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Loss of Both USP10 and p14ARF Protein Expression Is an Independent Prognostic Biomarker for Poor Prognosis in Patients With Epithelial Ovarian Cancer

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Abstract. *Background/Aim: The prognostic role of USP10 in epithelial ovarian cancer has been studied in various human cancers. Our aim was to evaluate the clinical and pathological significance of USP10 in epithelial ovarian cancer. Materials and Methods: Immunohistochemical analyses of the expression of USP10 and p14ARF by using tissue microarrays were performed in 336 ovarian tumours and the data were compared with clinicopathological variables. We examined their level of DNA methylation around the putative transcriptional start site in 5' CpG islands in fresh frozen tissues and ovarian cancer cells. Results: Expression of USP10 and p14ARF was significantly lower in cancer tissues than in normal epithelium. Low USP10 expression and a combined USP10/p14ARF low expression were revealed to be independent prognostic factors. A high degree of methylation in USP10 and p14ARF CpG islands was found by methylation specific PCR analysis in cancer than in normal tissues and cells. Conclusion: Decreased expression of USP10 or combined USP10/p14ARF decreased expression is a strong indicator of poor prognosis in patients with ovarian cancer.*

Epithelial ovarian cancer (EOC) is one of the most common gynaecological cancer. It is one of the most lethal diseases among the top five leading causes of cancer-related death in women (1). Advanced EOC patients exhibit only 40% of 5-year survival rate (2). Surgical cytoreduction followed by adjuvant combined chemotherapy is the standard treatment of EOC patients. Despite the improvement in treatment modalities, most women with EOC experience relapse and eventually die from the disease. One of the major limitations to successful treatment is development of acquired resistance against the chemotherapeutic agent. Consequently, understanding the molecular mechanisms of EOC and the availability of predictive biomarkers for chemosensitivity of EOC would lead to the development of more specific prognostic markers to improve patient survival.

Ubiquitination and de-ubiquitination are important post-translational modifications which regulate activation, localization and degradation of proteins through conjugating or deconjugating ubiquitin from substrate proteins (3). Ubiquitin-specific protease10 (USP10), a member of the ubiquitin-specific protease (USP) family, regulates crucial signalling factors for cellular growth and apoptosis (4, 5). Recently, several studies have reported USP10 as a novel regulator of p53 in cancers (6). It has also been reported that it contributes to tumorigenesis in several types of cancers, such as breast cancer, stomach cancer, and glioblastoma (7-9). p14ARF located in the *INK4a/ARF locus* at chromosome 9q21 is an alternative reading frame product that encodes p16protein (10). It is a potent tumour suppressor which stabilizes p53 that induces cellular senescence and prevents tumour cell growth. Down-regulation or deletion of p14ARF in various cancers, including breast, lung and gastric, has been reported (11-13). Recently, Ko *et al.* (14) demonstrated that USP10 and/or p14ARF are involved in tumorigenesis of non-small cell lung cancer, suggesting that c-Myc induced transcription of USP10 by deubiquitination-dependent stabilization of p14ARF and p53. Nevertheless, the

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prognostic and clinical significance of the expression of USP10 and p14ARF in EOC patients is limited.

In this study, we evaluated the prognostic value of the expression of USP10 and p14ARF in formalin-fixed paraffin-embedded (FFPE) EOC tissues by immunohistochemistry and quantitative image analysis. Moreover, we assessed the potential correlation between the loss of USP10 and p14ARF protein methylation and expression.

Materials and Methods

Patients and tumour specimens. Tumour samples from 212 EOC, 57 borderline ovarian tumours, 153 benign epithelial ovarian tumours, and 79 nonadjacent normal epithelial tissues were included in this study. The tumour specimens were gathered from patients who underwent primary surgery at Gangnam Severance Hospital between 1996 and 2012. Some paraffin blocks were supplied by the Korea Gynecologic Cancer Bank under Bio & Medical Technology Development Program of the Ministry of the National Research Foundation (NRF) funded by the Korean government (MSIT) (NRF-2017M3A9B8069610). All tumour tissues were histologically examined and only the specimens with a sufficient proportion of tumour cells were selected for tissue microarray (TMA) construction. Tumour staging was performed by the International Federation of Gynecology and Obstetrics (FIGO) classification. Clinical data including age at diagnosis, surgical procedure, survival period, and survival status were collected by reviewing medical records. Response to therapy was monitored according to Response Evaluation Criteria in Solid Tumors (RECIST; version 1.0) by computed tomography (15). Tumour grades and cell types were collected from the pathology report. All biological samples were acquired following informed consent from patients based on institutional review board (IRB) guidelines.

Tissue microarray and immunohistochemistry. Tissue microarray with one-millimetre cores was produced from archival FFPE tissue blocks. The TMA blocks were cut in to serial 5-μm-thick sections, and the sections were deparaffinized through xylene and rehydrated gradually from ethanol to distilled water. Then, to block endogenous peroxidase, 3% H_2O_2 solution in methanol was applied. Heat-induced antigen retrieval for USP10 and p14ARF was performed for 20 min in a pressure cooker containing an antigen retrieval buffer of pH 6.0 (Dako, Carpinteria, CA, USA) for USP10 and of pH 9.0 for p14ARF. The slides were then stained with anti-USP antibody (Abcam, Cambridge, MA, USA; rabbit polyclonal antibody, Cat. # Ab72486, 1:1000 dilution for 1 h at room temperature) and anti-p14ARF antibody (Cell Signaling Technology, Danvers, MA, USA; mouse antibody, Clone# no. 4C6/4; 1:000 dilution for 1 h at room temperature). Then, for antigen-antibody reaction, En vision+ Dual Link System-HRP (Dako) and DAB+ (3, 3'-diaminobenzidine; Dako) were applied. The stained sections were counterstained by haematoxylin and mounted with Faramount aqueous and mounting medium (Dako).

Evaluation of IHC staining. After staining, TMA sections were scanned by the high-resolution optical scanner NanoZoomer 2.0 HT (Hamamatsu Photonics K.K., Japan) at X20 objective magnification (0.5 μm resolution). The captured images were analysed by using Visiopharm software version 4.5.1.324 (VIS; Visiopharm, Hørsholm, Denmark). For assessment of USP10 and p14ARF staining, the intensity of the brown staining (0=negative, 1=weak, 2=moderate, and 3=strong) was scored by optimized settings with a predefined algorithm. The overall protein expression score (histoscore) was calculated by multiplying their staining intensity with the percentage of positive cells (possible range=0-300).

DNA methylation analysis. Genomic DNA from five primary EOC tissues and five normal tissues was isolated by a standard phenol-chloroform method for promoter methylation test. Sodium bisulphite modification of genomic DNA (2 μg) was performed using the EZ DNA Methylation Kit™ (Zymo Research, Orange, CA, USA). Gene promoter methylation analysis was performed with methylation-specific PCR (MSP) primer pairs placed near the putative transcription start site in the 5' CpG island using 2 μl of JumpStart REDTaq DNA Polymerase (Sigma-Aldrich Co. St. Louis, MO, USA) for amplification and bisulphite-treated DNA as template. The p14ARF and USP10 primers for bisulphite sequencing were used as described previously (16). For bisulfide sequence analysis, 2% agarose gel electrophoresis was used to separate PCR amplicons, which were purified with the Gel Extraction Kit (Qiagen GmbH, Hilden, Germany), and cloned by using the TOPO TA vector system (Invitrogen, Carlsbad, CA, USA). Isolation and purification of each clone was done with NucleoSpin Plasmid Isolation Kit (Macherey-Nagel, Düren, Germany). The positive clones were randomly selected (10-15 from each sample) and sequenced with the M13F primer. Finally, determination of methylation status of each CpG dinucleotide was performed.

Statistical analysis. Statistical analyses of USP10 and p14ARF expression were performed by Kruskal–Wallis test or Mann–Whitney when indicated. For survival analysis, expression values were analysed by the Kaplan–Meier method and log-rank test with the cut-off values demonstrating the best discrimination (histoscore: 157 for USP10 and 63 for p14ARF). For univariate analysis and multivariate analysis, the Cox proportional hazards model was applied to determine the significance among the following clinicopathological variables: age, cell type, tumour grade, FIGO stage, and CA125. Statistical analysis was performed by using the SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). All cases with p-value <0.05 were considered statistically significant.

Results

USP10 and p14ARF expression in normal ovarian/fallopian epithelial tissues, benign, borderline tumours, and ovarian cancers. We used TMA to examine by immunocytochemistry the expression of USP10 and p14ARF in 79 nonadjacent normal epithelial tissues, 153 benign, 57 borderline tumors, and 212 EOC tissues. Subsequently, their expression was analysed using quantitative image analysis software.

USP10 was expressed in the cytoplasm of nonadjacent normal epithelial tissues, benign, borderline tumors, and EOC tissues (Figure 1A). As shown in Figure 1B, UPS10 protein expression was down-regulated significantly in EOCs (p<0.001), borderline tumors (p=0.009), and benign tissues (p=0.004) compared to normal epithelial tissues. Moreover, USP10 expression was significantly decreased by the stage

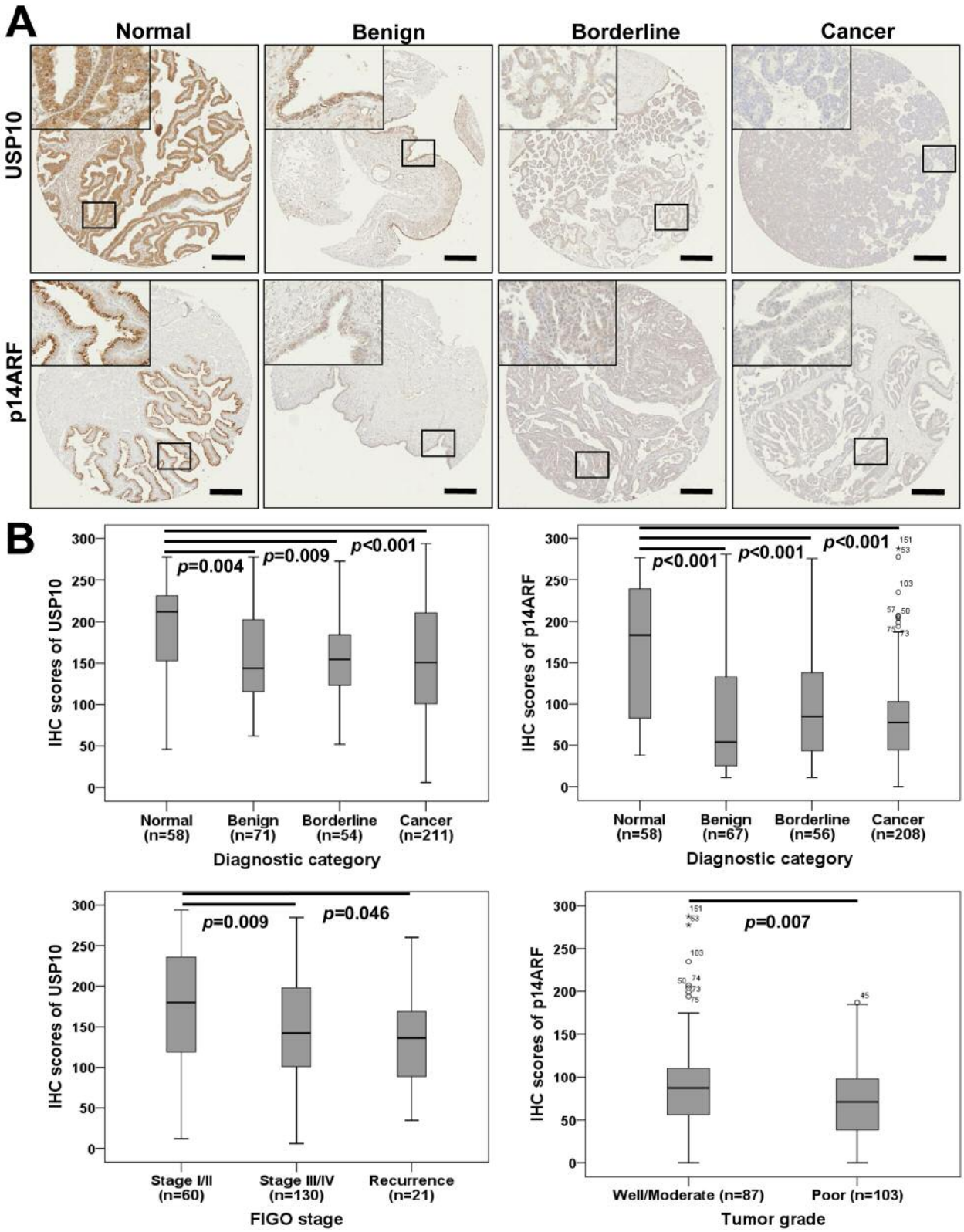

Figure 1. *USP10 and p14ARF expression in formalin-embedded normal, benign, borderline, and EOC tissues. Both USP10 and p14ARF proteins were expressed in cytoplasm. (A) Representative immunohistochemical image of USP10 and p14ARF in normal, benign, borderline, and EOC tissues. Scale bar: 250 μm. (B) IHC staining score of USP10 and p14ARF depended on diagnostic category, FIGO stage, and tumour grade.*

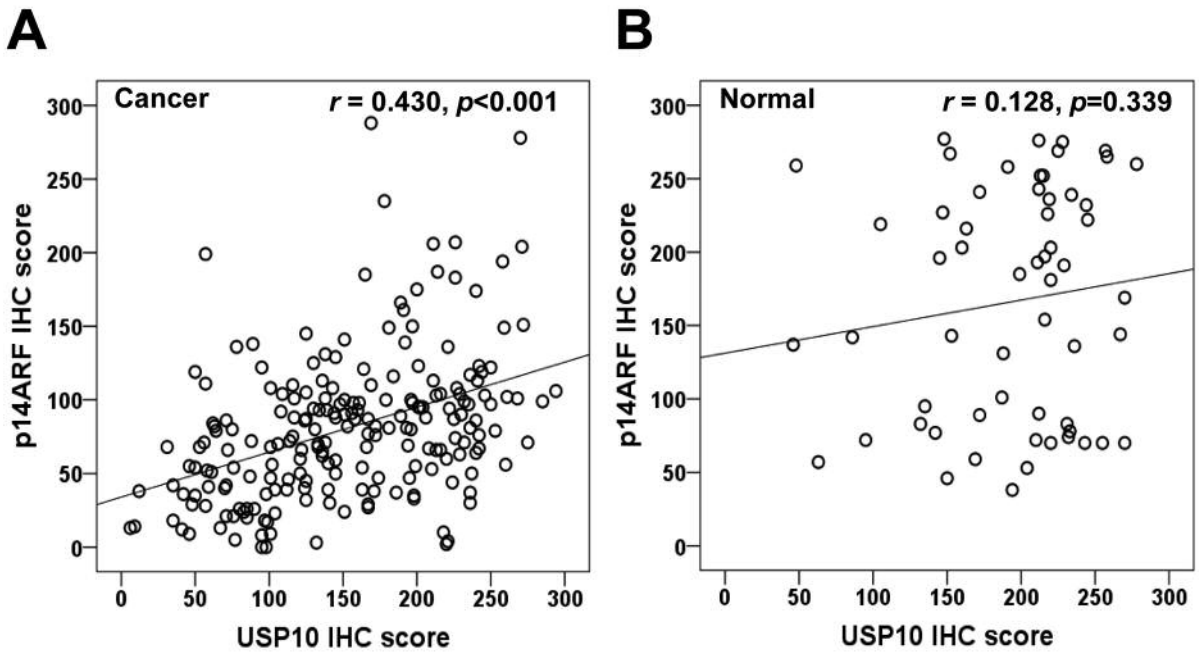

Figure 2. *Spearman's rank correlation analysis between USP10 and p14ARF expression. There was a significant positive correlation between USP10 and p14ARF expression in EOCs.*

of cancer as shown in Figure IB in following sequence: Stage I/II, Stage III/IV (p=0.009), and recurrence (p=0.046).

p14ARF followed a similar pattern in cytoplasmic expression as USP10 (Figure 1A). As Figure 1A and B show, the expression of p14ARF was significantly down-regulated in EOC tissues (p<0.001), borderline tumors (p<0.001), and benign (p<0.001) tissues compared to normal epithelial tissues. In addition, p14ARF immunoreactivity negatively correlated with poor grade (p=0.007; Figure 1B). USP10 expression status showed positive correlation with p14ARF expression status in EOC (Spearman's rho=0.430, p<0.001; Figure 2A). Meanwhile, a positive but not significant correlation was observed between USP10 and p14ARF in normal tissues (Spearman's rho=0.128, p=0.339; Figure 2B).

Clinicopathological characteristics according to USP10 and p14ARF expressions. The expression levels of USP10 and p14ARF and their association with some clinicopathological characteristics of EOC patients are summarized in Table I. Specifically, a significant association between low USP10 expression and diagnostic categories was observed. The USP10 was predominantly highly expressed in normal epithelium compared to benign, borderline tumours, and EOC (p<0.001, Table I, Figure 1B). In addition, USP10 expression was found to be significantly associated with serous cell type (p<0.023), lower stage (p<0.001), and chemosensitivity (p<0.001). However, there was no significant association between USP10 and tumour grade and CA125. In addition, down-regulation of p14ARF was associated with EOC (p<0.001) as well as lower tumour grade (p<0.007). Other clinicopathological parameters did not correlate with down-regulation of p14ARF.

Prognostic significance of USP10 and p14ARF protein expressions. To investigate the prognostic significance of USP10 and p14ARF expression in EOC, the prognostic value of USP10 and p14ARF expression on overall survival (OS) and disease-free survival (DFS) were assessed. The Kaplan–Meier plots demonstrated that down-regulation of USP10 protein was associated with poor DFS as well as OS (both p<0.001) (Figure 3A and B). The down-regulation of p14ARF expression was significantly associated with poor DFS (p=0.033) (Figure 3A). Low expression of p14ARF was associated with poor OS; however, it was not statistically significant (p=0.287) (Figure 3B). Moreover, low expression of both UPS10 and p14ARF proteins was associated with worse prognosis compared to high expression of both USP10 and p14ARF in terms of DFS and OS (both p<0.001) (Figure 3A and B).

The outcomes of univariate analysis and multivariate analysis of OS and DFS are presented in Table II. In multivariate analysis, low expression of USP10 was an independent poor prognostic factor for OS (HR=3.77, 95%CI=1.65-8.60, p=0.002) and dual USP10 and p14ARF

Table I. *Expression of USP10 and p14ARF with respect to clinicopathological characteristics of patients.*

	USP10				p14ARF			
	No.	%	Mean (95%CI)	*p*-Value	No.	%	Mean (95%CI)	*p*-Value
All	394	100	160 (153-166)		389	100	98 (91-105)	
Diagnosis				*p*<0.001				*p*<0.001
Normal	58	14.7	193 (178-207)		58	14.9	166 (145-186)	
Benign	71	18.0	155 (143-168)		67	17.2	92 (70-113)	
Borderline	54	13.7	156 (142-169)		56	14.4	101 (81-120)	
Cancer	211	53.6	153 (144-162)		208	53.5	80 (73-87)	
FIGO stage				*p*=0.005				*p*=0.189
I-II	60	28.4	176 (157-195)		58	27.9	88 (71-105)	
III-IV	130	61.6	145 (134-156)		129	62.0	75 (68-83)	
Recurrence	21	10.0	135 (108-163)		21	10.1	90 (73-107)	
Cell type				*p*=0.023				*p*=0.651
Serous	144	68.2	146 (135-156)		141	67.8	81 (73-89)	
Others	67	31.8	168 (151-186)		67	32.2	78 (65-91)	
Tumour grade								
Well/Moderate	88	45.6	159 (145-174)	*p*=0.090	87	45.8	91 (79-103)	*p*=0.007
Poor	105	54.4	143 (131-155)		103	54.2	72 (64-80)	
CA125								
Negative	35	16.8	151 (126-175)	*p*=0.821	32	15.6	84 (60-108)	*p*=0.749
Positive (>35 U/ml)	173	83.2	154 (144-163)		173	84.4	80 (73-87)	
Chemosensitivity				*p*<0.001				*p*=0.268
Sensitive	147	76.6	160 (148-171)		145	76.3	81 (72-89)	
Resistant	45	23.4	124 (108-139)		45	23.7	72 (61-82)	

FIGO: International Federation of Gynecology and Obstetrics. Protein expression was determined through analysis of an immunohistochemically stained tissue array, as described in the materials and methods section.

low expression showed high hazard ration indicating that it is a strong prognostic factor for OS (HR=4.35, 95%CI=1.58-11.90, *p*=0.004). However, low expression of p14ARF was not related to good OS (*p*=0.289). Analysing the effect on DFS, low USP10 expression was significantly associated with a good DFS (HR=2.35, 95%CI=1.42-3.87, *p*=0.001). Low expressions of both USP10 and p14ARF also showed similar result (HR=2.45, 95%CI=1.36-4.44, *p*=0.003). Moreover, FIGO stage (*p*=0.002) and tumour grade (*p*=0.026) were also independent poor prognostic factors for DFS.

Down-regulation of USP10 and p14ARF is regulated by hypermethylation of their promoter. We have systematically profiled CpG island promoter DNA methylation for USP10 and p14ARF since the association between gene silencing and promoter DNA methylation in human cancer is well known (17). In addition, it has also been reported for several cancer types that p14ARF gene silencing is regulated by promoter hypermethylation (18-20). However, the investigation of USP10 methylation level in various human cancers has not be done assertively. Therefore, the promoter methylation level for both USP10 and p14ARF in normal tissue compared to EOC tissues was investigated by bisulphite sequencing analysis.

Even though methylation of the promoter region of USP10 was identified in normal tissue, heavy hypermethylation in EOCs was observed (24-59% of total CpG sites) compared to normal tissues (0-3% of total CpG sites) (Figure 4A). In addition, promoter region of p14ARF genes also showed heavy hypermethylation in EOCs (59-87% of total CpG sites) compared to normal tissues (13-30% of total CpG sites) (Figure 4B). Similarly, some hypermethylation was detected for p14ARF gene in normal tissues. These data suggested that down-regulation of the expression of USP10 and p14ARF is regulated by promoter DNA methylation in EOC tissues.

Discussion

Despite the significant improvement in the clinical management of human cancers over the last several decades, there has been no much influence on survival in EOC. Moreover, the molecular and immunophenotyping markers that can help early diagnosis and treatment are confined. Therefore, it is paramount to identify possible molecular markers that have predictive and prognostic potential for EOC. In this study, we investigated the association between USP10 and p14ARF protein expression and their potential

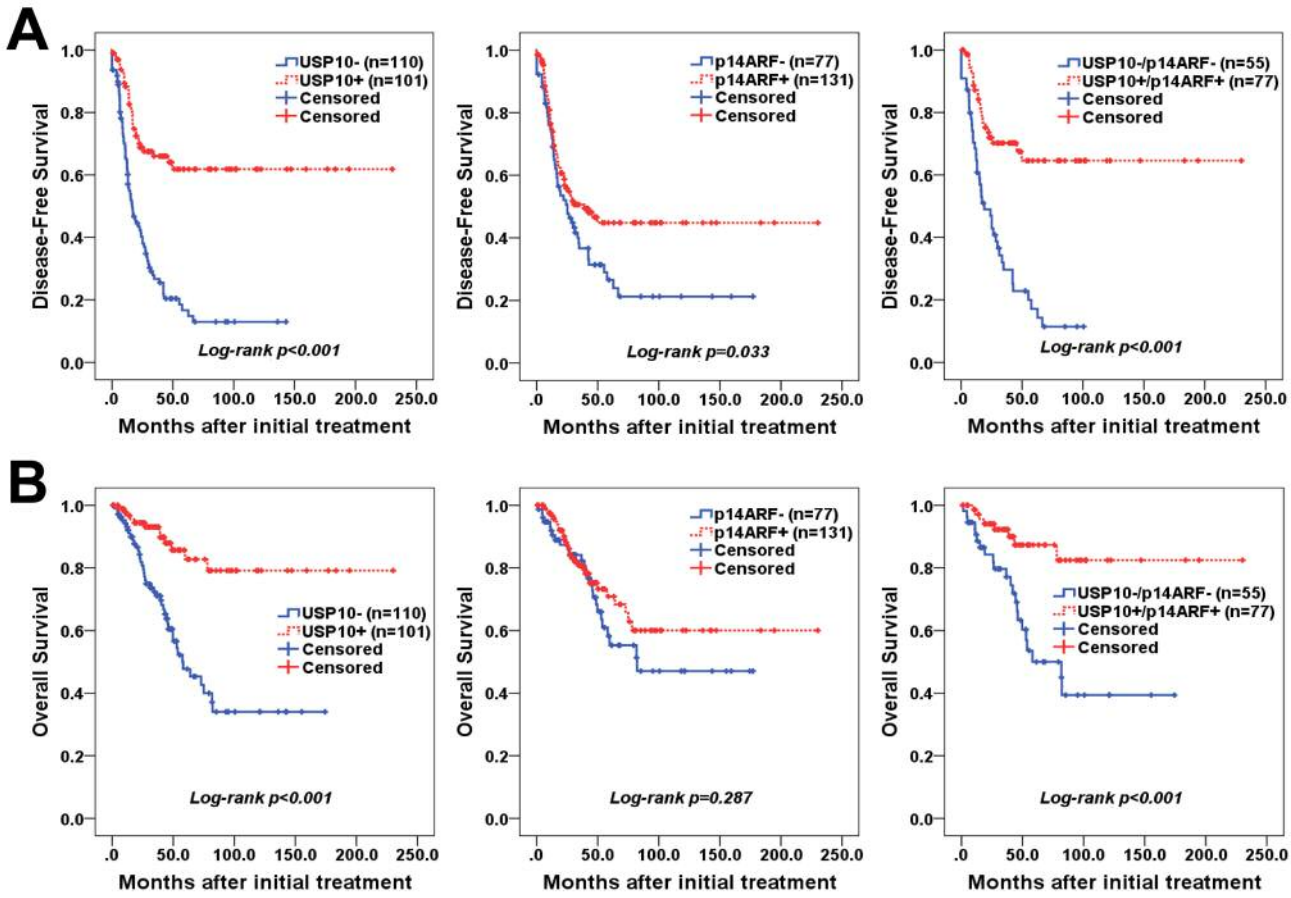

Figure 3. *Kaplan–Meier survival curves of USP10 and p14ARF expression in EOC. (A) EOC with negative USP10 expression exhibited poor DFS compared to USP10-positive groups (p<0.001). EOC with negative p14ARF expression exhibited poor DFS compared to p14ARF-positive group (p<0.033). When expression of both USP10 and p14ARF was negative, poor DFS was observed compared to the group positive for both USP10 and p14ARF (p<0.001). (B) When USP10 was not detected in EOCs, poor OS was observed compared to the USP10-positive group (p<0.001). The group in which neither USP10 nor p14ARF were expressed, poor OS was observed compared to the group positive for both USP10 and p14ARF (p<0.001).*

as prognostic makers for EOC. Also, we explored whether the low USP10 and p14ARF expression is associated with promoter hypermethylation.

DNA methylation is an important factor in epigenetic transcriptional control and genome stability (21). The hypermethylation of tumour suppressor genes and hypomethylation of oncogenes have been documented in various type of cancer (22). Previous studies have demonstrated p14ARF as a tumour suppressor gene and that it is associated with promoter hypermethylation in various human cancers including EOC (22). In addition, p14ARF has been reported to alter histone acetylation in melanoma (23). However, the association of USP10 methylation with EOC has not been established. The methylation status of USP10 was higher in EOC compared to control group (Figure 4A). Song *et al*. (24) have reported dense hypermethylation of promoter regions of both p14ARF and USP10 in small intestinal adenocarcinoma tissue compared to normal tissue, which suggests that DNA methylation analysis can be a useful tool for cancer-specific epigenetic therapy and diagnosis. In this study, we reported for the first time that down-regulation of p14ARF and USP10 could be associated with promoter hypermethylation in EOC.

DNA methylation associated with chemoresistance has also been found in various cancers. There are three types of DNA methyltransferases (DNMTs), DNMT1, DNMT3a and DNMT3b. Among them, DNMT1 has been reported as a target of adjuvant therapeutic approach to overcome chemoresistance in ovarian cancer (25). Even though a combination of platinum and taxene is the gold standard in chemotherapy for EOC, the majority of patients finally progress to platinum resistant disease. Therefore, it is important to investigate the mechanism of chemoresistance or develop new chemotherapeutic agents to overcome the drug resistance phenomenon. In our study, we found that the

Table II. *Univariate and multivariate analyses of the associations between prognostic variables and overall and disease-free survival in epithelial ovarian cancer.*

	Overall survival hazard ratio [95%CI]				Disease-free survival hazard ratio [95%CI]			
	Univariate	*p*-Value	Multivariate	*p*-Value	Univariate	*p*-Value	Multivariate	*p*-Value
Age (>50)	2.07 [1.19-3.62]	0.010	1.89 [0.98-3.64]	0.057	1.64 [1.13-2.40]	0.009	1.20 [0.78-1.85]	0.399
FIGO stage (≥III)	4.42 [1.74-11.22]	0.002	2.04 [0.78-5.32]	0.145	6.76 [3.39-13.46]	<0.001	3.38 [1.59-7.22]	0.002
Cell type (serous)	5.35 [2.13-13.43]	<0.001	2.46 [0.94-6.40]	0.065	3.28 [1.97-5.44]	<0.001	1.60 [0.87-2.92]	0.125
Tumour grade (poor)	2.28 [1.28-4.06]	0.005	1.91 [1.01-3.62]	0.046	2.15 [1.43-3.23]	<0.001	1.64 [1.06-2.54]	0.026
CA125 (>35 U/ml)	2.02 [0.86-4.73]	0.102	NA		2.12 [1.16-3.86]	0.014	1.46 [0.62-3.43]	0.382
USP10- (≤157)[a]	4.07 [2.14-7.71]	<0.001	3.77 [1.65-8.60]	0.002	3.40 [2.25-5.13]	<0.001	2.35 [1.42-3.87]	0.001
p14ARF- (≤63)[b]	1.32 [0.78-2.25]	0.289	NA		1.49 [1.02-2.16]	0.035	1.06 [0.68-1.66]	0.779
USP10 - /p14ARF -	3.94 [1.75-8.85]	0.001	4.35 [1.58-11.90]	0.004	3.61 [2.15-6.06]	<0.001	2.45 [1.36-4.44]	0.003

[a]Cut-off value of USP10 - is less than 220 of IHC score; [b]cut-off of p14ARF- is less than 63 of IHC score; CI: Confidence interval; FIGO: International Federation of Gynecology and Obstetrics; NA: not applicable.

expression level of USP10 is associated with chemoresistance (Table I). Even though, we need further evaluation whether DNMTs are involved, it is clear that inhibition of DNA hypermethylation of USP10 can be considered as an adjuvant therapeutic option for EOC.

USP10 expression was significantly inhibited and negatively correlated to tumour progression and stage in EOC. USP10 is a member of USP family which catalyses cleavage and hydrolysis of conjugated ubiquitin from target proteins (26). Up to now, p53 (6), BECN1 (5), SNX3 (27), and CFTR (28) proteins were identified as potential substrates of USP10. Even though in some cancers, such as prostate cancer (29), FLT3-ITD-positive AML (30), and glioblastoma multiforme (31), USP10 acts as a tumour initiator, in lung cancer (32), renal cell cancer (6), gastric carcinoma (33), and colon cancer (34), acts as a tumour suppressor similar to the current study in EOC. As a tumour suppressor, one of the important roles of USP10 is to catalyse deubiquitination and degradation and reverse translocation of p53 by MDM2, a known regulator of cellular p53 (6). USP10 acts as a tumour suppressor *via* the wild type *TP53* gene that encodes for p53 protein. However, with mutant p53, USP10 displayed oncogenic effect in renal carcinoma cell (6). Taken together, previous findings suggested that the role of USP10 as tumour suppressor or oncogenic protein depends on the tumour cell mutational status, such as *TP53* mutation. The limitation of this study is that while *TP53* mutation is reported in around 50% of human cancers, we did not evaluate the mutation of *TP53* in EOC. Further research about the role of *TP53* mutation in EOC is needed.

Different roles of USP have also been reported from another type of USP protein, USP7 (35). In addition, PTEN, which is a crucial tumour suppressor gene frequently lost in human malignancy, has been identified as a potential substrate protein of the deubiquitinase activity of USP10 in lung cancer (32, 36). Sun *et al*. (30) have reported that USP10 could directly interact with and stabilize PTEN by ubiquitin modifications. Moreover, both PTEN and USP10 have been proposed to antagonize c-Myc transcriptional activation *via* SIRT6 stabilization and to suppress tumour formation (37). Therefore, when both PTEN and p53 are lost, the genome is compromised, and the pathway downstream of Akt promotes cell survival and arrests cell cycle. The chromosomal alterations would be accumulated at a faster rate (38).

Since p53 protein is a substrate of USP10, we further evaluated *p14ARF/CDKN2A* which is a well-known tumour suppressor protein mediating oncogenic p53 activation. p14ARF binds to MDM2 and stabilizes p53 when there is DNA damage or cellular stress (39, 40). Although, there are numerous reports about transcriptional silencing of the p14ARF/CDKN2A gene through DNA promoter hypermethylation in many cancers, Ko *et al*. (14) have recently reported that c-Myc protein influences the stabilization of p14ARF by activating USP10 transcription. USP10 ablation destabilizes p14ARF and prevents c-Myc-induced cellular senescence. According to previous reports in non-small cell lung cancer, decreased expression of both USP10 and p14ARF have been reported to correlate with poor prognosis (14). In addition, similar findings were reported by Song *et al*. (24) in small intestinal adenocarcinoma. Song *et al*. (24) suggested that there would be a suppression effect on oncogene-induced senescence by disrupting transcriptional effect of c-Myc on USP10, and the c-Myc/USP10/p14ARF axis could be involved in the tumorigenesis. Similar to previous studies, we also found that low expression of USP10 and p14ARF correlate with tumorigenesis, and there was positive correlation between USP10 and p14ARF expression as shown in Figure 2A. The multivariable analysis also showed that when only low expression of USP10 was observed, there was significantly poor OS and DFS, but low p14ARF expression was not associated with poor OS and DFS. However, dual low expression of USP10 and p14ARF showed significant

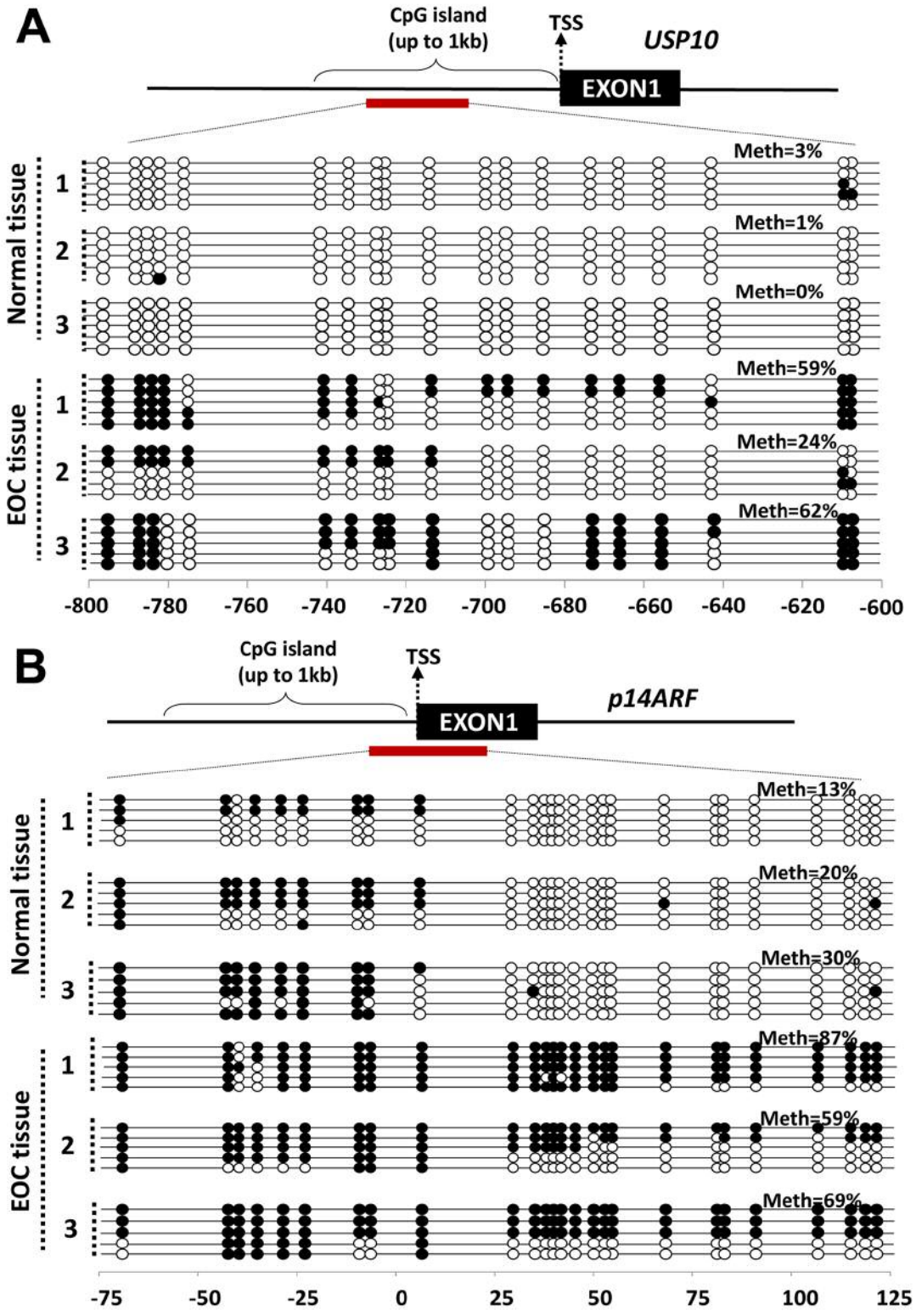

Figure 4. *The CpG island located in the USP10 and p14ARF promoter regions were analyzed by bisulphite sequencing. A schematic representation of USP10 or p14ARF CpG island is shown on the top. The red bar below CpG island represents the regions analysed by bisulphite sequencing. Vertical lines indicate each CpG site. Bisulphite sequencing analysis was performed for the (A) USP10 and (B) p14ARF genes in representative EOC (n=5) and normal (n=5) samples. Each circle represents a CpG dinucleotide. Black circles represent methylated cytosine while white circles represent unmethylated cytosines. TSS: Transcription start site; EOC: epithelial ovarian cancer.*

association with OS and DFS (Table II). Overall, the results strongly suggested that the c-Myc/USP10/p14ARF axis is an important pathway involved in the tumorigenesis of EOC.

In summary, we demonstrated that USP10 and p14ARF expression decreased in EOC. The expression of USP10 and p14ARF was highest in normal epithelial tissues, and gradually decreased in benign and borderline ovarian tumours. We also demonstrated that USP10 and p14ARF expression may be regulated by promoter hypermethylation suggesting that the p14ARF-USP10 axis affects cancer prognosis. Not only the prognostic significance of USP10 in EOC, but USP10 hypermethylation could also be associated with chemoresistance, which could be a new therapeutic target. In addition, USP10 and p14ARF protein expression could be a potential prognostic marker as the present study demonstrated their role in EOC.

Conflicts of Interest

The Authors report no conflicts of interest. The Authors alone are responsible for the content and writing of the article.

Authors' Contributions

HC, J-YC and J-HK conceived of the study and devised the experimental design. HC, J-YC and J-HK designed and build the tissuemicroarrays. GHH, HC, DBC and JMY performed experiments. GHH, DBC, HC, JMY, J-YC and J-HK performed data analysis for experiments or clinical records. GHH, HC and J-YC drafted the final version of the manuscript and figure legends. HC, J-YC and J-HK revised the figures, added critical content to the discussion and were responsible in revising all portions of the submitted portion of the manuscript. All Authors read and approved the final manuscript.

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