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Original Article

URI1 amplification in uterine carcinosarcoma associates with chemo-resistance and poor prognosis

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Abstract: Uterine carcinosarcoma (UCS) is a rare type of cancer and accounts for 5% of uterine malignancies. However, UCS patients suffer a high prevalence of chemo-resistance and a very poor prognosis compared to uterine cancer patients. URI is a chaperone with functions in transcription. We analyzed the somatic *URI1* copy number variation in 57 post-menopausal non-metastatic UCS patients in comparison to 363 uterine corpus endometrial carcinomas. *URI1* amplification was detected in 40% (23/57) of primary UCS and 5.5% (20/363) of uterine carcinomas. UCS patients with *URI1* amplification exhibited 13% (3/23) tumor-free survival compared to 41% (14/34) in the absence of *URI* amplification (P=0.023). *URI1* amplification (*OR*=6.54, P=0.027), weight (*OR*=1.068, P=0.024), hypertension (*OR*=3.35, P=0.044), and tumor stage (*OR*=2.358, P=0.018) associated with poor survival. Patients treated with hormone replacement therapy (*OR*=15.87, P=0.011) displayed enhanced overall survival. Combined radiation and chemotherapy improved patient survival (median survival=2043 days) compared to single (median survival=597 days) or no treatment (median survival=317 days, P=0.0016). Importantly, patients with *URI1* amplification had poor response to adjuvant treatment compared to control group (P=0.013). Tumors with *URI1* amplification displayed decreased transcription of genes encoding tumor suppressor and apoptotic regulators and increased expression of genes regulating oncogenesis, survival and metastasis. Overexpression of *URI1* in a cultured cell model induced *ATM* expression and resistance to cisplatin. Our findings suggest that high prevalence in UCS may associate with poor prognosis and worse response to adjuvant treatment.

Keywords: *URI1*, uterine carcinosarcoma, prognosis, survival

Introduction

Uterine carcinosarcoma (UCS), also known as malignant mixed Mullerian tumor (MMMT), is an undifferentiated uterine malignancy with characteristics of both carcinoma and sarcoma. The endometrial carcinoma occurs within the inner layer of tissue lining of the uterus, whereas the sarcoma arises from the outer layer of muscle of the uterus. USC makes up five percent of all uterine cancers [1]. In the United States, approximately 2 per 100,000 women develop UCS annually [2]. Because of the aggressive nature of UCS, only 35% of patients survive five years after diagnosis. UCS and endometrial carcinomas have similar risk factors. Both malignancies are associated with obesity, diabetes, hypertension, smoking, nulliparity, and use of estrogen ortamoxifen [3-9]. By contrast, progestin-containing contraceptives or postmenopausal hormone therapy reduces the risk of both types of cancers [10-13]. The primary management of UCS is surgical, although adjuvant radiotherapy treatment (RT) and/or chemotherapy are often used. While one study reported that adjuvant RT after surgery decreased the risk of pelvic cancer recurrence compared to surgery alone [14], it did not provide any overall survival benefit [14]. Additional studies have provided evidence to support the benefit of aggressive adjuvant chemotherapy that combines RT with DNA damage-based chemotherapy [15-17]. Currently, it remains challenging to predict outcomes for UCS patients and the mechanism underlying patient relapse is unclear. Therefore, a reliable preoperative biomarker that predicts primary surgical response and identifies patients who would benefit from aggressive adjuvant therapy is urgently needed.

Table 1. Clinicopathologic characteristics of the overall cohort

Characteristic	Value
Patient size	57
Age (y)	
Median	68
Range	51-90
Race	
White	44
Black	9
Asian	3
Unknown	1
Follow up (days)	
Median	497
Range	8-3115
Stage	
I	22
II	5
III	20
IV	10
Positive pelvic lymph nodes	15 (26%)
Positive aortic lymph nodes	9 (16%)
Tumor recurrence	29 (51%)
Surgical margin	12 (21%)
Depth of myometrial invasion %	
Median	40
Range	0-100
Residual tumor	
R0	34
R1	2
R2	10
Rx	11
Adjuvant treatment	
No	14
Radiation treatment	7
Chemotherapy	17
Radiation+chemo	19

The unconventional prefoldin RPB5 interactor 1 (URI1) was originally identified as a scaffold protein that binds RNA polymerase II [18]. URI plays important roles in regulating gene expression. URI interacts with several transcription factors including transcription factor IIF (TFII), and the androgen receptor (AR) in prostate cancer cells [19-21]. It also interacts with a chromatin remodeling complex (PAF1) and translation initiation factors [22, 23]. Current clinical findings have shown the correlation between *URI1* and human cancers. For example, one study reported *URI1* amplification in 10% of ovarian cancers and increased URI protein level correlates with tumor aggressiveness [24]. URI upregulation increases the expression of the "p53 and DNA damage-regulated gene 1" (PDRG1), suggesting a function for URI in DNA damage repair [19]. Decreased *URI1* expression inhibits cell proliferation and induces apoptosis in ovarian and liver cancer cells [25, 26]. In cervical cancer, increased URI expression is also associated with a high tumor grade [27].

Here, we hypothesize that *URI1* amplification is associated with poor clinical outcome in UCS patients. We also reconstituted *URI1* upregulation in a culture cell model and investigated URI overexpression in the DNA damage response.

Materials and methods

Patient cohort

We analyzed UCS and uterine corpus endometrial cacinoma samples with corresponding normal tissue from clinically annotated patients in The Cancer Genome Atlas (TCGA) Data Portal. All samples met freedom-to-publish criteria without restriction or limitations. The uterine corpus endometrial carcinomas cohort has been reported previously [28]. All UCS patients were postmenopausal (prior bilateral ovariectomy or >12 months since last menstrual period with no prior hysterectomy). All UCS specimens were surgically resected prior to systemic treatment and all patients received complete surgery as the primary treatment. UCS Patients did not have metastasis at the time of surgery. The clinical stage, age at diagnosis, tumor invasion percent, local lymphatic status, and surgical margin, were recorded. Patient race, weight, menopausal hormone therapy, hormonal contraceptives use, tamoxifen use, hypertension, diabetes, full-term pregnancies, and history of other malignancies were also recorded. Treatment response, time of disease relapse and date of death after initial diagnoses were recorded after the initial treatment. Lymph node positivity was determined by H & E staining and immunohistochemistry. We excluded patients who received neoadjuvant treatment. We also excluded patients with a history of tamoxifen use or with colorectal cancer, as some colon cancers can metastasize to the uterus. Using these criteria, we identified 57

Table 2. URI1 amplification associates with patient pathology and prognosis

URI1 amplification	No	Yes	*P*-value
Sample size	n=34	n=23	
Age (yrs)	69.2±9.9	70.0±8.4	0.57
Stage			0.2
I	16 (47%)	6 (26%)	
II	3 (9%)	2 (8%)	
III	9 (26%)	11 (48%)	
IV	6 (18%)	4 (17%)	
Primary therapy outcome success[1]	21 (61%)	10 (43%)	0.18
Tumor free survival	14 (41%)	3 (13%)	0.023
Tumor recurrence	18 (53%)	11 (48%)	0.7
Days of follow-up	743±206	800±208	0.79
Median survival days	771±142	685±198	0.51
Pelvic lymph nodes	7 (21%)	8 (35%)	0.23
Aortic lymph nodes	5 (15%)	4 (17%)	0.31
Depth of myometrial invasion %[2]	33±10.2	55±15.2	0.19
Surgical Margin	5 (15%)	7 (30%)	0.15

[1]Defined as complete or partial remission; [2]Depth of myometrial invasion divided by depth of myometrial thickness.

UCS patients and determined *URI1* copy number variation and mRNA expression.

Statistical analysis

Primary therapy outcome, tumor-free status, new tumor event, and surgical margins between *URI1* amplification and control groups were compared using the chi-square test. The differences in age at diagnosis, and follow up time between two groups were evaluated with an unpaired t-test. Tumor stage between control and *URI1* amplifed groups was evaluated with the Mann-Whitney test. Spearman's rank correlation coefficient was used to analyze pairwise correlation between patient characteristics. Disease-associated variables were analyzed by univariate and multivariate Cox regression to test correlation with survival. Pairwise correlation efficiency between variables was calculated by Spearman's rho. Somatic *URI1* copy number abnormalities were identified by SNP microarray with putative copy number=2 from GISTIC 2.0 and genome-wide exome sequencing as described previously [28, 29]. Overall survival was calculated using the Kaplan-Meier method, and the significance between groups was calculated by Wilcoxon test. Statistical analyses were performed using Prism version 6.0 (GraphPad Software, La Jolla, CA), cBioPortal [30], and SPSS version 13.0 (SPSS, Inc., Chicago, IL). All *in vitro* experiments were performed three times independently and the error bars represent the standard deviation with significance calculated by nonparametric t-test.

Cell survival assay and reagents

RL 95-2 cells (ATCC, Manasas, VA), a uterine/endometrial cancer line, were cultured in DMEM: F12 medium with 0.005 mg/ml insulin and 10% FBS. Cells were plated in 96-well plates at a concentration of 1,000 cells per well. After 48 hours of treatment, cell viability was measured using the CyQUANT assay (Life Technologies, Waltham, MA), as per manufacturer's instruction. Cisplatin is from Sigma-Aldrich (St. Louis, MO). ATM inhibitor is from Selleckchem (Houston, TX) (Catalog #S7136). Anti-URI1 antibody is from Santa Cruz Biotechnology (Santa Cruz, CA) (Catalog #376011). Anti-phospho-ATR (Catalog #2853), phospho-ATM (Catalog #5883), ATM (Catalog #2873), ATR (Catalog #2790), and phospho-γH2AX (Catalog #9718) antibodies are from Cell Signaling (Berverly, MA). Anti-tubulin antibody (Catalog #489P) is from Biolegend (San Diego, CA).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

qRT-PCR to quantitate *ATM* mRNA expression used SYBR Green (Applied Biosystem, Foster City, CA). Expression of *RPL-19* was used as the internal control. ATM primers are as following: F: AGACCGCGTGATACTGGATG. R: TCACTGTCACTGCACTCGGA.

Result

Patient cohort

This series of UCS (n=57) has a median age of diagnosis at 68 yrs, and median follow-up of 497 days (**Table 1**). In this cohort, stage I, II, III and IV cases are 39%, 9%, 35%, and 18% respectively. Pathology examination found 15 (26%) cases with positive pelvic lymph nodes involved and 9 (16%) cases with positive aortic lymph nodes involved (**Table 1**). Surgical margins were assessed in 12 (21%) cases (**Table**

Table 3. Multivariate logistic regression model of characteristics associated with survival in UCS

Variables	Univariant			Multivariant		
	P-value	*OR*	95% CI	*P*-value	*OR*	95% CI
*URI1*amp	0.551	1.240	0.612-2.513	0.027	6.543	1.234-34.7
Age at diagnosis	0.338	0.992	0.977-1.008	0.235	1.058	0.964-1.162
Weight (l b)	0.599	1.005	0.986-1.025	0.024	1.068	1.009-1.13
Hormonal contraceptives use	0.895	0.916	0.25-3.352	0.053	11.754	0.967-142.831
Menopausal hormone therapy	0.055	0.230	0.051-1.031	0.011	0.063	0.008-0.527
Hypertension	0.003	3.217	1.476-7.008	0.044	3.505	1.032-11.898
Diabetes	0.341	1.595	0.61-4.168	0.248	0.395	0.082-1.91
Pregnancies	0.171	0.769	0.528-1.12	0.24	0.708	0.398-1.259
Clinical stage	0.033	1.414	1.028-1.946	0.026	2.358	1.108-5.018
Pelvic lymph nodes positive	0.928	0.964	0.429-2.163	0.204	0.448	0.129-1.549
Aortic lymph nodes positive	0.090	2.021	0.897-4.555	0.697	0.774	0.214-2.807
Surgical margin	0.368	1.430	0.657-3.112	0.967	0.97	0.222-4.232
Radiation therapy	0.011	0.386	0.184-0.807	0.894	0.924	0.289-2.961
Chemotherapy	0.115	0.553	0.264-1.155	0.002	0.074	0.014-0.381

OR: Odds ratio, 95% CI: 95% confidence interval.

1). Among these 57 patients, 29 (51%) experienced tumor recurrence (**Table 1**). After surgery, 7 patients received only RT and 17 patients received only chemotherapy (**Table 1**). RT in combination with chemotherapy was given to 19 patients and 14 patients did not receive any adjuvant treatment (**Table 1**). In this cohort, 56 (98%) have *TP53* mutations, 22 (39%) have *PI3KCA* mutations, and 16 (28%) have *PTEN* mutations.

URI1 amplification in UCS associates with decreased tumor-free status

We first compared clinical and pathological characteristics in cases with normal or elevated *URI1* copy number. *URI1* amplification (URI1 amp) was detected in 23 (40%) patients (**Table 2**). Interestingly, we found *URI1* amplification in only 20 (5.5%) uterine corpus endometrial carcinomas (n=363) (Table S1). In UCS patients, the control group and the *URI1* amplified group have a similar age of diagnosis (69.2±9.9 vs. 70.0±8.4 yrs, P=0.57) and follow-up time (743±206 vs. 800±208 days, P=0.79) (**Table 2**). The *URI1* amp group contains 65% of tumors in III/IV stage, compared to 44% of the control group (P=0.2) (**Table 2**). Patients with *URI1* amplification trended toward deeper myometrial invasion (55%), higher chance of pelvic lymph node involvement (35%), and increased positive surgical margins (30%), compared to the control group (33%, 21%, and 15%, respectively) (**Table 2**). To determine the correlation of *URI1* copy number and patient outcome, we then compared disease progression in the control and *URI1* amp groups (**Table 2**). After primary therapy, 43% of patients in the *URI1* amp cohort had partial or complete response compared to 61% in the control group (P=0.18) (**Table 2**). Importantly, 41% of patients in the control group had tumor-free survival while only 13% of patients with *URI1* amplification remained tumor-free (P=0.023).

URI1 amplification, weight, hypertension, and tumor stage associate with UCS patient overall survival

Next, we investigated what pre-operative characteristics and adjuvant treatments associate with patient survival. Among the 14 morphologic variables in our study, *URI1* amplification (odds ratio (OR)=6.54, confidential interval (95% CI) 1.123-34.7, P=0.027), increasing weight (OR=1.068, 95% CI 1.009-1.13, P=0.024), hypertension (OR=3.35, 95% CI 1.032-11.898, P=0.044), and tumor clinical stage (OR=2.358, 95% CI 1.108-5.018, P=0.018) were significantly correlated with mortality (**Table 3**). A history of menopausal hormone therapy (OR=0.063, 95% CI 0.008-0.527, P=0.011) and chemotherapy (OR=0.074, 95% CI 0.014-0.381, P=0.002) were significantly associated with patient overall survival (**Table 3**). Correlation coefficient analysis revealed that *URI1* amplification does not associate with any preoperative factors except hormonal contraceptives (r_s=-0.521, P=0.011) (Table S2). Patients who have a history of using hormonal

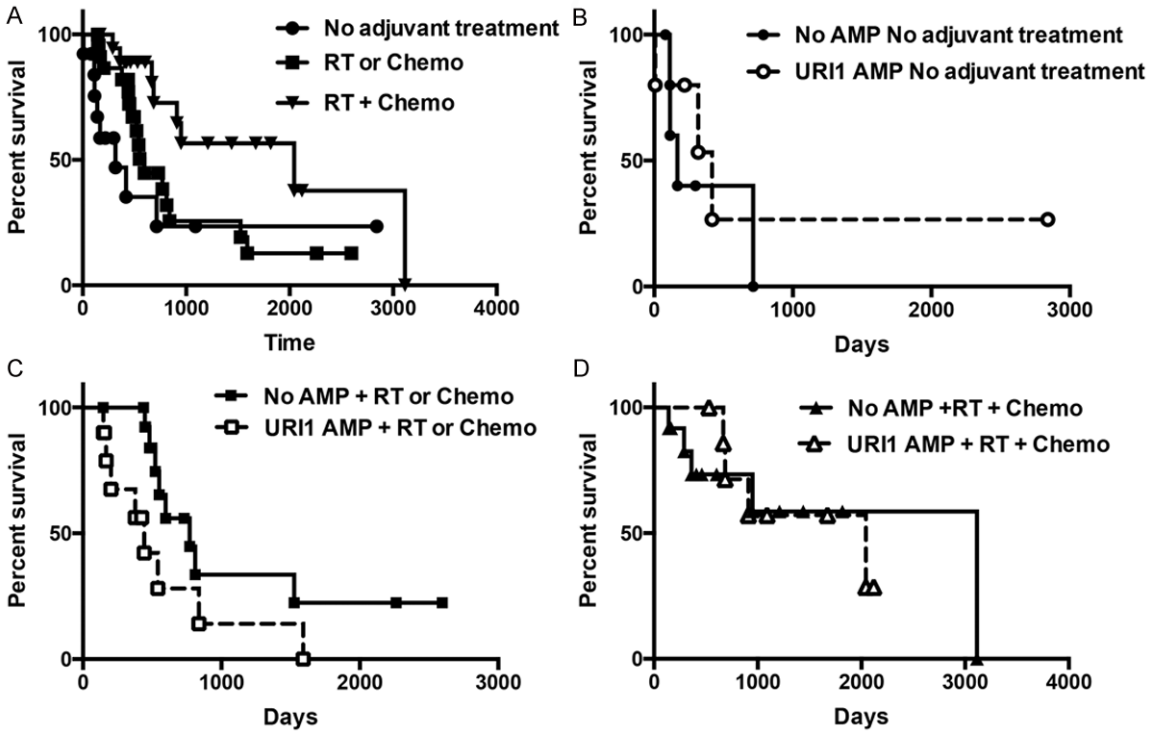

Figure 1. Association between *URI1* amplification and overall survival in UCS. A. Patients with adjuvant RT or chemotherapy (n=24) had significantly improved overall survival compared to patients without adjuvant treatment (n=14, P=0.026). Patients with combination of adjuvant RT and chemotherapy (n=19) have better overall survival compared to single treatment (P=0.029) and non-treated control (P=0.0024). B. Comparison of patient overall survival in control (n=8) and *URI1* amplified group (n=6) without adjuvant treatment (P=0.58). C. After adjuvant RT or chemotherapy, *URI1* amplified group (n=9) has the worse overall survival compared with control group (n=15, P=0.013). C. Comparison of *URI1* amplified (n=8) and control group (n=11) overall survival after adjuvant combination of RT and chemotherapy (P=0.53).

contraceptives tend to have a normal *URI1* copy number in USC malignancies. These data suggest that *URI1* amplification could be used as an independent factor to predict patient prognosis.

To analyze whether RT and chemotherapy provide benefit to patients with or without *URI1* amplification, we analyzed the overall survival in the control and *URI1* amp groups. Patients who received adjuvant RT or chemotherapy alone had significantly improved overall survival (median survival=597 days) compared to the untreated group (median survival=317 days, P=0.026) (**Figure 1A**). Patients with combined RT and chemotherapy had the best overall survival (median survival=2043 days) compared to single adjuvant treatment (P=0.029) or the non-treated group (P=0.0024) (**Figure 1A**). *URI1* amplification in non-treated patients did not significantly affect overall survival (P=0.58) (**Figure 1B**). Among UCS patients who received single adjuvant treatment, the *URI1* amp group had a much worse prognosis (median survival=442 days) compared to control patients (median survival=771 days, P=0.013) (**Figure 1C**). A combination of chemotherapy and RT dramatically improved overall survival in both URI1 amp (median survival=2043 days) and control group (median survival=3115 days) and the difference between these groups was not significant (P=0.053) (**Figure 1D**). Thus, our data indicates that patients with *URI1* amplification are less responsive to single adjuvant treatments while RT-chemotherapy combination can significantly improve patients overall survival in both control and URI1 amp patients.

URI1 amplification correlates with alteration in cancer-related gene expression and induces DNA damage-resistance through ATM upregulation

To elucidate the potential mechanism underlying the association between *URI1* amplification and poor prognosis, we compared the expres-

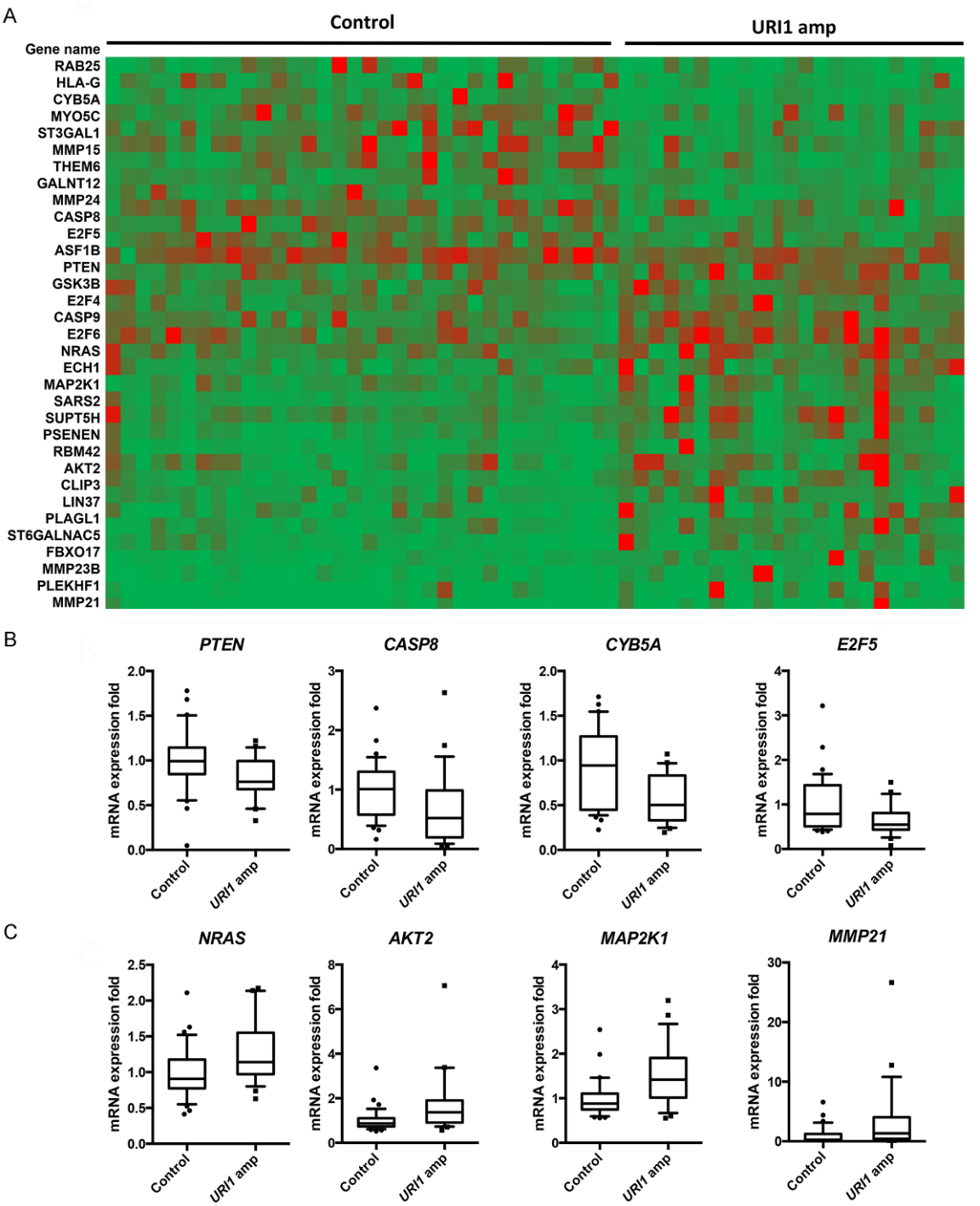

Figure 2. Gene expression alteration in UCS with *URI1* amplification. A. After analyzing 220 cancer related genes expression in control (n=34) and *URI1* amplified tumors (n=23), heat map representation shows 34 genes with significant expression alternation. B. UCS tumors with *URI1* amplification have decreased levels of *PTEN* (P=0.0082), *CASP8* (P=0.0035), *CYB5A* (P=0.0022), and *E2F5* (P=0.031). C. UCS tumor with URI1 amplification have increased expression of *NRAS* (P=0.0044), *AKT2* (P=0.0024), *MAP2K1* (P=0.0020), and *MMP21* (P=0.0081).

sion of 220 genes that regulate cell cycle, proliferation, apoptosis, cancer metastasis, and differentiation. We specifically excluded genes on chromosome 19 where the *URI* gene resides to avoid genome linkage effects as a result of *URI* amplification. We identified 33 genes with significant mRNA expression alteration, among which 13 genes were downregulated and 20

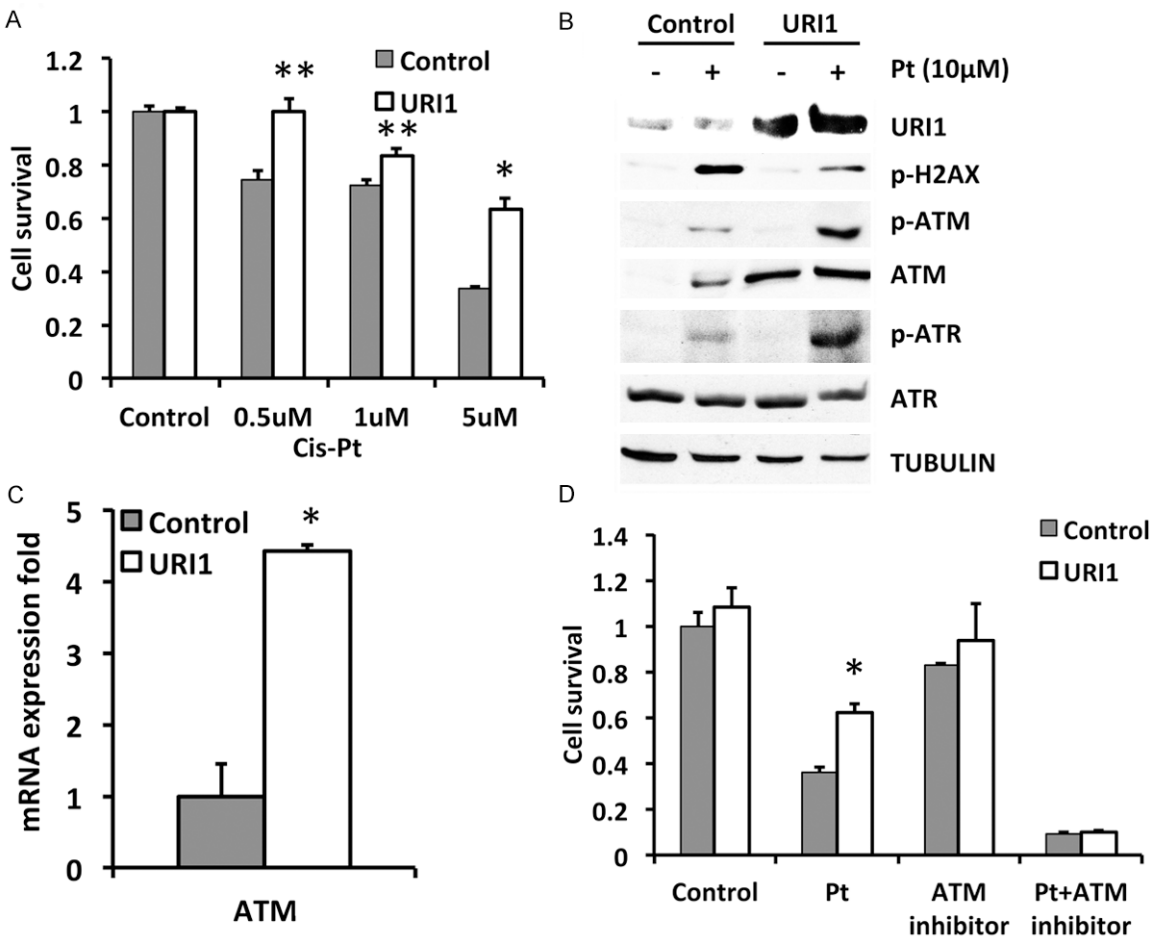

Figure 3. URI1 induces ATM expression and promotes DNA damage resistance. A. RL 95-2 cells were transfected with control plasmid or URI1. After 48 hours of cisplatin treatment, cell survival was measured using the CyQUANT assay. Error bars represent standard deviation from three independent experiments. (*P<0.0001, **P<0.05). B. RL 95-2 cells were transfected with URI1 and treated with cisplatin for 48 hours. Cell lysates were subjected to western bot analysis. C. RL 95-2 cells were transfected with URI1. After 48 hours, cell mRNA was extracted and reversely transcribed to cDNA. *ATM* mRNA expression was quantified by q-RT PCR. (*P<0.0001). D. RL 95-2 cell were transfected with control plasmid or URI1. After 48 hours of cisplatin (5 μM) or/and ATM inhibitor (100 nM) treatment, cell survival was measured using the CyQUANT assay. (*P<0.001).

were upregulated (**Figure 2A** and Table S3). Functional cluster analysis showed significant alteration in apoptosis and cell proliferation pathways. For example, *URI1* amp tumors have decreased expression of the pro-apoptotic enzyme *CASP8* (P=0.0035), the tumor suppressor *PTEN* (P=0.0082), the membrane-bound cytochrome with tumor suppressor and autophagy functions *CYB5A* (P=0.0022), and the transcriptional activator of proliferative genes *E2F5* (P=0.031. **Figure 2B**). UCS tumors with amplified *URI1* also express increased levels of the anti-apoptotic factor *AKT2* (P=0.0024), the proto-oncogene *NRAS* (P=0.0044), the metastatic promoting matrix metalloproteinase *MMP21* (P=0.0081), and the MAPK activator and mediator of cell growth *MAP2K1* (P=0.0020, **Figure 2C**).

Our data above showed that patients with *URI1* amplification were less responsive to adjuvant therapies that induce DNA damage (**Figure 1C**). To elucidate the mechanism underlying such treatment-resistance, we investigated cell response to DNA damage upon URI1 upregulation. Overexpression of URI1 significantly increased cell survival after cisplatin treatment of the uterine/endometrial cancer cell line RL95-2 (**Figure 3A**). Cells with increased *URI* expressed a much higher level of phospho-ATM and phospho-ATR after cisplatin-induced DNA damage (**Figure 3B**). However, URI overexpressing cells also displayed dramatically

lower γH2AX phosphorylation levels compared to controls cells after cisplatin treatment, suggesting less DNA-damage accumulation and therefore a URI1-mediated genome protective effect (**Figure 3B**). In a time course, we found that the phospho-γH2AX levels in URI-overexpressing cells reached peaks after 3 hours of cisplatin treatment and diminished quickly, while the DNA damage in control cells kept accumulating for over 24 hours (Figure S1). This suggests that although DNA damage pathways are activated, the resultant γH2AX foci are more rapidly resolved in *URI1* amplified cells. Therefore, *URI1* amplification promotes resistance to DNA damage. We also found that URI-induced ATM upregulation was at the level of transcription with mRNA expression increasing over 4-fold (**Figure 3C**). Importantly, treatment with cisplatin and ATM inhibitor together resulted in cell death in *URI1* overexpressing cells compared to cisplatin alone, suggesting that URI-induced chemo-resistance is ATM-dependent and this combination could be useful for the treatment of chemo-insensitive patients with *URI* amplification (**Figure 3D**).

Discussion

URI was first identified as a chaperone protein and a component of the RNA polymerase complex [18]. In later studies, we and others have demonstrated its function in both cytoplasm and nucleus, regulating proliferation and apoptosis. For example, URI has proliferative and anti-apoptotic effects in hepatitis, breast and colorectal cancer cell lines, but not in immortalized but non-transformed renal and liver cells [18, 26]. Studies in prostate cancer, however, revealed an anti-proliferative role of URI [31, 32]. Further, a recent study reported *URI1* amplification in 10% of ovarian cancer [24]. In this cohort of ovarian cancer patients, increased URI expression associated with larger tumor size, higher grade and chemo-resistance [24]. Ovarian cancer cell lines carrying *URI1* copy number variations are rapamycin-resistant and more proliferative [24]. Two more recent publications showed increased mRNA expression of *URI1*, along with several other potential oncogenes such *GAB2* and *PAK4*, in endometrial cancer [27, 33]. The *TP53* mutation rate (98%) in the cohort we analyze is much higher than a previously reported 32%, from a study in 25 UCS cases [34]. This may be due to the high percentage (53%) of stage III and IV carcinosarcoma in our cohort, compared to other reported cases (27%) [6].

Although UCS presents with undifferentiated features and a poor prognosis, risk factors from our analysis include body weight, history of hypertension, tumor stage, and local lymph node involvement, which all correlated with poor survival. Our analysis showed that *URI1* amplification negatively associates with tumor-free survival after primary treatment (P=0.023) and increases patient risk of death by more than 6.5 fold. In contrast, a history of menopause hormone therapy appeared to have a dramatic protective effect in UCS overall survival by over 15-fold. Besides standard surgical treatment, UCS patients were often given adjuvant RT, or chemotherapy depending on the tumor stage. Despite the fact that UCS patients with *URI1* amplification have a worse response to RT or chemotherapy compared to control patients, our analysis suggests that combined RT and chemotherapy might provide significant benefit to patient overall survival with *URI1* amplification. It is interesting that progesterone or progestin use in hormone contraception is inversely associated with *URI1* amplification (r_s=-0.52, P=0.011). A number of studies suggest that current use of oral contraceptives appears to increase the risk of breast cancer, and cervical cancer [35, 36]. In contrast, women who use oral contraceptives have reduced risks of ovarian and endometrial cancer [37, 38]. It requires further study to elucidate the potential mechanism underlying the association between progesterone use and *URI1* amplification.

Our *in vitro* data indicated that increased URI promotes ATM expression in uterine cancer cells. As a result, cells became less responsive to cisplatin treatment. Importantly, the combination of cisplatin and ATM inhibitor reversed URI-induced DNA damage-resistance, suggesting the potential benefit of including ATM inhibitor with current chemo- and radiation-therapy in patients with *URI1* amplification. Other mechanisms could also contribute to URI-induced chemo-resistance. For example, most UCS tumors carry *TP53* mutation or deletion. Although DNA damage pathways are activated and upregulated, they appear to be ignored and γH2AX foci are more rapidly resolved in *URI1* amplified cells. This could result in URI-dependent protection from DNA damage induced cell death.

One limitation in our study is the lack of reliable UCS models. As an alternative, we used the RL 95-2 cell line, which is derived from patients with moderately differentiated uterine carcinoma. An UCS patient derived cell line would be a better model to investigate this disease at molecular and cellular level. A further retrospective study utilizing a larger patient cohort may provide a better understanding of the impact of *URI1* copy number variation in UCS. Overall, our study indicates that *URI1* amplification in UCS strongly associates with a poor prognosis as a result of protection from DNA damage. The idea that *URI1*-amplification dependent chemo-resistance can be overcome by inhibiting ATM is a potential translational off shoot of our study.

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Disclosure of conflict of interest

None.

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Table S1. *URI1* CNV in human cancers

Cancer Types	Case	Cases with CNV	Amplification	Deletion	Percent case altered
Uterine Carcinosarcoma	57	23	23	0	40.3%
Uterine Corpus Endometrial Carcinoma	363	20	20	0	5.5%

Table S2. Pairwise Spearman's rank correlation coefficient between patient characteristics

		URI1 amp	Menopausal hormone therapy	Hormonal contraceptives	Hypertension	Diabetes	Pregnancies	Weight	Pelvic positive	Aortic positive	Age	Clinical stage	Positive margins
URI1 amp	r_s	1											
	p	.											
Menopausal hormone therapy	r_s	0.237	1										
	p	0.076	.										
Hormonal contraceptives	r_s	**-0.521**	0.046	1									
	p	**0.011**	0.735	.									
Hypertension	r_s	0.18	-0.128	-0.199	1								
	p	0.18	0.343	0.137	.								
Diabetes	r_s	0.067	0.046	-0.118	0.03	1							
	p	0.618	0.735	0.383	0.824	.							
Pregnancies	r_s	0.128	0.142	0.267	-0.12	-0.093	1						
	p	0.39	0.34	0.069	0.424	0.534	.						
Weight	r_s	-0.239	-0.101	-0.165	0.204	0.099	0.009	1					
	p	0.098	0.49	0.257	0.161	0.498	0.951	.					
Pelvic positive	r_s	0.158	0.019	-0.205	0.013	0.184	-0.002	-0.008	1				
	p	0.24	0.887	0.126	0.926	0.17	0.99	0.957	.				
Aortic positive	r_s	0.036	-0.162	0.008	-0.01	-0.149	0.006	0.035	**0.397**	1			
	p	0.79	0.229	0.951	0.94	0.27	0.967	0.81	**0.002**	.			
Age	r_s	0.085	-0.237	-0.125	0.257	0.113	0.012	-0.207	**0.274**	0.042	1		
	p	0.53	0.075	0.353	0.053	0.402	0.937	0.153	**0.039**	0.754	.		
Clinical stage	r_s	0.171	-0.153	-0.14	0.118	0.046	-0.07	-0.023	**0.375**	**0.283**	0.038	1	
	p	0.203	0.256	0.3	0.383	0.734	0.638	0.878	**0.004**	**0.033**	0.779	.	
Positive margins	r_s	0.189	-0.193	-0.037	-0.127	0.103	-0.124	-0.103	0.082	0.13	**0.338**	**0.376**	1
	p	0.158	0.15	0.785	0.345	0.444	0.406	0.481	0.543	0.333	**0.01**	**0.004**	.

Table S3. Gene expression changes in UCS with URI1 amplification

Gene	Fold changes (URI1 amp/control)	*P*-value
RAB25	0.44	0.0452
HLA-G	0.47	0.0264
CYB5A	0.56	0.0069
MYO5C	0.57	0.0212
ST3GAL1	0.57	0.0260
MMP15	0.58	0.0109
THEM6	0.61	0.0131
GALNT12	0.64	0.0341
MMP24	0.64	0.0485
CASP8	0.65	0.0215
E2F5	0.66	0.0205
ASF1B	0.77	0.0388
PTEN	0.80	0.0178
GSK3B	1.25	0.0243
E2F4	1.25	0.0401
CASP9	1.30	0.0249
E2F6	1.30	0.0249
NRAS	1.32	0.0046
ECH1	1.37	0.0292
MAP2K1	1.48	0.0016
SARS2	1.52	0.0084
SUPT5H	1.64	0.0092
PSENEN	1.66	0.0028
RBM42	1.70	0.0021
AKT2	1.74	0.0067
CLIP3	1.75	0.0278
LIN37	1.91	0.0007
PLAGL1	2.00	0.0093
ST6GALNAC5	2.27	0.0106
FBXO17	2.43	0.0007
MMP23B	2.43	0.0045
PLEKHF1	2.87	0.0035
MMP21	3.28	0.0350
PEG3	3.43	0.0459
RYR1	5.49	0.0262

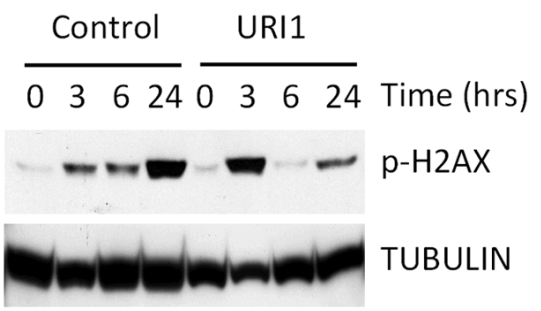

Figure S1. URI1 overexpression decreased DNA damage accumulation in RL 95-2 cells. RL 95-2 cells were transfected with URI1 and treated with cisplatin for 48 hrs. Cell lysates were harvested at different time point for western blots.