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Expression of DNA repair genes in ovarian cancer samples: Biological and clinical considerations

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ABSTRACT

The purpose of this study was to investigate retrospectively the mRNA expression of genes involved in different DNA repair pathways implicated in processing platinum-induced damage in 171 chemotherapy-naïve ovarian tumours and correlate the expression of the different genes with clinical parameters. The expression of genes involved in DNA repair pathways (*PARP1*, *ERCC1*, *XPA*, *XPF*, *XPG*, *BRCA1*, *FANCA*, *FANCC*, *FANCD2*, *FANCF* and *PolEta*), and in DNA damage transduction (*Chk1* and *Claspin*) was measured by RT-PCR in 13 stage I borderline and 77 stage I and 88 III ovarian carcinomas. *ERCC1*, *XPA*, *XPF* and *XPG* genes were significantly less expressed in stage III than in stage I carcinoma; *BRCA1*, *FANCA*, *FANCC*, *FANCD2* gene expressions were low in borderline tumours, higher in stage I carcinomas and lower in stage III samples. High levels of *ERCC1*, *XPA*, *FANCC*, *XPG* and *PolEta* correlated with an increase in Overall Survival (OS) and Progression Free Survival (PFS), whilst high *BRCA1* levels were associated with PFS on univariate analysis. With multivariate analyses no genes retained an association when adjusted by stage, grade and residual tumour. A tendency towards a better PFS was observed in patients with the highest level of *ERCC1* and *BRCA1* after platinum-based therapy than those given both platinum and taxol. The expression of DNA repair genes differed in borderline stage I, stage I and stage III ovarian carcinomas. The role of DNA repair genes in predicting the response in ovarian cancer patients seems far from being established.

1. Introduction

Epithelial ovarian cancer (EOC) ranks fifth in cancer incidence in Western women and has the highest mortality rate amongst gynaecological cancers.[1,2] The 5-year survival rate of ovarian cancer ranges from 30% to 92% depending on the spread of disease at diagnosis. The relatively asymptomatic nature of early-stage disease and the lack of adequate

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screening test have resulted in the fact that 75% of patients are diagnosed with evidence of metastatic spread beyond the ovary with FIGO (the International Federation of Gynecology and Obstetrics) stages III and IV. Standard treatment for late-stage disease involves cyto-reductive surgery followed by chemotherapy.[3,4] More than 70% of the patients will respond to this front line chemotherapy regimen, but the majority of them will eventually relapse in 2–3 years and die from chemo-resistant disease. Borderline ovarian tumours form a separate entity in the group of ovarian tumours as they are characterised by a degree of cellular proliferation and nuclear atypia in the absence of infiltrative destructive growth or obvious stromal invasion; the 5 year OS rate for early-stage disease is approximately 98% and varies from 86% to 92% in more advanced stages.[5]

Cis-platinum (DDP) is one of the most active agents in ovarian cancer, having greatly improved the EOC OS, with 70% of patients achieving complete remission after a first-line platinum-based therapy.[6,7] Unfortunately though, more than half develop recurrent disease.[8]

The identification of molecular markers to predict the sensitivity or resistance to platinum-containing regimens could help identify patients who would benefit from a platinum-based therapy. DDP is a DNA damaging agent, causing DNA lesions processed by the nucleotide excision repair (NER) pathway, Fanconi anaemia (FA) pathway, translesion repair (TLR), mismatch repair (MMR) and homologous recombination repair (HRR).[9–12] Experimental data suggest the importance of DNA repair in the cellular response to DDP and related compounds like carboplatin. Cells lacking *ERCC1* and *XPG*, genes coding for NER proteins, are extremely sensitive to DDP, like cells lacking a functional HRR, whilst over-expression of these proteins renders the cells resistant to the treatment.[13,14] A number of papers have analysed the role of the protein and/or mRNA expression of genes involved in DNA repair as markers predictive of response to platinum-based therapy in different tumour types, including ovarian cancer, with contrasting results.[15–24] The present study investigated retrospectively the significance of the mRNA expression of genes involved in different DNA repair pathways implicated in processing platinum-induced damage in 171 chemotherapy naïve ovarian tumours and correlated the tumour expression of the different genes with clinical parameters.

2. Materials and methods

2.1. Patients and tumour samples

Biopsies collected at primary surgery at the San Gerardo Hospital (Monza, Italy) were immediately frozen and stored at –80 °C. The collection and use of tumour samples were approved by the local Ethics Committee. Samples comprised >70% of tumour cells, as examined after haematoxylin and eosin staining. A pathologist confirmed the diagnosis of ovarian cancer, the histological subtype, according to the WHO system, and the clinical stage. According to FIGO staging, 13 tumours were diagnosed as borderline stage I, 77 as stage I and 81 as stage III ovarian carcinomas. Their histopathological features are summarised in Table 1. All patients were treated according to FIGO guidelines. Standard treatments included cyto-reductive surgery and chemotherapy depending on disease stage according to current guidelines.

2.2. RNA isolation and real time PCR

Tumour fragments were homogenised in RNA lysis buffer in ice with an Ultra-turrax and RNA was purified using the SV Total RNA Purification Kit (Promega). Retro-transcription to cDNA was done using the High Capacity cDNA Archive Kit (Applied Biosystem). Two 96-well plates were obtained, each containing a different set of samples (stage I, stage III and borderline). Some samples were present in both plates as calibrators. Genes selected have a key role in the BER (*PARP1*); in the NER pathway (*ERCC1*, *XPA*, *XPF* and *XPG*); the FA pathway (*BRCA1*, *FANCA*, *FAN C*, *FANCD2* and *FANCF*); in TLR (*PolEta*) and DNA damage checkpoint sensors (*Claspin* and *Chk1*). Optimal primer pairs (see Supplementary Table 1) were chosen, spanning splice junctions, using PRIMER-3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and the specificity was verified by detecting single-band amplicons of the PCR products. Absolute copy numbers of mRNA were determined by real time RT-PCR (ABI-7900, Applied Biosystems) with the SYBR Green technique, using an EP Motion 5075 robot (Eppendorf). Standard curves for each gene were included for absolute quantification of mRNA.

2.3. Data and statistical analysis

RT-PCR data were quality-controlled using SDS 2.3 software (Applied Biosystem) and thresholds and baselines for each gene were manually adjusted. The text files were loaded using a dedicated web platform and processed by a range of scripts written in R language for further quality control. Normalisation was done using the absolute copy number of the housekeeping gene *Actin*. Two acquisitions were done for each gene, using different samples, so to correct for inter-run technical variation, common samples were chosen as inter-run calibrators. The calibrated and normalised values were evaluated, displaying a non-normal distribution. A non-parametric ANOVA was chosen as samples were divided into three groups (borderline, stage I and stage III).

The association of gene expression in stage I and stage III carcinomas with clinical parameters was investigated in SAS using the χ^2 statistic and each gene distribution was split into three groups defined by the two tertiles calculated on patients who did not die at the end of the study. OS and PFS were classified as outcome measures and defined as the length of time from the first surgery to the last follow-up date or death (irrespective of the cause) or to the first relapse event. OS and PFS curves were plotted with the Kaplan–Meier method. The log-rank test and Cox proportional hazard models were used to compare time-to-event distributions between pre-defined groups. The estimates from the Cox regression model are presented as Hazard Ratios (HRs) and 95% confidence intervals (CIs).

Table 1 – Patients' main characteristics.

Clinical parameters	Borderline	Carcinomas	Stage I	Stage III
Number of total patients (%)	13 (100)	158 (100)	77 (100)	81 (100)
Age at diagnosis (years)				
Median (range)	41.5 (23.7–87.2)	54.4 (13.2–81.8)	52.7 (21.1–81.8)	55.8 (13.2–77.3)
Mean ± SD	47.0 ± 20.9	54.3 ± 12.6	52.7 ± 12.0	55.7 ± 13.1
FIGO stage (%)				
Stage Ia	8 (61.5)	23 (14.5)	23 (29.9)	
Ib		5 (3.2)	5 (6.5)	
Ic	5 (38.5)	49 (31)	49 (63.6)	
Stage III		81 (51.3)		81 (100)
Tumour grade (%)				
NA[a]		1 (0.6)		1 (1.2)
Borderline	13 (100)			
1		27 (17.1)	19 (24.7)	8 (9.9)
2		34 (21.5)	23 (29.9)	11 (13.6)
3		96 (60.8)	35 (45.4)	61 (75.3)
Histotype (%)				
Serous	6 (46.1)	94 (59.5)	27 (35.1)	67 (82.7)
Mucinous	6 (46.1)	18 (11.4)	16 (20.8)	2 (2.5)
Endometroid		23 (14.6)	17 (22)	6 (7.4)
Clear cell		19 (12)	16 (20.8)	3 (3.7)
Other	1 (7.8)	4 (2.5)	1 (1.3)	3 (3.7)
Residual tumour (%)				
NA[a]	0 ( )	1 (0.6)		1 (1.2)
<2 cm	13 ( )	111 (70.3)	77 (100)	34 (42)
>2 cm	0 ( )	46 (29.1)		46 (56.8)
Vital status at last follow-up (%)				
Alive WED[b]	11 (84.6)	81 (51.3)	62 (80.5)	19 (23.5)
Alive WT[c]	1 (7.7)	5 (3.2)	1 (1.3)	4 (4.9)
Progressive death	0	68 (43)	12 (15.6)	56 (69.1)
Dead for other reason	1 (7.7)	4 (2.5)	2 (2.6)	2 (2.5)
Chemotherapy (%)				
NA[a]	1 (7.7)	2 (1.3)		2 (2.5)
No	12 (92.3)	31 (19.6)	31 (40.3)	
Platinum[d]		83 (52.5)	45 (58.4)	38 (46.9)
Platinum[d] and taxol		42 (26.6)	1 (1.3)	41 (50.6)

[a] Not available.
[b] Alive without evidence of disease.
[c] Alive without tumour.
[d] Patients treated with cisplatinum or carboplatinum.

3. Results

3.1. Patients' characteristics

We selected a cohort of 171 ovarian cancer biopsies obtained at the time of diagnosis, with a median follow-up of 6.3 years. Their histopathologic and clinical parameters are summarised in Table 1. Germline mutations for *BRCA1* were unknown at the time of selection. Borderline ovarian tumours (low malignant potential) were allocated to a separate category by the WHO in 1973 considering their significantly better prognosis. Thirteen borderline ovarian cancers (all stage I) were included in the study as we were interested in a molecular characterisation of this type of tumour, being aware that there were too few to allow correlations with clinical parameters.

3.2. mRNA expression of the 13 genes involved in DNA repair pathways

Genes involved in different DNA repair pathways were studied by RT-PCR and Table 2 summarises the data for the three sub-classes of patients. There was considerable variability in expression levels in all the genes. Take the median as proxy for the expression level of all samples, the NER genes appeared to be the least expressed and *XPG* absolute levels were the lowest. *ERCC1*, *XPA*, *XPF* and *XPG* genes were significantly less expressed in stage III than in stage I carcinoma, but there was no difference between borderline cases and carcinomas. Amongst the FA genes, only *FANCF* was not differently expressed in the three groups; patterns of expression were similar in *BRCA1*, *FANCA*, *FANCC*, *FANCD2* genes, with low levels

Table 2 – Normalised and calibrated values (mean ± SD and median) of the different DNA repair genes in the tumour samples.

Pathway	Genes	Borderline (Bl)		Stage I (SI)		Stage I (SIII)		ANOVA	
		Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Mediaq	*p* value	Significant contrasts
BER	*PARP1*	0.92 ± 0.69	0.69	2.50 ± 2.10	1.82	2.02 ± 1.44	1.81	3.42E–04	SI versus Bl, SIII versus Bl
NER	*ERCC1*	1.36 ± 0.54	1.18	2.34 ± 3.54	1.59	1.38 ± 1.06	1.11	5.72E–03	SI versus SIII
	XPA	3.14 ± 2.04	2.45	2.85 ± 3.26	1.81	1.34 ± 1.24	0.89	7.52E–06	SI versus SIII, SIII versus Bl
	XPF	1.67 ± 0.68	1.43	2.55 ± 2.33	2.33	1.52 ± 1.09	1.29	3.01E–05	SI versus SIII
	XPG	3.17 ± 3.83	1.58	3.46 ± 5.90	1.45	1.74 ± 3.21	0.9	3.63E–03	SI versus SIII
FA	*BRCA1*	1.48 ± 1.61	1.19	5.05 ± 11.17	2.33	2.64 ± 3.14	1.55	2.29E–03	SI versus SIII, SI versus Bl
	FANCA	0.48 ± 0.38	0.33	3.44 ± 5.26	1.53	2.18 ± 2.23	1.46	1.85E–04	SI versus SIII, SI versus Bl
	FANC C	1.82 ± 1.03	1.52	3.42 ± 3.02	2.59	2.32 ± 1.73	1.86	1.91E–03	SI versus SIII, SI versus Bl
	FANC D2	1.07 ± 0.89	0.61	6.93 ± 12.83	3.29	4.35 ± 7.37	1.77	1.97E–04	SI versus SIII, SI versus Bl
	FANC F	1.21 ± 0.84	1	2.40 ± 2.09	2.13	2.03 ± 1.70	1.66	6.80E–02	–
TLR	*POLETA*	1.90 ± 0.66	2.11	3.43 ± 3.66	2.44	2.30 ± 2.02	1.68	5.27E–03	SI versus SIII
SENSORS	*CHK 1*	0.73 ± 0.62	0.53	3.06 ± 3.72	1.79	2.84 ± 3.28	2.22	1.77E–04	SI versus Bl, SIII versus Bl
	CLASPIN	0.73 ± 0.72	0.34	7.66 ± 16.69	2.22	6.57 ± 10.50	3.03	7.82E–05	SI versus Bl

in borderline tumours, higher levels in stage I and a lower level in stage III samples. The differences reached statistical significance value (see Table 2). *PolEta* expression levels were significantly lower in stage III than stage I. A significant increase was observed in *Claspin*, *Chk1* and *PARP1* levels between stage I than in borderline, whilst levels were similar in stages I and III samples.

3.3. *Correlations between mRNA expression and clinico-pathological*

The association between the levels of the genes and the different clinico-pathological parameters was investigated only in the 158 patients with carcinoma, excluding the 13 with borderline tumours. Table 3 reports the univariate associations that reached statistical significance. Lower levels of *ERCC1*, *XPA*, *XPG*, *XPF*, *BRCA1* and *FANCC* were associated with more advanced disease (stage III) and higher grade (grade 3). A lower level of *PolEta* was associated with advanced stage and a lower level of *Claspin* with higher grade. Higher levels of *ERCC1*, *XPA* and *FANCC* correlated with the vital state. Stratifying according to the stage, high grade was associated with a lower level of *XPG* and the clear cell histotype with low levels of *XPA* and high levels of *BRCA1* in stage I. In stage III patients, lower levels of *ERCC1* and *FANCF* were associated with higher tumour grade, and higher levels of *ERCC1*, *XPA* and *FANCC* with a residual tumour $\leqslant$ 2 cm. Correlation analyses with responses were also done in stage III patients and only the highest *BRCA1* levels associated with response ($p = 0.0357$).

Table 4 reports the univariate analysis on carcinomas with OS and with PFS. The highest tertile of *ERCC1*, *XPA*, *FANCC* and *PolEta* levels were correlated with an increase in both OS and PFS, both the intermediate and highest levels of *XPG* were associated with OS and PFS, whilst intermediate *BRCA1* levels associated with both OS and PFS and its highest level only with PFS. These correlations were lost in stage I patients, after adjusting for stage (see Supplementary Table 2). In stage III patients, the highest levels of *ERCC1* retained the association with longer OS and PFS even with a higher *p* value ($p = 0.056$

Table 3 – Significant *p* values for the association between gene expression and clinico-pathological characteristics.

Genes	Carcinomas				Stage I		Stage III		
	Stage	Grade	Serous histology	Vital state	Grade	Clear cell	Grade	Residual tumour	Response to therapy[a]
PARP1									
ERCC1	0.0028	0.0013		0.0149			0.0014	0.0175	
XPA	0.0002	0.001		0.0109		0.0489		0.0057	
XPF	0.0007	0.0233							
XPG	0.0112	0.001			0.0503				
BRCA1	0.0094	0.0439	0.007			0.0122			0.0357
FANCA					0.0236				
FANCC	0.0092	0.0296		0.0077				0.0409	
FANCD2									
FANCF		0.003					0.0024		
POLETA	0.0117			0.058					
CHK1									
Claspin		0.0191							

[a] Complete response (clinical and pathological) after the first six cycles of platinum-based chemotherapy.

Table 4 – Overall-Survival (OS) and Progression-free Survival (PFS) in ovarian patients by mRNA expression of the different genes.

Gene	Carcinomas			
	OS		PFS	
	HR	*p* value	HR	*p* value
PARP1	0.872	0.6105	1.045	0.8604
	0.61	0.1174	0.656	0.1601
ERCC1	1.005	0.9836	0.999	0.9976
	0.284	**0.0029**	**0.364**	**0.005**
XPA	0.937	0.7949	0.924	0.7383
	0.397	**0.0143**	**0.362**	**0.0044**
XPF	0.903	0.7044	0.977	0.9238
	0.594	0.1015	0.496	**0.0247**
XPG	**0.524**	**0.0278**	**0.572**	**0.0369**
	0.579	**0.0579**	**0.529**	**0.0218**
BRCA1	**0.479**	**0.0176**	**0.533**	**0.0241**
	0.704	0.1984	**0.564**	**0.0315**
FANCA	0.636	0.1074	0.716	0.2045
	0.573	0.0628	0.743	0.2826
FANCC	0.748	0.2553	0.901	0.663
	0.377	**0.0069**	**0.432**	**0.0119**
FANCD2	0.794	0.4025	0.868	0.5845
	0.737	0.3096	0.763	0.3366
FANCF	0.825	0.4963	0.899	0.6877
	0.698	0.2097	0.811	0.4363
POLETA	0.738	0.2533	0.884	0.622
	0.48	**0.0272**	**0.491**	**0.0267**
CHK1	1.05	0.8685	1.353	0.2822
	1.001	0.9987	1.219	0.4934
Claspin	0.89	0.6831	1.083	0.7644
	0.941	0.8334	0.875	0.6296
Clinical parameter (*p* value)				
Stage (III/I)	<0.0001		<0.0001	
Grade (III/I)	0.0007		0.0002	

The mRNA expression distribution of all the genes was split into three groups, as described in Section 2. Data are Hazard Ratios (HR) and CI (95% confidence interval) of the intermediate (first line) and highest expression tertile (second line) compared to the lowest tertile. Figures in bold are significant.

and $p = 0.061$), and the intermediate level of *BRCA1* was associated with increased OS and PFS ($p = 0.022$ and $p = 0.056$). The fact that no similar association could be detected in the highest tertile raises doubts about the real value of the association. Multivariate analyses using Cox regression, however, showed that none of the DNA repair gene expressions retained a significant association either on the whole patient population adjusted by stage and grade, or in stage III patients adjusted for residual tumour (Table 5).

The levels of the genes were then correlated with the response to therapy (platinum based or platinum plus taxol) for stage III patients. There was a tendency, though not statistically significant, towards longer PFS in patients with the highest level of *ERCC1* and *BRCA1* treated with platinum-based therapy ($p = 0.104$ and $p = 0.066$ respectively), compared to patients treated with platinum-based plus taxol therapy ($p = 0.683$ and $p = 0.720$) (Fig. 1 panels A and B and Supplementary Table 3). In addition, higher levels of both *ERCC1* and *BRCA1* predicted longer PFS in patients treated with the platinum-based regimen than in those given platinum plus taxol (Fig 1, panel C; $p = 0.085$ versus $p = 0.685$).

4. Discussion

This study investigated the mRNA expression of genes involved in different DNA repair pathways in 171 ovarian tumours. The analysis focused on genes involved in BER, NER, FA, TLR pathways and genes with a checkpoint function (*Chk1* and *Claspin*) as all of these have been variably involved in the repair of platinum-induced DNA lesions.

This is the first attempt to study many DNA repair genes in a big cohort of ovarian cancer patients. The data clearly show that genes involved in the FA pathway and in checkpoint function were significantly more expressed in carcinoma stage I than in borderline stage I (Table 2). This finding is open to different interpretations. As borderline ovarian tumours are a benign condition,[25] the data suggest that malignant transformation is associated with an up-regulation of genes involved in DNA repair and in maintaining genomic stability (i.e. *Chk1*). The recent proposal that increased DNA repair gene expression is associated with a metastatic phenotype in both melanomas and breast cancer would go along with our data.[26–29] Gene expression profiling showed that primary

Table 5 – Multivariate analysis on all carcinomas and stage III samples.

Genes	Carcinomas				Stage III			
	OS		PFS		OS		PFS	
	HR	*p* value	HR	*p* value	HR	*p* value	HR	*p* value
PARP1	0.985	0.9548	1.159	0.5595	0.832	0.5399	0.813	0.4788
	0.751	0.3695	0.816	0.5003	0.783	0.4971	0.797	0.5121
ERCC1	1.152	0.5743	1.17	0.5114	1.057	0.8407	1.017	0.9501
	0.601	0.2514	0.673	0.2908	0.416	0.1583	0.483	0.1823
XPA	1.135	0.6204	1.204	0.4365	0.778	0.379	0.99	0.9691
	0.807	0.5861	0.684	0.3058	1.197	0.7164	1.007	0.9887
XPF	1.212	0.4789	1.275	0.3297	1.055	0.857	1.185	0.5432
	1.277	0.4625	0.991	0.9779	1.398	0.3745	1.381	0.3885
XPG	0.675	0.1852	0.771	0.3379	0.617	0.148	0.745	0.3364
	1.195	0.5529	1.027	0.9263	1.366	0.342	1.233	0.5155
BRCA1	0.579	0.079	0.633	0.1042	0.596	0.1513	0.666	0.2087
	1.161	0.5918	0.837	0.5126	1.163	0.6602	0.934	0.8363
FANCA	0.808	0.4552	0.84	0.5159	0.766	0.4196	0.792	0.4547
	0.715	0.2693	0.896	0.6973	0.747	0.3713	0.874	0.6643
FANCC	1.093	0.7315	1.403	0.1671	0.971	0.9195	1.283	0.3614
	0.627	0.2043	0.683	0.26	1.31	0.5191	1.119	0.7786
FANCD2	1.11	0.7153	1.112	0.6905	0.748	0.3706	0.857	0.6086
	1.016	0.9579	0.893	0.6926	1	0.9999	0.746	0.3602
FANCF	0.854	0.5774	0.874	0.6137	1.167	0.6225	1.102	0.7416
	1.139	0.6638	1.284	0.374	1.043	0.9022	1.042	0.901
POLETA	1.095	0.7409	1.216	0.4418	0.836	0.5511	0.821	0.4924
	0.836	0.601	0.84	0.5957	0.689	0.3166	0.832	0.6048
CHK1	1.115	0.7168	1.494	0.1665	1.006	0.9868	1.651	0.1271
	0.895	0.719	1.094	0.7594	0.887	0.7289	0.948	0.8745
Claspin	0.541	0.0383	0.702	0.1952	0.775	0.4407	0.964	0.9048
	0.745	0.3205	0.604	0.0778	1.005	0.9891	0.689	0.2567

The mRNA expression distribution of all the genes was split into three groups, as described in Section 2. Data are Hazard Ratios (HR) and CI (95% confidence interval) of the intermediate (first line) and highest expression tertile (second line) compared to the lowest tertile. Multivariate Cox regression analysis was done adjusting by stage and grade for all carcinomas and by residual tumour for stage III samples.

tumours that metastasize over-expressed a number of genes involved in genomic surveillance and in recovery of stalled DNA replication forks, compared to primary tumours that are not likely to metastasize. Genes involved in NER were not implicated in the metastatic risk and this supports our own data.[26] Ovarian borderline tumours have a low malignant potential and a different pathogenesis from high grade carcinomas.[25] Expression profile studies cluster them[30] separately and our data reinforce the fact that they are two different entities and that the malignant phenotype seems to associate early in the clinical history of ovarian tumours with an up-regulation of DNA repair genes (compare borderline tumours stage I and carcinoma stage I). The fact that almost all the DNA repair genes were less expressed in stage III suggests that with the progression of the disease, whilst maintaining a metastatic potential, the different regulation of these genes leads to a decrease in expression. The down-regulation of genes involved in the same DNA repair pathways (NER and FA) suggests a sort of coordinated regulation. The existence in the 5′-flanking regions of the NER genes of common binding sites for proteins acting on transcription has been associated with coordinated expression.[31–33] Both loss of the proteins with transcriptional activity and/or epigenetic modification might be responsible for the down-regulation of the genes. Alterations of the methylation and acetylation status of the chromatin are frequent epigenetic events in tumours[34–36] and hypermethylation of genes involved in DNA repair has been reported in ovarian cancer (*BRCA1*, *FANCF* and *MTMG*)[37–40] and other tumour types.[41–44] The methylation status of these genes is now being analysed closely in this sample population.

Univariate analysis correlating the expression levels of the genes with different clinico-pathological parameters clearly showed that higher levels of *ERCC1*, *XPA* and *FANCC* were associated with better OS and PFS, and intermediate levels of *BRCA1* with better PFS. When the analysis was done adjusting for stage, associations were lost in stage I patients, and in stage III only patients with higher *ERCC1* still had the association with OS and with PFS (Supplementary Table 2). In the Cox multivariate analysis none of the genes maintained independent predictive power suggesting that both stage and residual tumour are overriding prognostic factors that should be accounted for in all studies looking for predictive or prognostic biomarkers in ovarian cancer.

The present findings disagree with a number of preclinical and clinical studies. Preclinical evidence has been put forward that cell lines over-expressing *BRCA1* and *ERCC1* were more resistant to the cytotoxicity of DDP,[13,45] and that the lack of *BRCA1*[46] and *ERCC1*[9] or their down-regulation by siRNA or antisense strategies sensitised cells to the same drug.[47,48] Clinical studies on the predictive/prognostic roles of some of the genes suggested that lower levels of *ERCC1* and *BRCA1*,

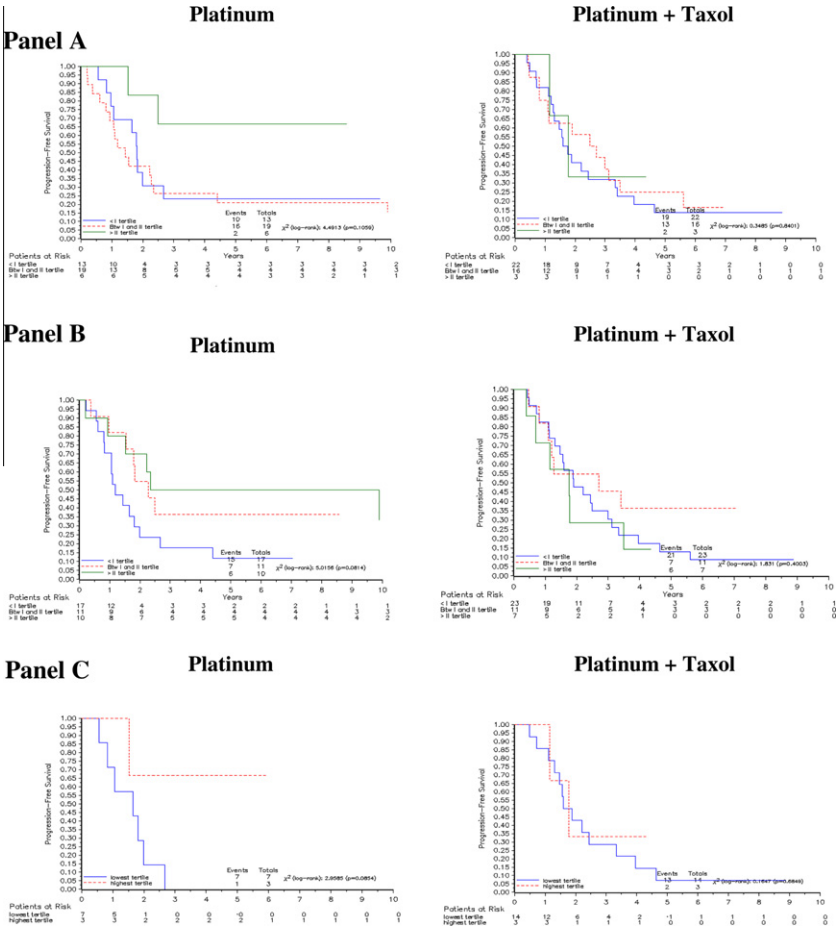

Fig. 1 – Kaplan–Meier progression-free survival estimates in stage III ovarian cancer patients given platinum-based chemotherapy (left hand panels) or platinum plus taxol (right hand panels) by *ERCC1* expression (panel A) and *BRCA1* expression (panel B) or by both highest and lowest *BRCA1* and *ERCC1* levels (panel C).

the genes most studied, were associated with longer PFS in patients given the platinum-based therapy for different tumour types, but their value as predictive markers is not yet clear.[15–20,22] In ovarian cancer, low levels *ERCC1* and *BRCA1* correlated with improved survival[20] and patients with low/intermediate levels of *BRCA1* had significantly better OS than patients with high levels.[21] In both studies the levels of the genes were quantified by RT-PCR and the possible reasons for the differences from our data might be the cut-off values used to stratify the patients, not always reported, and the numbers of patients. *XPG* (*ERCC5*), but not *ERCC1*, has been recently reported to have prognostic value in ovarian cancer; patients with a down-regulated gene have longer PFS than those patients with up-regulation.[49] However, we found the opposite, a higher level of XPG mRNAs being associated with better survival, but the association was lost in stage-adjusted analysis.

When we analysed the predictive role of the gene expression levels in response to therapy in stage III patients, there was a tendency towrds shorter PFS in patients with higher levels of *ERCC1*, *BRCA1* or both, in patients treated with taxol plus platinum-based therapy than in those given platinum compounds alone. These data clearly need to be confirmed in larger series of patients and suggest not only that the activity of taxol seems to be independent of the levels of these genes, but that adding taxol to a platinum-based chemotherapy in patients with the highest levels of *ERCC1* and *BRCA1* might interfere with the activity of platinum. These latter data corroborate the report by Smith et al.[50] in which PSF and OS did not vary amongst patients with different mRNA ERCC1 levels treated with DDP and taxol; whereas in patients treated with DDP alone, there was an association between high ERCC1 levels and shorter OS. Again, patients with higher BRCA1 levels showed a tendency towards longer OS when treated with platinum plus taxol than patients given only platinum.[21] These contrasting results suggest that the DDP-taxol combination merits further *in vitro* and *in vivo* experiments considering that both drugs are now the standard for ovarian cancer.

We studied the mRNA expression profile of genes involved in different DNA repair pathways important for the activity of cisplatinum in a large cohort of ovarian patients. Interestingly, we found a different expression of the genes involved in DNA repair pathways among borderline stage I, stage I and stage III ovarian carcinomas. When we correlated the gene expression levels with the clinico-pathological parameters, we found that high levels of *ERCC1*, *XPA*, *FANCC*, *XPG* and *PolEta* correlated with an increase in Overall Survival

(OS) and Progression Free Survival (PFS), whilst high *BRCA1* levels were associated with PFS on univariate analysis; however, these correlations were lost in multivariate analysis. Shorter Progression Free Survival (PFS) in patients with higher levels of *ERCC1*, *BRCA1*, or both genes was observed in patients treated with taxol plus platinum-based therapy than in those given platinum compounds alone, even this trend did not reach a statistically significant value. These data suggest that the predictive role of DNA repair genes in predicting the response in ovarian cancer patients to a platinum-based therapy is far from being established and more studies are indeed needed.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.11.029.

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