Human antigen R as a predictive marker for response to gemcitabine-based chemotherapy in advanced cisplatin-resistant urothelial cancer

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Abstract. In patients with advanced urothelial cancer (UC), a combination of cisplatin (CDDP) and gemcitabine (GEM) is the most commonly used first-line systematic chemotherapy regimen. Although no standard regime for the treatment of CDDP-resistant UC has been established, GEM-based regimens are frequently used in these patients. In other types of cancer, human antigen R (HuR) status in cancer cells is closely associated with patient response to GEM. The aim of the present study was to establish the predictive potential of HuR expression for disease progression and survival in patients with UC who were treated with GEM-based regimens as a first or second-line chemotherapy. A total of 50 patients with advanced UC were enrolled in the current study. As first-line chemotherapy, methotrexate, vinblastine, epirubicin and CDDP (MVEC) combination therapy and GEM and CDDP combination therapy were administered in 34 (68.0%) and 16 patients (32.0%), respectively. Following progression, 45 patients (90.0%) were treated with combined GEM and paclitaxel therapy, and 5 patients (10.0%) were treated with GEM monotherapy. Cytoplasmic and nuclear HuR expression was evaluated using immunohistochemical techniques. The associations between HuR expression levels and local tumor response and treatment outcomes were analyzed. In first-line chemotherapy, no anticancer effects were observed to be significantly associated with nuclear or cytoplasmic HuR expression. In second-line chemotherapy nuclear HuR expression also exhibited no significant association with anticancer effects; however, the local tumor response was significantly improved if positive cytoplasmic HuR expression was present (P=0.002). Multivariate analyses revealed that cytoplasmic HuR expression levels were a significant predictive marker for longer OS (hazard ratio, 0.22; 95% confidence interval, 0.09-0.56; P=0.001). No significant association was observed between nuclear HuR expression levels and the overall survival. Therefore, cytoplasmic HuR expression is a significant predictive marker of response to GEM-based chemotherapy in patients with CDDP-resistant UC. Despite the limitations of a small and retrospective study, the results of the present study may facilitate the development of novel treatment strategies and provide a focus for additional basic and clinical studies.

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Introduction

Regulation of mRNA decay is an important mechanism underlying the control of gene expression (1). The control of mRNA stability depends on sequences in the transcript, and on the RNA-binding proteins that dynamically bind to these sequences (2). Human antigen R (HuR) is a member of the embryonic lethal abnormal visual family of RNA-binding proteins. HuR has numerous functions, but one of the best characterized is the regulation of mRNA turnover and stability (3). Various molecules that are associated with cell proliferation, migration, immune response and angiogenesis have previously been identified as targets of HuR (3-5). Increased expression levels of HuR are significantly associated with malignant aggressiveness and poor survival in various types of cancer, including urothelial cancer (UC) (6-10).

Another important function of HuR is to increase the protein expression of deoxycytidine kinase (dCK), a key enzyme involved in metabolizing the prodrug GEM into its active metabolites through phosphorylation (11). Consequently, increased expression levels of dCK may be associated with certain anticancer effects of GEM. A role for dCK in activating GEM cytotoxicity has also been indicated by the finding that suppression of dCK activity is associated with resistance to GEM in various types of cancer (12,13). Increased expression levels of HuR may increase the cytotoxicity of GEM and reduce chemoresistance to this drug in a variety of malignant cell types. A previous *in vitro* study demonstrated that the modulation of dCK expression via HuR overexpression markedly sensitized pancreatic cancer cells to GEM (11). Similar results were identified in human gallbladder cancer cells (14), and an *in vivo* study has also demonstrated that the status of

HuR expression in various cancer cells is closely associated with response to GEM and GEM-based chemotherapy in patients with pancreatic cancer (11,15). Thus, HuR may have an important role in the GEM-based chemotherapy of patients with cancer (16).

In patients with advanced UC, a cisplatin (CDDP)-based regimen is the most commonly used first-line chemotherapy (17). Combined chemotherapy with methotrexate, vinblastine, doxorubicin and CDDP (MVAC) has also been established as one of most useful regimens for the treatment of patients with advanced UC since the 1980s (18). From 2000, combined gemcitabine (GEM) and CDDP therapy (GC) has become another standard chemotherapy regimen for the treatment of patients with UC, as it has been demonstrated to exert similar antitumor effects with reduced toxicity, as compared to MVAC therapy (19). However, GC therapy is limited with respect to the degree and duration of its anticancer effects, particularly in patients with metastatic UC (20,21). Furthermore, the efficacy and safety of this regimen as a second line chemotherapy approach, following CDDP-based therapy, has yet to be established, despite numerous clinical trials of various drugs and regimens (22-25). A number of previous studies have, therefore, investigated the potential of non-CDDP agents, including GEM, as second- or third-line chemotherapy agents (24,25); single-drug therapy with GEM, and combination therapy with GEM and paclitaxel (PTX), have been reported (24,25). Therefore, GEM is an essential chemotherapeutic agent for the treatment of patients with advanced and recurrent UC, and chemosensitivity to GEM is an important determinant of tumor suppression, treatment outcomes and survival in these patients.

Based on the results of these previous studies, it was hypothesized that HuR expression may be a useful predictive marker of antitumor effects in patients with advanced UC; however, there is currently limited evidence to support this hypothesis. The primary purpose of the present study was to clarify the prognostic role of HuR expression in first- and second-line chemotherapy, with respect to tumor size and progression free survival. The association between anticancer effects and HuR intracellular localization (nuclear or cytoplasmic) and the use of GEM in the therapeutic regimen, was also evaluated. Finally, the predictive potential of HuR expression levels were analyzed, with respect to the treatment outcomes of patients with advanced UC who were treated with a GEM-based chemotherapy regimen, using multivariate analyses that included pathological features.

Materials and methods

Patients. A total of 50 patients with advanced UC (male, 32; female, 18), who were treated with chemotherapy in Nagasaki University Hospital (Nagasaki, Japan), were analyzed retrospectively. These patients were selected as they had received a CDDP-based first-line chemotherapy regimen, followed by a GEM-based second-line chemotherapy regimen. For first-line chemotherapy, MVEC and GC regimens were administered to 34 (68.0%) and 16 patients (32.0%), respectively. Following a diagnosis of tumor progression, 45 patients (90.0%) were treated with combined GEM and PTX therapy, and 5 patients (10.0%) were treated using GEM monotherapy. The relevant clinicopathological features are presented in Table I. The median patient age was 70 years (range, 39-88 years), and 27 (54.0%), 22 (44.0%) and 1 (2.0%) patient(s) had UC of the urinary bladder, upper urinary tract and both locations, respectively. With regard to pathological features, 43 (86.0%) and 41 (82.0%) patients were diagnosed as having muscle invasive and metastatic disease, respectively. A total of 7 patients (14.0%) with upper urinary tract cancer were determined to have non-muscle invasive disease. However, these patients were unable to receive radical surgery as they were elderly or had metastatic disease.

Chemotherapy. The MVEC regimen consisted of methotrexate (30 mg/m^2 on days 1, 15 and 22), vinblastine (3 mg/m^2 on days 2, 15 and 22), epirubicin (30 mg/m^2 on day 2) and CDDP (70 mg/m^2 on day 2), administered by intravenous infusion over a 28-day cycle. GC therapy consisted of GEM (1,000 mg/m^2 on days 1, 8 and 15) and CDDP (70 mg/m^2 on day 2) and was also administered by intravenous infusion over a 28-day cycle. For second-line chemotherapy, GP therapy consisted of GEM (700 mg/m^2) and paclitaxel (70 mg/m^2), administered by intravenous infusion on day 1 and 8 of each 28-day cycle. GEM monotherapy (1,000 mg/m^2 on day 1 and 8) was also administered by intravenous infusion over a 28-day cycle. In the current study, all patients received ≥2 cycles of first-line chemotherapy, and the median number of treatment cycles was two for MVAC and GC therapy.

Immunohistochemistry. HuR expression was evaluated as previously described (8). Briefly, immunohistochemical analyses were performed using formalin-fixed, paraffin-embedded tissue sections. The tissue sections (5 μm) were deparaffinized in xylene and rehydrated in solutions of graded ethanol. Antigen retrieval was performed by heating at 100˚C for 15 min in 0.01 M sodium citrate buffer (pH 6.0). All tissue sections were then immersed in 3% hydrogen peroxide for 30 min to block endogenous peroxidase activity. The tissue sections were incubated overnight with the primary antibody [HuR (H-280): sc-20694, 1:100; Santa Cruz Biotechnology, Dallas, TX, USA] at 4˚C, followed by washing in 0.05% Tween-20 in phosphate-buffered saline. Subsequently, the tissue sections were incubated with peroxidase using the labeled polymer method with Dako EnVision+™ Peroxidase (Dako North America, Inc., Carpinteria, CA, USA) for 60 min at room temperature according to the manufacturer's instructions. The peroxidase reaction was visualized using a liquid 3,3'-diaminobenzidine tetrahydrochloride substrate kit (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The tissue sections were then counterstained using hematoxylin. As previously described (8), formalin-fixed and paraffin-embedded liver tissue samples (comprising resected and stored specimens obtained from Nagasaki University Hospital between January 2012 and December 2014) were used as the positive controls. A consecutive section from each tissue sample was processed without the primary antibody to be used as the negative controls.

HuR expression was evaluated based on an immunoreactive staining score, as previously reported (8,11). HuR expression was evaluated separately in cancer cell cytoplasm and nuclei. Briefly, HuR immunostaining in cytoplasm of cancer cells was scored as follows: 0, no staining; 1, weak or

Table I. Clinicopathological features according to the first-line chemotherapy regimen.

Clinicopathological feature	Total (n=50)	First-line chemotherapy		P-value
		GC (n=16)	MVEC (n=34)	
Gender, n (%)				0.157
Male	32 (64.0)	8 (50.0)	24 (70.6)	
Female	18 (36.0)	8 (50.0)	10 (29.4)	
Age, years				0.205
Median (range)	70 (39-88)	66 (39-88)	73 (45-80)	
Site of primary tumor, n (%)				0.603
Bladder	27 (54.0)	10 (62.5)	17 (50.0)	
Upper tract	22 (44.0)	6 (37.5)	16 (47.1)	
Bladder and upper tract	1 (2.0)	0 (0.0)	1 (2.9)	
Grade, n (%)				0.138
Low or grade 1+2	9 (18.0)	1 (6.3)	8 (23.5)	
High or grade 3	41 (82.0)	15 (93.7)	26 (76.5)	
T stage, n (%)				0.361
T1	7 (14.0)	4 (25.0)	3 (8.8)	
T2	18 (36.0)	6 (37.5)	12 (35.3)	
T3	18 (36.0)	5 (31.3)	12 (35.3)	
T4	7 (14.0)	1 (6.3)	6 (17.6)	
Metastasis, n (%)				0.094
Absence	9 (18.0)	5 (31.3)	4 (11.8)	
Presence	41 (82.0)	11 (68.7)	30 (88.2)	

GC, combination therapy of gemcitabine and cisplatin; MVEC, combination therapy of methotrexate, vinblastine, epirubicin and cisplatin; T, tumor.

focal staining in <10% of cells; 2, moderate or intense staining in 10-50% of cells; 3, moderate or intense staining in >50% of cells. Nuclear HuR expression was scored as follows: 0, no staining; 1, <10% of cells stained; 2, 10-50% of cells stained; 3, >50% of cells stained. A score of 0 or 1 was considered to indicate low HuR expression, whereas a score of 2 or 3 was determined to indicate high HuR expression. This evaluation was performed by two independent investigators blinded to the clinical features and survival data. The tissue sections were observed using an E-400 light microscope (Nikon Corporation, Tokyo, Japan) to obtain digital images. In addition, the computer-aided image analysis system WinROOF version 5.0 (Mitani Corporation, Fukui, Japan) was used to evaluate HuR expression.

Treatment response. Between 8 and 16 weeks following chemotherapy, all patients underwent a computed tomography scan or magnetic resonance imaging to determine the in-field tumor response. The local response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines version 1.1 (26). Based on these guidelines, the complete response (CR) was defined as the disappearance of all target lesions and the reduction in size of any pathological lymph nodes to <10 mm in the short axis. Partial response (PR) was defined as a decrease in the sum of the longest tumor diameters by ≥30%. Stable disease was defined as insufficient tumor shrinkage to qualify as PR, or as an insufficient increase in tumor size to qualify as progressive disease (PD). PD was defined as an increase in the sum of the longest tumor diameter by ≥20%, and an absolute increase in tumor size of ≥5 mm. The appearance of new lesions was also considered to indicate disease progression. The association between HuR expression levels and progression-free survival (PFS) was investigated, in addition to its association with overall survival (OS) following the initiation of second-line chemotherapy. The study protocol was approved by the Human Ethics Review Committee of Nagasaki University Hospital (Nagasaki, Japan), and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients involved in the present study prior to their enrollment.

Statistical analysis. Data are expressed as the median and the range. The Mann-Whitney U test was used for the analysis of continuous variables. The χ^2 test and Fisher's exact test were used for comparisons of categorical data. Survival analysis was conducted using Kaplan-Meier analysis and the log-rank test. In addition, univariate and multivariate Cox proportional hazard analyses were used to obtain a hazard ratio (HR) with a 95% confidence interval (CI), and a P-value for survival analyses. All statistical tests were two-sided and all statistical analyses were performed using StatView for Windows version 5.0 software (Abacus Concepts, Berkeley,

Table II. Associations between HuR expression and response to first-line chemotherapy.

			First-line chemotherapy			
	Total (n=50)		GC (n=16)		MVEC (n=34)	
Expression	Negative	Positive	Negative	Positive	Negative	Positive
Nuclear HuR, n (%)	11 (22.0)	39 (87.0)	4 (25.0)	12 (75.0)	7 (20.6)	27 (79.4)
Response, n (%)						
Complete response	3 (27.3)	2 (5.1)	1 (25.0)	1 (8.3)	2 (28.6)	1 (3.7)
Partial response	1 (9.1)	11 (28.2)	1 (25.0)	2 (16.7)	0 (0.0)	9 (33.3)
Stable disease	3 (27.3)	12 (30.8)	1 (25.0)	2 (16.7)	2 (28.6)	10 (37.0)
Progressive disease	4 (36.4)	14 (35.9)	1 (25.0)	7 (58.3)	3 (42.9)	7 (25.9)
P-value	0.136		0.670		0.076	
Cytoplasmic HuR, n (%)	14 (28.0)	36 (72.0)	5 (31.3)	11 (68.8)	9 (26.5)	25 (73.5)
Response, n (%)						
Complete response	1 (7.1)	4 (11.1)	0 (0.0)	2 (22.2)	1 (11.1)	2 (8.0)
Partial response	2 (14.3)	10 (27.8)	0 (0.0)	3 (33.3)	2 (22.2)	7 (28.0)
Stable disease	2 (14.3)	13 (35.1)	1 (20.0)	2 (22.2)	1 (11.1)	11 (44.0)
Progressive disease	9 (64.3)	9 (25.0)	4 (80.0)	4 (44.4)	5 (55.6)	5 (20.0)
P-value	0.077		0.310		0.170	

GC, combination therapy of gemcitabine and cisplatin; MVEC, combination therapy of methotrexate, vinblastine, epirubicin and cisplatin; HuR, human antigen R.

CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

HuR expression patterns and patient response to first-line chemotherapy. The immunoreactivity of HuR was detected in the nucleus and cytoplasm of bladder cancer cells. In contrast to the normal urothelium, moderate-intense cytoplasmic HuR expression levels were frequently detected in cancer cells. Overall, 78.0% (39/50) and 72.0% (36/50) of tumors were determined to have positive nuclear and cytoplasmic HuR expression, respectively (Table II). With regard to the characteristics of HuR expression in UC cells, no significant difference (P=0.529) was observed between bladder cancer cells and upper urinary tract cancer cells.

The anticancer effects of first-line chemotherapy were evaluated according to the RECIST guidelines and are summarized in Table II. Of a total of 50 patients, 5 (10%) and 12 (24%) were determined to exhibit a CR and PR, respectively. The response rates of MVAC therapy demonstrated no significant difference (P=0.442) from those of GC therapy (35.3 and 31.3%, respectively). The anticancer effects observed in individual patients were not significantly associated with the localization of HuR expression [nuclear staining (P=0.136), as compared with cytoplasmic staining (P=0.076)]. When similar analyses were performed for the first-line chemotherapy regimen, nuclear and cytoplasmic HuR expression levels were not determined to be significantly associated with the anticancer effects of MVAC or GC therapy (Table II). The median of PFS following the initiation of first-line MVEC (6 months) and GC therapy (4 months) was also similar (P=0.172; data not shown). In addition, nuclear and cytoplasmic HuR expression was not observed to be significantly associated with PFS following first-line chemotherapy (P=0.213 and 0.277, respectively; Fig. 1A and B).

HuR expression and response to second line chemotherapy. In second-line GEM-based chemotherapy, nuclear HuR expression was not observed to be significantly associated with anticancer effects in the first-line MVEC or GC therapy groups (Table III). Furthermore, as presented in Fig. 2A and B, nuclear HuR expression levels were not associated with the OS rate from second-line therapy in patients who had received first-line MVEC therapy (P=0.116) or first-line GC therapy (P=0.975). However, the tumor size in patients with positive cytoplasmic HuR tumor expression was significantly reduced (P=0.002), compared with patients with negative HuR tumor expression (Table III). Such anticancer effects, evaluated according to the RECIST guidelines, were also observed in patients treated with first-line MVAC therapy (P=0.025). A similar trend was also observed in patients treated with first-line GC therapy, but this difference was not statistically significant (P=0.053; Table III). Furthermore, OS in patients with positive cytoplasmic HuR tumor expression was significantly longer, compared to those with negative expression, in patients who received first-line MVAC (P<0.001; Fig. 2C) and first-line GC therapy (P=0.029; Fig. 2D). Notably, the prognostic indications of HuR cytoplasmic and nuclear expression for OS following the initiation of second-line GEM-based chemotherapy were contrary (Fig. 2A and D).

Table III. Associations between HuR expression and response in second-line chemotherapy.

			First-line regimen			
	Total (n=50)		GC (n=16)		MVEC (n=34)	
Expression	Negative	Positive	Negative	Positive	Negative	Positive
Nuclear HuR, n (%)	11 (22.0)	39 (87.0)	4 (25.0)	12 (75.0)	7 (20.6)	27 (79.4)
Response, n (%)						
Complete response	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Partial response	2 (18.2)	6 (15.4)	2 (50.0)	3 (25.0)	0 (0.0)	3 (11.1)
Stable disease	8 (72.7)	22 (56.4)	2 (50.0)	5 (41.7)	6 (85.7)	17 (62.9)
Progressive disease	1 (9.1)	11 (28.2)	0 (0.0)	4 (33.3)	1 (14.3)	7 (25.9)
P-value	0.136		0.371		0.467	
Cytoplasmic HuR, n (%)	14 (28.0)	36 (72.0)	5 (31.3)	11 (68.8)	9 (26.5)	25 (73.5)
Response, n (%)						
Complete response	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Partial response	0 (0.0)	8 (22.2)	0 (0.0)	5 (45.5)	0 (0.0)	3 (12.0)
Stable disease	6 (42.9)	24 (66.7)	2 (40.0)	5 (45.5)	4 (44.4)	19 (76.0)
Progressive disease	8 (57.1)	4 (11.1)	3 (60.0)	1 (9.1)	5 (55.6)	3 (12.0)
P-value	0.002		0.053		0.025	

GC, combination therapy of gemcitabine and cisplatin; MVEC, combination therapy of methotrexate, vinblastine, epirubicin and cisplatin; HuR, human antigen R.

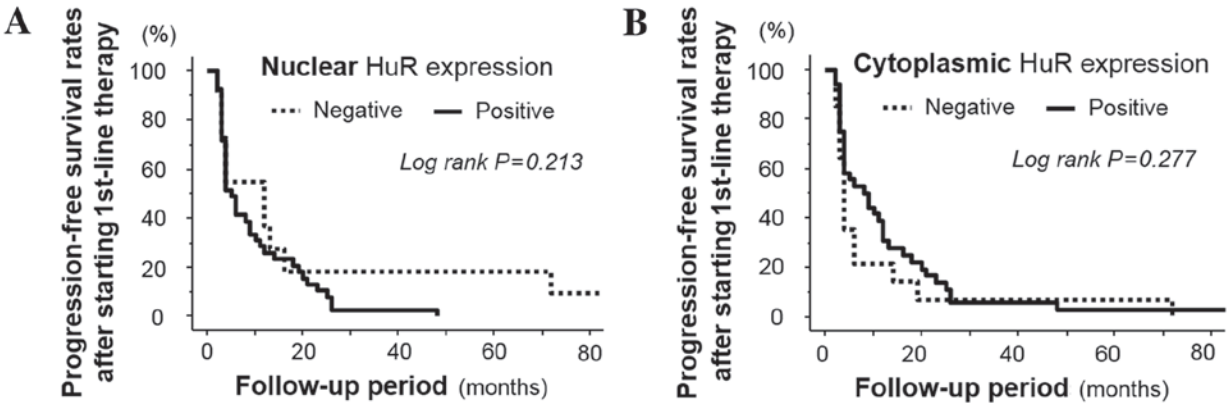

Figure 1. Kaplan-Meier survival curves of progression-free survival following the initiation of first-line chemotherapy, according to (A) HuR nuclear and (B) cytoplasmic expression levels. Although they were not observed to significantly differ, the progression-free survival rates demonstrated opposing trends. HuR, human antigen R.

Independent associations between HuR expression and patient survival from the initiation of second-line chemotherapy, in univariate and multivariate analysis models that included clinicopathological features and first-line chemotherapy regimens, are summarized in Table IV. Cytoplasmic HuR expression was identified as a significant predictive factor for longer OS (HR, 0.22; 95% CI, 0.09-0.56; P=0.001), whereas nuclear HuR expression was not (HR, 1.21; 95% CI, 0.43-3.39; P=0.724, Table IV). However, this was only determined to be for patients who received second-line GP therapy (HR, 0.33; 95% CI, 0.12-0.92; P=0.034). When similar analyses were performed according to tumor type, significant and independent associations were detected in bladder cancer (HR, 0.31; 95% CI, 0.10-0.96; P=0.042) and upper urinary tract cancer (HR, 0.14; 95% CI, 0.09-0.69; P=0.019).

Discussion

HuR is primarily detected in the nucleus under normal physiological conditions, but relocates to the cytoplasm in response to various stimuli, including certain signaling pathways activated during carcinogenesis (27). Similar findings have also been observed in patients with various malignancies, including bladder cancer (8). Therefore, the pathological role and biological characteristics of HuR in

Table IV. Predictive value for overall survival from commencement of second-line chemotherapy.

	Univariate analysis			Multivariate analysis		
Clinical feature	HR	95% CI	P-value	HR	95% CI	P-value
Male gender	0.54	0.26-1.12	0.096	0.29	0.11-0.78	0.014
Age, years	0.99	0.97-1.03	0.958	1.00	0.96-1.03	0.787
High grade/grade 3	1.01	0.42-2.42	0.983	0.88	0.31-2.50	0.814
Tumor stage 4	1.35	0.39-4.68	0.641	0.57	0.14-2.37	0.443
Presence of metastasis	1.42	0.54-3.70	0.478	1.48	0.45-4.65	0.523
First-line MVEC	1.77	0.82-3.90	0.153	2.48	0.95-6.78	0.063
Positive C-HuR	0.27	0.12-0.59	0.001	0.22	0.09-0.56	0.001
Positive N-HuR	1.56	0.66-3.69	0.308	1.21	0.43-3.39	0.724

HR, hazard ratio; CI, confidence interval; MVEC, combination therapy of methotrexate, vinblastine, epirubicin, and cisplatin; C, cytoplasmic; N, nuclear; HuR, human antigen R.

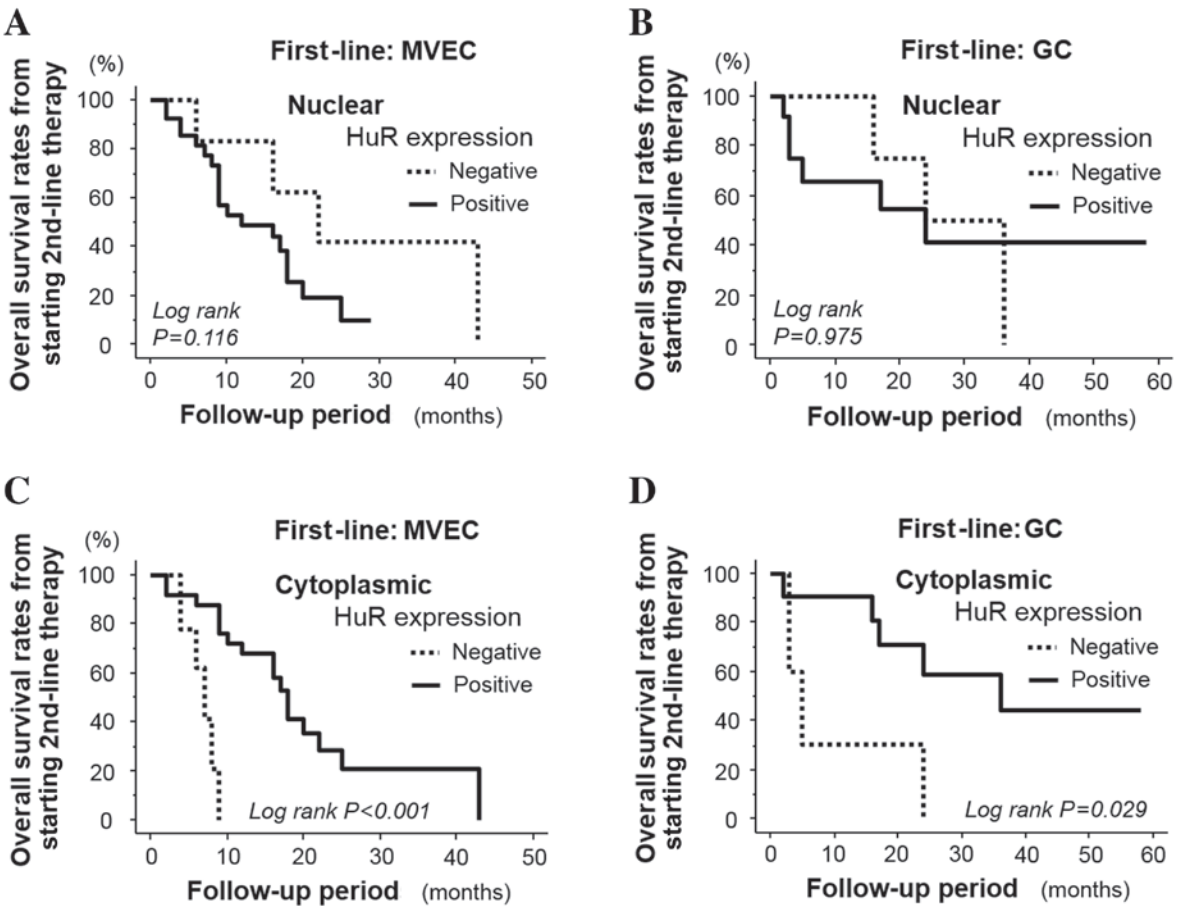

Figure 2. Kaplan-Meier survival curves of overall survival following the initiation of second-line chemotherapy, with respect to (A and B) nuclear HuR expression levels. For first-line chemotherapy, no significant difference in overall survival was observed in the (A) MVEC or (B) GC therapy groups. (C and D) Similar survival curves according to cytoplasmic HuR expression status were observed in patients undergoing (C) MVEC and (D) GC therapy. HuR, human antigen R; GC, combination therapy of gemcitabine and cisplatin; MVEC, combination therapy of methotrexate, vinblastine, epirubicin and cisplatin.

numerous types of malignancy have been investigated *in vivo* and *in vitro* (5-10,28).

To the best of our knowledge, the first study that identified upregulated HuR expression as a significant marker for an improved response to GEM-based chemotherapy in patients with pancreatic cancer was published in 2009 (11). The study also observed that high HuR expression levels predicted a favorable prognosis in these patients (11). This result was notable as increased HuR expression was previously considered to predict progression and shorter survival in numerous types of malignancy (9). Subsequently, the molecular mechanisms underlying the anticancer effects of GEM were identified in pancreatic

cancer cells (29). Although high HuR expression levels were significantly associated with a high T stage, it was also indicated to be a potent marker of clinical outcomes for patients with resected pancreatic cancer who were undergoing GEM therapy (15). These results demonstrated that HuR expression had conflicting prognostic indications with respect to malignant potential and the response to GEM-based chemotherapy in pancreatic cancer. In addition to pancreatic cancer, HuR has been reported to have important roles in the chemosensitivity of human gallbladder cancer cells to GEM (14). Therefore, it was hypothesized in the current study that HuR expression in UC cells may be a useful predictive marker for the efficacy of GEM-based therapy in patients with UC.

To the best of our knowledge, the present study is the first to assess the association between HuR expression levels and specific chemosensitivity in human UC tissues. The results demonstrated that cytoplasmic HuR expression levels are significantly associated with the anticancer effects of a second-line GEM-based regimen, but not with a first-line chemotherapy that included a GC regimen. In addition, cytoplasmic HuR expression levels were a useful predictive marker for OS from the initiation of second-line GEM-based chemotherapy, but not for progression-free survival following first-line chemotherapy. However, it remains to be elucidated why cytoplasmic HuR expression was significantly associated with the anticancer effects of a second-line GEM-based chemotherapy regimen, but not of a first-line GC therapy, and the design of the present study did not permit this to be elucidated. However, there are a number of possible reasons for these findings. Firstly, in GC therapy administered to chemo-naïve patients with UC, the most effective component may be CDDP, rather than GEM (30). Among CDDP-based regimens, the GC regimen is administered at a reduced frequency and causes fewer severe adverse effects, compared with the MVAC regimen; however, these two regimens have similar anticancer effects and prognostic implications in patients with advanced UC (21). Therefore, the predictive value of cytoplasmic HuR expression levels for the anticancer effects of certain first-line chemotherapy regimens is relatively low, and its effects were primarily determined by chemosensitivity towards CDDP. Secondly, there is a possibility that HuR inhibited the anticancer effects of CDDP in first-line chemotherapy based on a previous report (22). It was identified that, in patients with UC receiving GEM-based second-line chemotherapy, cytoplasmic HuR expression levels were positively associated with prolongation of OS periods. These findings were concordant with the results of previous studies of other types of cancer (11,14). By contrast, it has also been suggested that increased HuR expression levels are associated with decreased sensitivity to CDDP in ovarian cancer (31). As aforementioned, CDDP is the principal agent in first-line chemotherapy (21,30). If this phenomenon also occurs in UC cells, HuR expression may reduce their sensitivity to GC therapy. Therefore, HuR is able to mediate and suppress the anticancer effects of GEM. Furthermore, in addition to GEM, high HuR expression levels may regulate the response to paclitaxel via regulation of chemoresistance-associated factors including microRNA (31,32). A previous *in vitro* study demonstrated that cytoplasmic HuR expression was associated with the efficacy of various anticancer agents (33-35); however, it has also been revealed that HuR is able to mediate chemoresistance in numerous types of cancer (36,37). Further studies are required in order to investigate and elucidate the complex mechanisms underlying the interaction between HuR expression levels and the response of patients to chemotherapy. Although numerous previous studies have reported that the expression of HuR in the cytoplasm has important roles in tumor aggressiveness, prognosis and the modulation of chemosensitivity-associated factors (6,8,28,31,32). It remains to be elucidated whether this is also true of nuclear HuR expression. Nuclear HuR expression was demonstrated to inhibit the chemoresistance-associated protein, tubulin beta class 3 (TUBB3), resulting in a improved prognosis, whereas the expression of cytoplasmic HuR enhanced TUBB3 expression and was associated with an improved treatment outcome in patients with ovarian cancer (31). Furthermore, the association between HuR expression levels and chemoresistance depends on the presence of certain binding partners, including acidic leucine-rich nuclear phosphoprotein 32 family member A (38,39). Therefore, the localization and availability of co-factors for HuR in various cancer cells may influence its pathological and biological characteristics.

The pathological aggressiveness and molecular characteristics of UC are regulated by complex underlying mechanisms, including external factors (40). A limitation of the present study was its relatively small study cohort; in addition, the variation and non-uniformity of the treatment regimens and the patients' clinical backgrounds must be noted. Therefore, in order to determine the prognostic role of HuR expression with respect to patient response to GEM-based chemotherapy and the treatment outcomes in advanced UC, further detailed *in vitro* and *in vivo* studies, including clinical trials, are essential (15,16).

In conclusion, the present study identified that HuR expression levels were not significantly associated with antitumor effects or improved PFS following first-line chemotherapy, including with GC therapy. By contrast, cytoplasmic HuR expression was identified to be significantly associated with antitumor effects, as determined by the RECIST criteria, and it may also predict the OS of patients with UC who have undergone second-line GEM-based chemotherapy. This is important with respect to the selection of treatment approaches for second-line chemotherapy in these patients. Further and similar studies are required, with a larger study population, in order to corroborate these results. In addition, prospective and randomized clinical trials are necessary to clarify the clinical potential of cytoplasmic HuR expression as a predictive marker in patients with advanced UC.

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