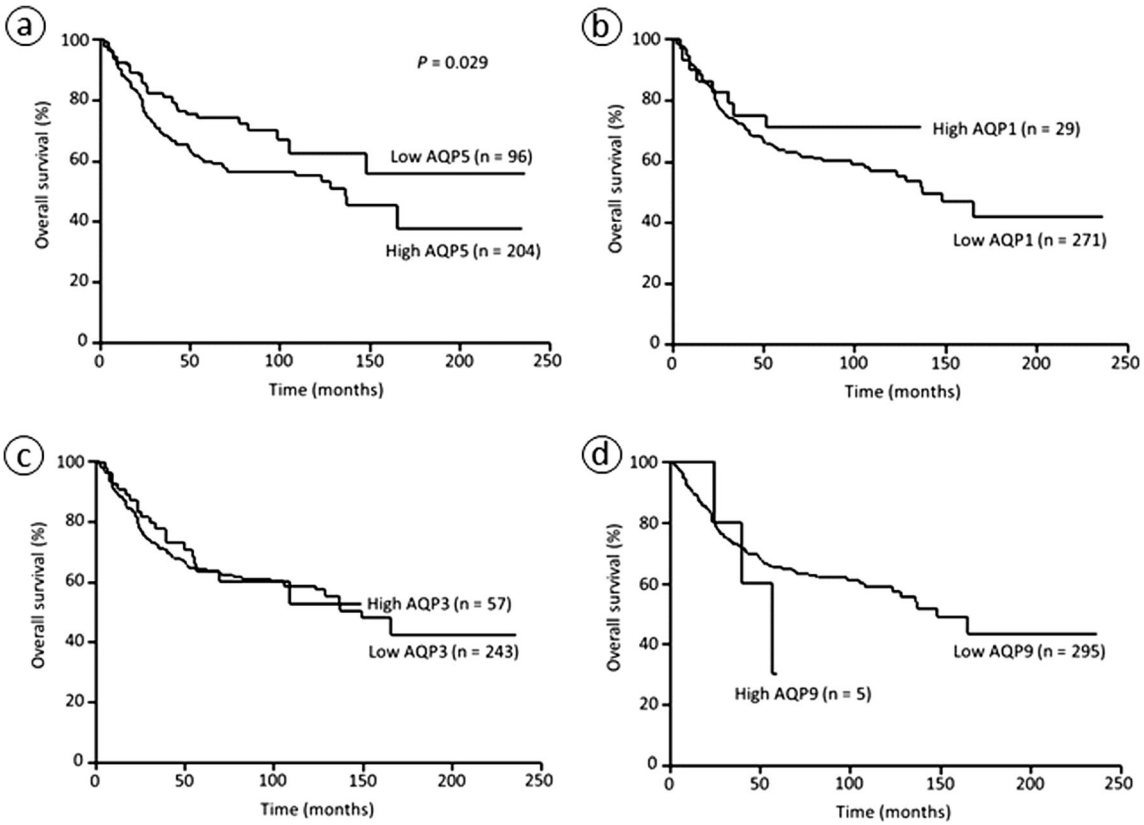

Fig. 2 Overall survival curves for patients with ovarian carcinomas with high and low expressions of AQP5 (**a**), 1 (**b**), 3 (**c**) and 9 (**d**). **a** Low AQP5 expression was significantly ($P = 0.029$) associated with better patient prognosis than high AQP5 expression. **b** Low AQP1 expression was associated with poorer prognosis than high AQP1 expression, but the difference was not statistically significant. **c** Low and high expression of AQP3 were not associated with prognosis. **d** High AQP9 expression was correlated with poorer prognosis, but the difference between the two curves was not statistically significant

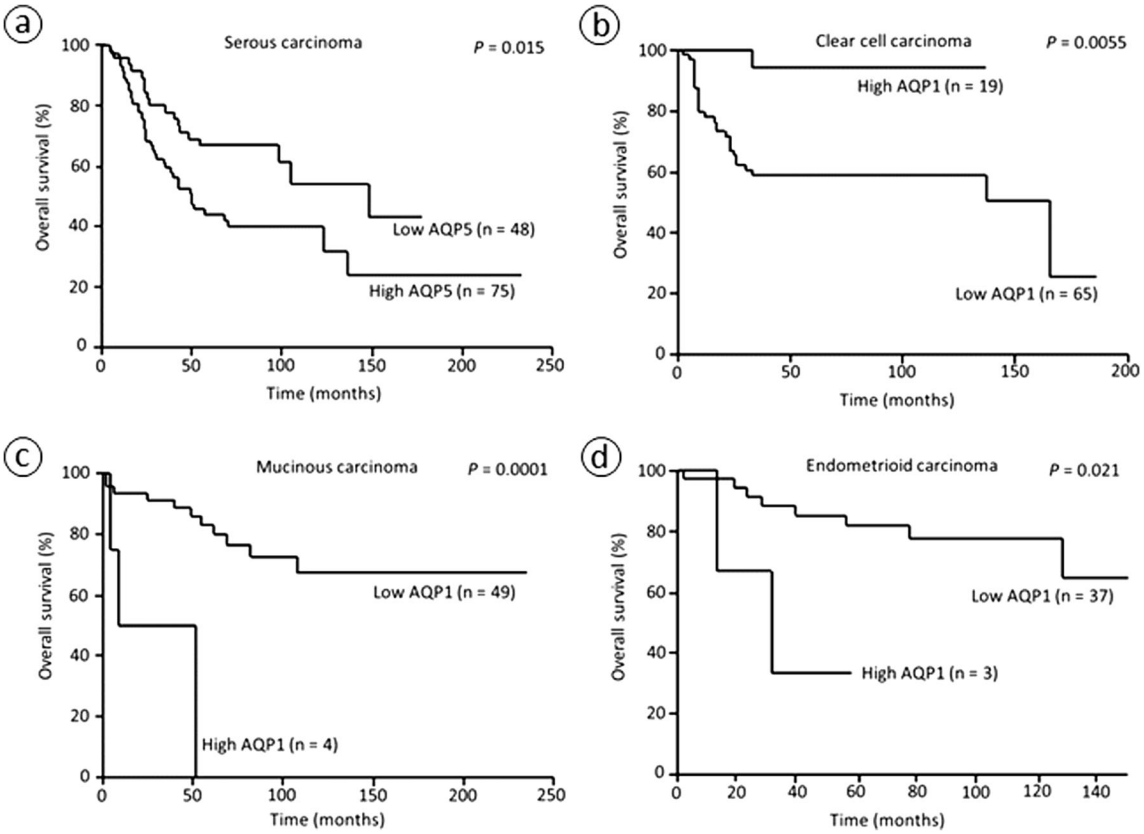

Fig. 3 Overall survival curves for patients with ovarian cancers of different histological type with high and low AQP5 and AQP1 expression. **a** Curves for patient groups with high and low AQP5 expression in serous carcinoma. The high AQP5 expression group showed poorer prognosis than the low AQP5 expression group ($P = 0.015$). **b** Curves for patient groups with low and high AQP1 expression in clear cell carcinoma. The patient group with low AQP1 expression showed significantly poorer prognosis than the group with high AQP1 expression ($P = 0.0055$). **c** Curves for patient groups with high and low AQP1 expression in mucinous carcinoma. **d** Curves for patient group with high and low AQP1 expression in endometrioid carcinoma

Discussion

In the present study, we examined the expression patterns of AQP1, 3, 5, and 9 proteins in 300 ovarian carcinoma tissues using immunohistochemical analysis. Increased expression of AQP5 was significantly associated with poorer prognosis in patients with ovarian carcinoma, and especially with SC. Multivariate analysis confirmed that high AQP5 expression was an independent prognostic factor for predicting poor OS. We also observed that high expression of AQP1 was

Table 3 Univariate and multivariate analyses of parameters associated with the overall survival of patients with ovarian carcinoma

Parameters	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age (≥ 50 years vs. < 50 years)	1.112 (0.760–1.650)	0.588		
FIGO stage (III/IV vs. I/II)	4.521 (2.976–7.083)	< 0.0001*	3.027 (1.802–5.156)	< 0.0001*
Suboptimal surgery (yes vs. no)	3.863 (2.643–5.712)	< 0.0001*	2.059 (1.307–3.317)	0.002*
Postoperative chemotherapy (yes vs. no)	2.192 (0.919–7.156)	0.081		
AQP1 (low vs. high)	1.431 (0.743–3.199)	0.305		
AQP3 (high vs. low)	0.951 (0.570–1.510)	0.839		
AQP5 (high vs. low)	1.600 (1.057–2.496)	0.026*	1.694 (1.117–2.649)	0.013*
AQP9 (high vs. low)	1.642 (0.404–4.367)	0.433		

*Statistically significant

CI, confidence interval; FIGO, The International Federation of Gynecology and Obstetrics; AQP, aquaporin

Table 4 Univariate and multivariate analyses of parameters associated with the overall survival of patients with serous carcinoma of the ovary

Parameters	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age (≥ 50 years vs. < 50 years)	0.941 (0.557–1.643)	0.826		
FIGO stage (III/IV vs. I/II)	2.902 (1.352–7.548)	0.005*	2.079 (0.884–5.732)	0.097
Suboptimal surgery (yes vs. no)	2.488 (1.412–4.638)	0.001*	1.810 (0.978–3.576)	0.059
AQP1 (high vs. low)	2.049 (0.335–6.605)	0.371		
AQP3 (high vs. low)	0.525 (0.086–1.687)	0.322		
AQP5 (high vs. low)	1.969 (1.148–3.518)	0.013*	1.859 (1.080–3.331)	0.025*
AQP9 (high vs. low)	1.541 (0.252–4.972)	0.574		

*Statistically significant

CI, confidence interval; FIGO, The International Federation of Gynecology and Obstetrics; AQP, aquaporin

associated with poorer prognosis in EC and MC, and better prognosis in CCC. Multivariate analysis confirmed that high AQP1 expression was an independent poor prognostic factor in EC, and low AQP1 expression was an independent poor prognostic factor in CCC. Our results suggest that expression of AQP1 protein may have different clinical significance in different histological types of ovarian carcinoma.

AQPs are water channel proteins that facilitate transcellular water movement. Accumulating evidence suggests that AQPs are involved in cell migration and proliferation, processes that play important roles in the pathogenesis of cancer [3–8]. The molecular mechanism(s) by which AQPs influence these processes in cancer biology is not fully understood. One proposed mechanism for AQP1-modulated tumor cell migration is that AQP1 permits water flow across the plasma membrane in response to an osmotic gradient created by cytosolic actin depolymerisation, and active solute influx at the leading edge of migrating cells [4, 7]. Resistance to apoptosis has been proposed to be part of the mechanism underlying enhanced proliferation of AQP1 expressing cells [23, 24]. Potential downstream effectors in signaling pathways that are also implicated in AQP1-mediated tumor progression include TGF-β, FAK, β-catenin, RhoA and Rac [24–26]. AQP5 may promote cell proliferation and metastatic potential by activating the EGFR/Ras/ERK/p38 MAPK pathway [27, 28].

Previous studies showed various aquaporins expressed in ovarian carcinoma [16–22]. Yang et al. suggested that overexpression of AQP5 played an important role in tumorigenesis of ovarian carcinomas, which might be related to ascites formation by ovarian carcinoma [16]. In addition, AQP5 expression was associated with proliferation and migration of ovarian cancer cells in vitro and in vivo [29, 30]. To date, relationships between expression of aquaporins and patient prognosis have not been reported. In this study, we revealed that high AQP5 expression is associated with poorer prognosis of patients with ovarian carcinoma, especially those with SC. The high frequency of strong AQP5 expression in ovarian carcinoma, and its tendency to correlate with patient prognosis suggest that AQP5 may be a therapeutic target for ovarian carcinoma.

In our study, strong expression of AQP1 correlates with improved prognosis for patients with CCC, but poorer prognoses for patients with EC and MC. It is possible that the prognostic value of AQP1 expression varies depending on the histological type of ovarian carcinoma. In most studies, AQP1 expression has been correlated

Table 5 Univariate and multivariate analyses of parameters associated with the overall survival of patients with clear cell carcinoma of the ovary

Parameters	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age (≥ 50 years vs. < 50 years)	0.641 (0.304–1.388)	0.253		
FIGO stage (III/IV vs. I/II)	5.367 (2.422–13.05)	< 0.0001*	3.415 (1.285–9.307)	0.014*
Suboptimal surgery (yes vs. no)	4.682 (2.154–10.11)	0.0002*	2.364 (0.969–6.008)	0.059
AQP1 (low vs. high)	9.682 (2.056–172.8)	0.001*	9.124 (1.925–163.2)	0.002*
AQP3 (high vs. low)	1.345 (0.579–2.899)	0.473		
AQP5 (high vs. low)	1.477 (0.605–4.412)	0.413		

*Statistically significant

CI, confidence interval; FIGO, The International Federation of Gynecology and Obstetrics; AQP, aquaporin

with poor prognosis and increased aggressiveness of various cancers [9, 10, 23]. However, a few studies focused on cholangiocarcinoma and renal cell carcinoma have indicated that decreased AQP1 was associated with poorer patient prognosis [31–33]. Although the prognostic implications of AQP1 expression appear to differ among cancer types and among histological types even within a single cancer type, the number of studies is insufficient to draw conclusions. Nonetheless, it might be of interest to note that low AQP1 expression correlated with poorer patient outcome for patients with ovarian and renal CCC, most of which are clear cell adenocarcinomas. The relationship between AQP1 and glycogen metabolism may be relevant, as hypoxia-induced glycolysis enhances transcription of AQP1 through E-box/ChoRE [34]. It is possible that hypoxia and/or high levels of glycolysis in cancer cells might be associated with high AQP1 expression. Because high AQP1 expression was characteristically more frequent in CCC than in other histological types of ovarian carcinoma, quantitation of AQP1 expression in CCC might be a practical aid to prognostication and determination of treatment regimens in patients with CCC.

Although AQP3 expression was not associated with prognosis, low AQP3 expression was more frequent in the FIGO stage III and IV group than in the stage I and II group in this study. High AQP3 expression was observed relatively frequently in MC, CCC and EC, but was rare in SC. In previous studies, epidermal growth factor (EGF) and estrogen have been suggested to be upstream regulators of AQP3 expression [35, 36]. In cultured ovarian cancer cells, EGF treatment increased AQP3 expression [35]. In breast cancer cells expressing estrogen receptor, stimulation with estrogen transcriptionally upregulated expression of AQP3 [36]. Therefore, it is possible that these factors influence AQP3 expression to various degrees in each histological type.

Most cases of ovarian carcinoma (98%) showed low AQP9 expression. Only three cases of SC and two cases of EC showed high AQP9 expression. Because of the small number of cases, the clinicopathological significance of high AQP9 expression in ovarian carcinoma remains unclear.

In conclusion, our data suggest that strong expression of AQP1 and AQP5 proteins correlates with prognosis in patients with ovarian carcinoma. Overexpression of AQP5 may be an unfavorable prognostic factor for ovarian carcinoma. The relationship of AQP1 expression to prognosis varies with histological type of ovarian carcinoma. Two limitations of this study are that it only performs immunohistochemistry, and that it is retrospective. Although the role of AQPs in human tumor pathology has been explored extensively, their molecular mechanisms in different tumor types have not been fully elucidated. Further studies are needed to investigate the precise mechanisms of AQP1 and AQP5 in progression of ovarian carcinoma.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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