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DNA Damage Repair Proteins (PARP1, XRCC1 and POLβ) have Unfavorable Potential Prognostic Role in Primary Ovarian Cancer

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Abstract

Background: DNA Damage Repair (DDR) proteins have crucial roles and can modify tumor behavior. XRCC1, PARP1 and polβ are important driving biomarkers that could enhance OC development and progression. They are key proteins in DDR. Although different studies have investigated their role in different cancerous organs, there remains a lack of knowledge on how the interaction between these proteins can influence the ovarian tumor cases. This study evaluates the biological and prognostic significance of the above proteins, in addition, to identify the one that has the dominating function in primary OC patients.

Methods: PARP1, XRCC1 and polβ expression was assessed immunohistochemically in a large primary OC cohort (n=525). Outcome analysis was evaluated using Progression Free (PFS) and Overall Survival (OS).

Results: High PARP1and XRCC1 expression was associated with features of high risk OC, including serous histology, advanced grade/stage, and higher residual tumor following surgery. Similarly, polβ overexpression was linked to serous histology and higher stage tumors. On the other hand, XRCC1 negative/low expression improved patients' platinum sensitivity. Interestingly, overexpression of all the surrogate biomarkers was associated with poor outcome, not just in the whole cohort but also in platinum sensitive tumors. Importantly, the multivariate analysis revealed that XRCC1 is an independent factor for poor PFS and PARP1 for OS.

Conclusion: The DDR proteins (PARP1, XRCC1 and polβ) have crucial roles in OC progression and can potentially predict patients' outcome. The data presented here reveal a novel network between the investigated proteins with vital clinical applications in OC. Therefore, this current study highlighted the critical role of this network, which could be utilized as a future therapeutic target in primary OC.

Keywords: DNA Repair; XRCC1; PARP1; polβ; Ovarian cancer; Prognosis

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Introduction

To date and in spite of the availability of personalized cancer therapeutic agents and the advances in platinum-based chemotherapy, the survival in advanced Ovarian Cancer (OC) patients remains poor. Platinum agents' can induce intra and inter-strand DNA adducts in cells, which if unrepaired, can promote the development of DNA Double Strand Breaks (DSBs) during replication. This induced DNA damage, is detected and processed through the DNA repair mechanisms [1,2].

The enzyme Poly-(ADP)-Ribose Polymerase 1 (PARP1) is critically involved in DNA repair. The formation of PAR (Poly-ADP-Ribose) polymers occurs when PARP1 is activated through binding to DNA single-strand breaks. As a result, other DNA repair factors (including XRCC1) will be recruited at the DNA damage sites resulting in efficient DNA repair [1,3,4]. Interestingly, in solid tumors, it has been demonstrated that PARP1 is vital and strongly related to promoting platinum chemo-resistance and it gets up regulated in different types of cancers, which could contribute to resistance against anticancer treatments [5-7]. Therefore, this led to the development of PARP1

inhibitors as anticancer drugs [8-11]. However, multiple intrinsic or acquired factors can contribute to development of resistance to PARP inhibitors [1,12].

X-ray Repair Cross-Complementing protein 1 (XRCC1) has essential roles in DNA repair. It is a scaffolding protein and its N-terminal domain binds to DNA strand breaks [2,13,14]. XRCC1 is composed of three domains, BRCT I domain that interacts with PARP1, BRCT II domain which binds to Ligase III and the N-terminal domain which links to POLβ [14]. XRCC1 is involved in coordination of DNA Base Excision Repair (BER) and Single-Strand Break Repair (SSBR). In addition, XRCC1 also has roles in alternative Non-Homologous End Joining (alt-NHEJ) pathway for Double Strand Breaks (DSBs). Delays in SSB re-joining leading onto SSBs and ultimately if unrepaired, to double DSBs [15,16]. Moreover, XRCC1 deficiency potentiates chemotherapy cytotoxicity including platinum sensitivity in OC cells [15,17]. Furthermore, studies in OC patients have demonstrated a significant association between XRCC1 positive expression and clinical outcome [17-19].

Mitochondria in mammalian cells contain an efficient BER system that can employ DNA Polymerase β (POLβ) in order to remove some of the oxidative DNA damage products [20]. Therefore, POLβ has a key role in maintaining the genomic stability [20]. Polβ is a distributive polymerase that devoid any proof reading activity. It interacts with several components of the BER machinery such as XRCC1, PARP1 and ligase III to accomplish its biochemical functions [21-23]. About 30% of human tumors appear to express polβ variant proteins, which are associated with aggressive tumor phenotype [24]. Moreover, an increase level of polβ was measured in different types of cancer, including prostate, breast, and colonic [25]. Nevertheless, both Polβ levels and activity were noted to increase in chronic myeloid leukemia patients [24]. Similarly, in OC, higher levels of POLβ were also detected in cancerous tissue specimens [26]. Importantly, POLβ deficiency can promote sensitivity of tumor cells to alkylating agents, induced apoptosis, and chromosomal breaking [25,27-29].

PARP1, XRCC1 and Polβ interact with each other resulting in divergent associations in OC patients. Here, we aimed to explore their prognostic and biological significance in primary OC tissues using the large well-characterized primary OC series. Our interpretation of the prognostic and altered expression of PARP1, XRCC1 and POLβ may reveal new insights into the prognostic knowledge of ovarian tumors.

Methods

Study cohort

This study was carried out on a consecutive series of 525 primary OC cases diagnosed between 1997 to 2010 at Nottingham University Hospitals (NUH) NHS Trust, United Kingdom (UK). The clinicopathological data included: Histology subtype, International Federation of Obstetricians and Gynecologists (FIGO) stage, grade, tumor surgical debulking, chemotherapy regimen used. Whereas all patients received platinum-based chemotherapy, platinum sensitivity/resistance (defined as patients who had progression during first-line platinum chemotherapy or relapse within 6 months after completing platinum treatment), and tumor relapse with survival status were retrieved from the electronic patients' records in the hospital computer systems. Survival was calculated from the operation date until the 1st of October 2016 when any remaining survivors were censored. Progression-free survival was calculated from the date of the initial surgery to disease progression or from the date of the initial surgery to the last date known to be progression-free for those censored. Supplementary Table 1 summarizes the clinicopathological parameters of the study cohort.

Immunohistochemistry

Tissue Microarrays (TMAs) were prepared from primary OC cohorts as described previously [30]. The TMA was constructed using a TMA GRAND MASTER 2.4-UG-EN MACHINE, using 1 mm punch sets. To reduce the impact of tumor heterogeneity, three separate areas from each case were cored.

The primary antibody specificity of surrogate biomarkers was validated using Western blot on whole cell lysates of A2780, and A2780C is human OC cell lines (obtained from the American Type Culture Collection; ATCC, Manassas, USA). All the surrogate proteins supported a specific band at the expected molecular weight

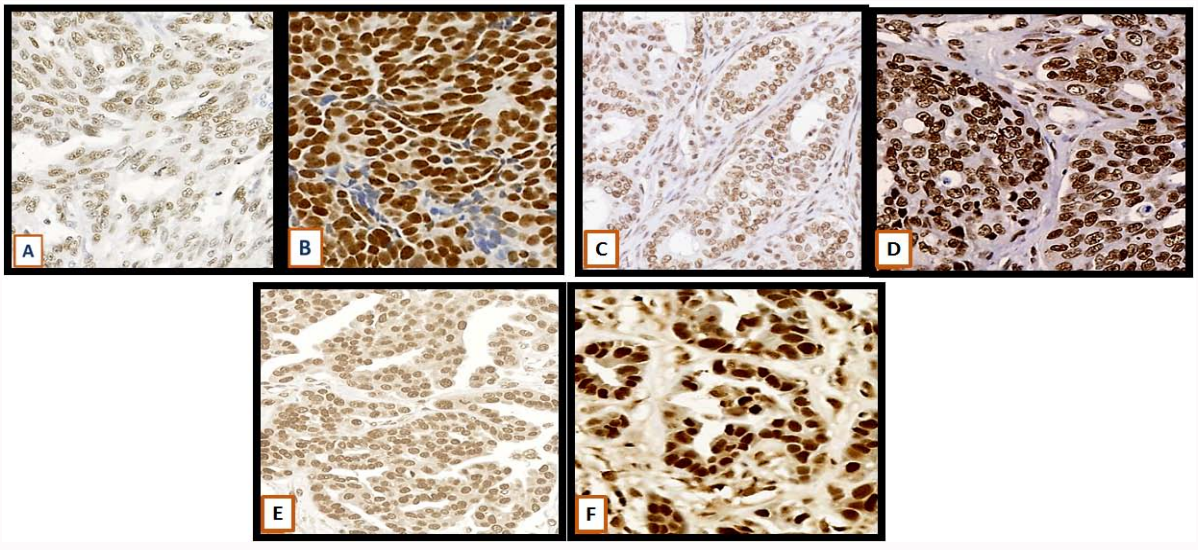

Figure 1: Immunohistochemistry expression of PARP1, XRCC1 and polβ in tissue microarray images.
(A and B) represent the expression of PARP1 in cancer cells; A) weak staining, B) strong staining. (C and D) represent the expression of XRCC1 in cancer cells; C) weak staining, D) strong staining. (E and F) represent the expression of polβ in cancer cells; E) weak staining, F) strong staining. Images were captured at 20-times magnifications.

Table 1: Clinicopathological significance of PARP1, XRCC1 and polβ proteins expression in the patients of primary ovarian carcinoma. Significant values are in bold.

Parameter	PARP1- No. (%)	PARP1+ No. (%)	*P- value* (X^2)	XRCC1- No. (%)	XRCC1+ No. (%)	P- value (X^2)	Polβ - No. (%)	Polβ+ No. (%)	P- value (X^2)
Pathological Type									
Serous	84 (35.7)	151 (64.3)		66 (24.7)	201 (75.3)		113 (48.5)	120 (51.5)	
Mucinous	37 (78.7)	10 (21.3)		23 (47.9)	25 (52.1)		32 (74.4)	11 (25.6)	
Endometrioid	40 (58.0)	29 (42.0)	***<0.0001* (-54.305)**	30 (41.7)	42 (58.3)	***<0.0001* (-28.104)**	49 (69.0)	22 (31.0)	***0.001* (-21.876)**
CCC	29 (80.6)	7 (19.4)		24 (58.5)	17 (41.5)		26 (70.3)	11 (29.7)	
Mixed	3 (21.4)	11 (78.6)		4 (28.6)	10 (71.4)		6 (50.0)	6 (50.0)	
Other	6 (40)	9 (60)		3 (25)	9 (75)		4 (33.3)	8 (66.7)	
FIGO Stage									
I	78 (52.3)	71 (47.7)		70 (44.9)	86 (55.1)		101 (67.3)	49 (32.7)	
II	29 (44.6)	36 (55.4)	0.443 (-2.685)	18 (26.5)	50 (73.5)	***0.001* (-15.581)**	25 (41.0)	36 (59.0)	***0.003* (-14.273)**
III	74 (43.5)	96 (56.5)		58 (29.7)	137 (70.3)		87 (52.4)	79 (47.6)	
IV	12 (48.0)	13 (52.0)		5 (16.7)	25 (83.3)		15 (55.6)	12 (44.4)	
Grade									
G1	43 (75.4)	14 (24.6)		27 (46.6)	31 (53.4)		30 (61.2)	19 (38.8)	
G2	31 (46.3)	36 (53.7)	***<0.0001* (-21.148)**	24 (31.6)	52 (68.4)	0.105 (-4.515)	45 (64.3)	25 (35.7)	0.072 (-5.259)
G3	104 (41.8)	145 (58.2)		90 (32.5)	187 (67.5)		124 (50.4)	122 (49.6)	
Measurable Disease									
Non	138 (52.1)	127 (47.9)	***0.024* (-5.121)**	138 (52.1)	127 (47.9)	***0.002* (-9.362)**	148 (57.6)	109 (42.4)	0.522 (-0.41)
Measurable	48 (39.7)	73 (60.3)		48 (39.7)	73 (60.3)		66 (54.1)	56 (45.9)	
Platinum sensitivity									
Sensitive	154 (49.7)	156 (50.3)	0.397 (-0.717)	127 (38.6)	202 (61.4)	***0.005* (-7.79)**	157 (52.0)	145 (48.0)	0.076 (-3.157)
Resistant	29 (43.9)	37 (56.1)		17 (21.8)	61 (78.2)		25 (39.7)	38 (60.3)	

and as instructed by their supplier, 116 KDa for the PARP1 protein, 85 KDa for the XRCC1 protein and 38 KDa for the polβ protein, Supplementary Figure 1.

Optimization of the surrogate antibodies was performed to identify the optimal concentration and incubation for representative staining, detailed in the Supplementary Table 2, 3. Then, after selecting the optimal antibody concentration, expression of PARP1, XRCC1 and polβ proteins in OC was assessed by IHC using the Novocastra Novolink TM Polymer Detection Systems kit (Code: RE7280-K, Leica, Biosystems, UK). TMA sections (4 µm) were stained with the primary antibodies diluted with Bond™ primary antibody diluent to reach. The optimized concentration of the recombinant Ag solution required us to totally neutralize the binding capacity of the primary mouse anti-human PARP1 antibody was 1:600 with 60 min incubation at room temperature. TMA sections were incubated for 30 min at room temperature with 1:250 of the second antibody, anti-XRCC1 mouse monoclonal. Finally, the optimized dilution factors for the anti-polβ rabbit polyclonal antibody were 1:200 with 60 min incubation time at room temperature. OC tissue was utilized as a negative control where the primary antibody step was omitted from the IHC protocol, Supplementary Figure 2.

Protein expression scoring

Cores were scored independently on two different occasions by the Main Author (MA) and a histopathologist (IM and MT); then, the average score was used as a final score. The sub-cellular localization of each marker was identified (nuclear, cytoplasm, cell membrane). Intensities of sub cellular compartments were each assessed and grouped as follows: 0= no staining, 1= weak staining, 2= moderate staining, 3= strong staining. The percentage of tumor cells in each category was estimated (0% to 100%). H-score (range 0 to 300) was calculated by multiplying the staining intensity and the percentage of staining [31]. Not all TMA cores were suitable for IHC analysis due to missing cores or absence of tumor cells.

Statistical analysis

The analysis was performed using SPSS v. 22 (IBM, Chicago, IL, USA) for Windows. Association with clinical and pathological parameters using categorized data was examined using a Chi-squared test. All tests were 2-tailed. The median was utilized to define the single optimal cut-off point for H score. They were at <80 H-score for PARP1, >100 H-score for XRCC1 and ≤ 180 H-score for polβ. Survival rates were determined using Kaplan–Meier method and compared by the log-rank test. A p value of less than 0.05 was identified as statistically significant. This study was carried out in accordance with the declaration of The Helsinki and ethical approval, which was obtained from the Nottingham Research Ethics Committee (REC Approval Number 06/Q240/153).

Results

Immunohistochemical results

All the surrogate markers used have shown a unique localized distribution among the different cellular compartments. The protein expressions of PARP1 was mainly nuclear in the tumor cells with variable intensities, although there were some faint cytoplasmic staining; it was not taken into consideration, (Figure 1A, 1B). Similarly, both XRCC1 and polβ revealed mainly nuclear expression in invasive OC cells, (Figures 1C-1F). After excluding uninformative

Table 2: Clinicopathological significance of the combined PARP1, XRCC1 and polβ positive expression in primary OC, significant values are in bold.

Parameter	PARP1-XRCC1-No. (%)	PARP1-XRCC1-No. (%)	PARP1+XRCC1-No. (%)	PARP1+XRCC1+ No. %)	*P- Value* (X^2)	Polβ-/PARP1-No. (%)	Polβ-/PARP1+ No. (%)	Polβ+/PARP1-No. (%)	Polβ+/PARP1+ No. (%)	P- value (X^2)	Polβ-/XRCC1-No. (%)	Polβ-/XRCC1+ No. (%)	Polβ+/XRCC1-No. (%)	Polβ+/XRCC1+ No. (%)	P- value (X^2)
Pathological Type															
Serous	32 (14.3)	45 (20.2)	27 (12.1)	119 (53.4)		44 (21.3)	55 (26.6)	27 (13.0)	81 (39.1)		37 (16.3)	73 (32.2)	20 (8.8)	97 (42.7)	
Mucinous	18 (45.0)	14 (35.0)	1 (2.5)	7 (17.5)		22 (61.1)	5 (13.9)	4 (11.1)	5 (13.9)		18 (45.0)	13 (32.5)	1 (2.5)	8 (20.0)	
Endometrioid	21 (34.4)	14 (23.0)	6 (9.8)	20 (32.8)	***<0.0001*** **(-69.891)**	27 (42.9)	16 (25.4)	8 (12.7)	12 (19.0)	***<0.0001*** **(-60.959)**	22 (32.8)	24 (35.8)	5 (7.5)	16 (23.9)	***<0.0001*** **(-44.099)**
CCC	19 (59.4)	7 (21.9)	1 (3.1)	5 (15.6)		22 (66.7)	2 (6.1)	4 (12.1)	5 (15.2)		18 (54.5)	5 (15.2)	2 (6.1)	8 (24.2)	
Mixed	1 (7.7)	2 (15.4)	3 (23.1)	7 (53.8)		1 (9.1)	5 (45.5)	1 (9.1)	4 (36.4)		3 (25.0)	3 (25.0)	1 (8.3)	5 (41.7)	
Other	0 (0.0)	3 (27.3)	2 (18.2)	6 (54.5)		3 (27.3)	1 (9.1)	0 (0.0)	7 (63.6)		1 (9.1)	3 (27.3)	2 (18.2)	5 (45.5)	
FIGO Stage															
I	46 (34.3)	22 (16.4)	13 (9.7)	53 (39.6)		54 (40.3)	34 (25.4)	12 (9.0)	34 (25.4)		53 (37.6)	43 (30.5)	8 (5.7)	37 (26.2)	
II	11 (18.3)	14 (23.3)	7 (11.7)	28 (46.7)	0.1 (-14.761)	16 (28.6)	7 (12.5)	7 (12.5)	26 (46.4)	0.077 (-15.525)	12 (20.3)	12 (20.3)	6 (10.2)	29 (49.2)	***0.004*** **(-24.301)**
III	30 (18.9)	40 (25.2)	20 (12.6)	69 (43.4)		39 (26.9)	38 (26.2)	22 (15.2)	46 (31.7)		32 (20.0)	52 (32.5)	15 (9.4)	61 (38.1)	
IV	4 (16.7)	7 (29.2)	1 (4.2)	12 (50.0)		8 (33.3)	5 (20.8)	3 (12.5)	8 (33.3)		3 (11.5)	11 (42.3)	2 (7.7)	10 (38.5)	
Grade															
G1	22 (44.0)	15 (30.0)	1 (2.0)	12 (24.0)		23 (51.1)	5 (11.1)	9 (20.0)	8 (17.8)		21 (43.8)	9 (18.8)	1 (2.1)	17 (35.4)	
G2	15 (24.2)	13 (21.0)	7 (11.3)	27 (43.5)	***0.002*** **(-20.273)**	21 (36.2)	15 (25.9)	4 (6.9)	18 (31.0)	***0.005*** **(-18.34)**	18 (26.5)	26 (38.2)	3 (4.4)	21 (30.9)	***0.007*** **(-17.57)**
G3	48 (20.6)	47 (20.2)	30 (12.9)	108 (46.4)		58 (26.1)	54 (24.3)	30 (13.5)	80 (36.0)		52 (22.2)	66 (28.2)	27 (11.5)	89 (38.0)	
Measurable Disease															
Non	73 (30.5)	47 (19.7)	23 (9.6)	96 (40.2)	***0.004*** **(-13.179)**	88 (38.3)	42 (18.3)	27 (11.7)	73 (31.7)	***0.009*** **(-11.552)**	74 (30.2)	68 (27.8)	23 (9.4)	80 (32.7)	0.068 (-7.128)
Measurable	15 (13.0)	30 (26.1)	16 (13.9)	54 (47.0)		24 (22.4)	34 (31.8)	14 (13.1)	35 (32.7)		22 (18.6)	41 (34.7)	8 (6.8)	47 (39.8)	
Platinum sensitivity															
Sensitive	77 (27.4)	58 (20.6)	34 (12.1)	112 (39.9)	0.107 (-6.101)	92 (34.1)	63 (23.3)	35 (13.0)	80 (29.6)	0.636 (-1.706)	88 (30.6)	81 (28.1)	23 (8.0)	96 (33.3)	***0.023*** **(-9.525)**
Resistant	9 (14.8)	17 (27.9)	5 (8.2)	30 (49.2)		15 (26.3)	14 (24.6)	7 (12.3)	21 (36.8)		7 (11.5)	23 (37.7)	7 (11.5)	24 (39.3)	

Table 3: Multivariate Cox regression analysis for prediction of ovarian cancer progression free and overall survival with altered protein expression and platinum sensitivity, significant values are in bold.

	Progression Free Survival				Overall Survival			
	P-value	HR	95% CI		P-Value	HR	95% CI	
			Lower	Upper			Lower	Upper
XRCC1	***0.028***	0.402	1.046	2.136	0.237	0.213	0.869	1.761
PARP1	0.91	0.02	0.725	1.434	***0.015***	0.415	1.082	2.118
Polβ	0.345	1.167	0.842	1.616	0.788	-0.05	0.69	1.326
Tumour stage	***<0.0001***	2.15	1.756	2.64	***0.0001***	1.537	4.754	9.656
Platinum sensitivity	***<0.0001***	2.683	9.837	21.74	***<0.0001***	1.917	4.782	9.677

cores (they were randomly excluded due to loss of the cores during TMA construction or antigen retrieval, folded tissue during processing or cores containing<15% tumor cells), High PARP1, XRCC1 and POLβ expressions were observed in 52.1%, 66.7% and 43.4% of cases, respectively.

Correlation of PARP1, XRCC1 and polβ positive expression with clinicopathological variables

High PARP1 nuclear expression was highly positively associated with serous adenocarcinomas, (p-value <0.0001). On the other hand, low level of this protein was associated with lower grade disease, (p-value <0.0001) compared with high expressers. In addition, if compared with low expression cases, high levels of this protein was associated with higher risk of post-surgical residual disease, (p-value =0.024). XRCC1 protein expression showed strong features as a poor prognostic biomarker for OC patients. Up-regulated protein expression of XRCC1 was strongly associated with serous histology type, (p-value <0.0001). Moreover, the positive expression of same biomarker was also correlated with advanced tumor stage, (p-value =0.001) and an increased likelihood of having post-operative measurable disease, (p-value =0.002) indicating sub-optimal debulking at surgery when compared with low XRCC1 expressers. On the other hand, the absence/low expression of XRCC1 protein significantly improved the response to platinum chemotherapeutic agents, (p-value =0.005). Similar to PARP1 and XRCC1, Polβ overexpression was linked to aggressive epithelial OC characteristics. Higher levels of polβ protein expression was significantly correlated with serous tumor histology, (p-value =0.001). However, low level of this protein was significantly associated with lower stage disease, (p-value =0.003) when compared with high tumor expressers. In addition, both platinum resistance and higher-grade disease was more common in tumors with high polβ expression, although it was in borderline significance, (p-value =0.076) and (p-value =0.076) respectively (Table 1).

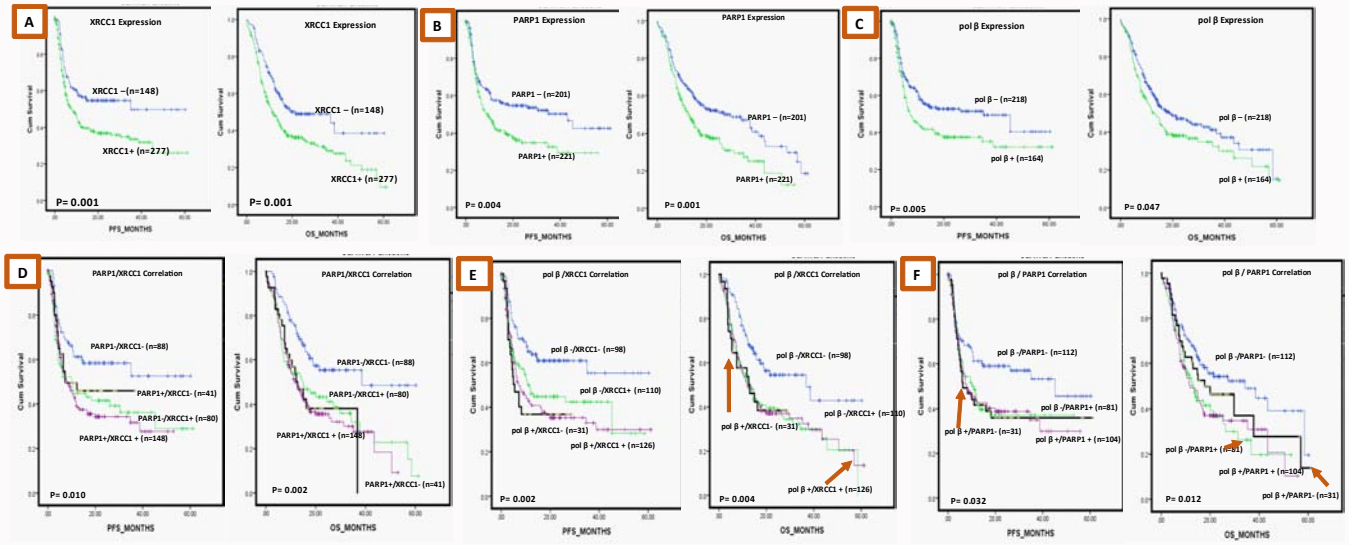

Figure 2: Kaplan Meier plots illustrating the associations between the altered expression of A) PARP1, B) XRCC1 and C) polβ with PFS and OS in primary ovarian carcinoma (Whole cohort).
D-F) illustrating the associations between the combined altered expression of: D) PARP1 and XRCC1, E) polβ and XRCC1 and F) polβ and PARP1 proteins with PFS and OS in primary ovarian carcinoma (Whole cohort).

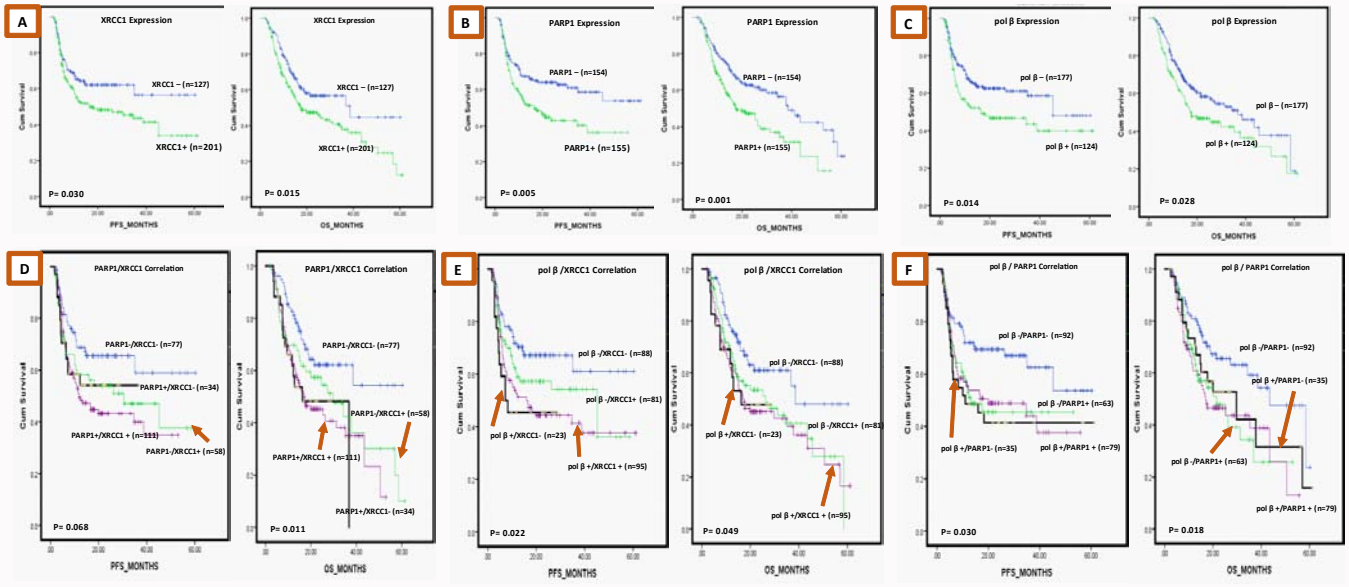

Figure 3: Kaplan Meier plots illustrating the associations between the altered expression of A) PARP1, B) XRCC1 and C) polβ with PFS and OS in primary ovarian carcinoma (Platinum sensitive cohort).
D, E, F) illustrating the associations between the combined altered expression of: D) PARP1 and XRCC1, E) polβ and XRCC1 and F) polβ and PARP1 proteins with PFS and OS in primary ovarian carcinoma (Platinum sensitive cohort).

Analyzing the significance of the combined expression of the surrogate proteins and then, correlating their expression with the clinicopathological data. Tumors with combined high expression of both PARP1 and XRCC1 had a highly positive significant association with serous adenocarcinomas histology subtype, (p-value <0.0001), higher-grade disease, (p-value =0.002) and increased risk of measurable tumor after surgery, (p-value =0.004), if compared with low expressers of both biomarkers. Similarly, high expression of both polβ and PARP1 was correlated with serous tumor histology and higher grade, (p-value <0.0001) and (p-value =0.005) respectively. On the other hand, low/negative expression tumors of both biomarkers had lower chance of post-operative residual disease, (p-value =0.009) when compared with the high expression cases. In addition, when the both XRCC1 and polβ high co-expression was analyzed together with the clinicopathological variables, high expression of both proteins was highly significantly correlated with serous histology type, (p-value <0.0001). Furthermore, a positive association between high expression of both biomarkers and advanced tumor stage and grade was noted, (p-value =0.004) and (p-value =0.007) respectively. On the other hand, XRCC1/polβ negative/low expression significantly improved the response to platinum agents, (p-value =0.023). Furthermore, polβ/XRCC1 overexpression was in borderline significance with higher residual disease post-surgery, (p-value =0.068) (Table 2).

Outcome analysis

Univariate analysis in the whole cohort: In general, high

expression of all the surrogate proteins, XRCC1, PARP1 and polβ showed unfavorable prognosis. Compared with low protein expression ovarian tumors, high nuclear expression of XRCC1 was associated with shorter both Progression Free Survival (PFS), (p-value =0.001) and Overall Survival (OS), (p-value =0.001) (Figure 2A). Similarly, high PARP1 expression showed an adverse clinical outcome with poor recurrence outcome, (p-value =0.004) and OS, (p-value =0.001) (Figure 2B), compared with tumors that had low PARP1 expression. In addition, cases with high polβ expression showed an adverse clinical outcome with poor OS, (p-value =0.047) and PFS, (p-value =0.005) (Figure 2C), when compared with tumors that had low polβ level.

Additionally, investigating XRCC1 expression with the other proteins revealed that low combined expression of both XRCC1 and PARP1 was associated with better outcome in terms of both recurrence and overall survival, (p-value =0.010) and (p-value =0.002) respectively, (Figure 2D), compared with high protein expression tumors. Similarly, combined negative/low level of both XRCC1 and polβ also showed improved outcome with longer both OS, (p-value =0.002) and PFS, (p-value =0.004), (Figure 2E), compared with the over-expressers. On the other hand, high significance with worse outcome was calculated with the combined high expression of polβ and PARP1, when compared with low expression samples, PFS (p-value =0.032) and OS (p-value =0.012), (Figure 2F).

Univariate survival analysis in platinum sensitive cohort: The KM estimates of both the PFS and OS for patients with OC who were treated primarily by surgery showed that the overexpression of XRCC1 had a significant worse outcome if compared with low expressers, (p-value =0.030) and (p-value =0.015) respectively, (Figure 3A). Similarly, high PARP1 and polβ nuclear expression in tumors showed adverse clinical outcome with poor PFS, (p-value =0.005), (p-value =0.014) and OS, (p-value =0.001) and (p-value =0.028) respectively, (Figure 3B, 3C).

Furthermore, investigating the effect of the combined expression of all the three tested biomarkers with each other on the survival outcome, combined high level of both XRCC1 and PARP1 showed inverse significant relation with OS, (p-value =0.014) and borderline significance with worse PFS, (p-value =0.068) (Figure 3D). On the other hand, combined negative/low expression of XRCC1 and polβ revealed interestingly, that it was associated with the improved outcome in terms of both PFS, (p-value =0.022) and OS, (p-value =0.049) compared with protein overexpression tumors, (Figure 3E). Moreover, combined low expression of both PARP1 and polβ showed significant association with improved PFS, (p-value =0.030) and OS, (p-value =0.018) (Figure 3F), compared with high protein expressers.

Univariate survival analysis in platinum resistant cohort: Apart from a significant correlation of increased XRCC1 expression with worse PFS, (p-value =0.040), there was no significance with patients' outcome noted with the other biomarkers in platinum resistant ovarian tumors.

Multivariate analysis

To further test the influence of XRCC1, PARP1 and polβ proteins overexpression on OC patients' prognosis, a multivariate analysis (Cox multivariate model) of the above biomarkers positive expression together with the platinum sensitivity and tumor stage were tested. The results revealed that XRCC1 was independent prognostic factors for worse PFS, (p-value =0.028). Concerning OS, PARP1, (p-value =0.015) was independent factor for poor OS, (Table 3).

Discussion

Different studies have emphasized the role of DDR proteins (XRCC1, PARP1 and polβ) in cancer progression. Moreover, they linked their positive expression to altered response to platinum therapeutic agents [13,32-39]. However, the clinical and biological implications of the positive expression of those biomarkers in human primary OC tissue have remained largely unexplored. In this current study, the biological and prognostic role of XRCC1, PARP1 and polβ was investigated in a big cohort of primary OC cases in order to determine the influence of the high expression of those proteins not only on the patients' pathological variables and their response to chemotherapeutic agents, but also on their survival. In our IHC data, in general we showed that XRCC1, PARP1 and polβ are positively associated with poor tumor prognostic variables, chemo-resistance and poor outcome.

The scaffolding protein, XRCC1 is a key player in BER, SSBR, and alt-NHEJ. XRCC1 deficiency in tumor cells delays the SSB rejoining and results in elevated levels of sister chromatid exchanges [13,14]. Interestingly, XRCC1 deficiency or mutation can also result in PARP1 hyper-activation [16]. Moreover, depletion of XRCC1 increases not only the effect of ionizing radiation on tumor cells, but also the sensitivity of the cancer cells to chemotherapy agents [13,14]. Furthermore, genetic polymorphisms in XRCC1 may influence cancer risk and the response to platinum-based chemotherapy [40-43]. These polymorphisms in XRCC1 may also have an effect on patients' clinical outcome in OC through the modifying role on the response to chemotherapeutic agents [42]. In a previous IHC study on primary OC, XRCC1 positive expression was mainly sub-localized in the nucleus of the tumor cells. In addition, XRCC1 overexpression was linked to platinum resistance and increased risk of tumor recurrence [17]. Moreover, the same study linked XRCC1 overexpression to serous histology type, higher stage disease and residual tumor post-surgery. In addition and regarding the patients' outcome, XRCC1 low expressers showed a significant relation to prolonged survival [17]. Similarly, a study conducted by Ang et al. [44] on primary head and neck squamous carcinoma, both poor patients' outcome and advanced stage disease had significant association with increased XRCC1 level. Moreover, low XRCC1 improved the patients' survival [44]. In our large cohort of primary ovarian tumors, XRCC1 high expression was linked to poor prognostic clinicopathological variables, increased tumor relapse rate, and reduced survival. On the flip side, tumors with low XRCC1 level showed improved response to platinum chemotherapy.

PARP1 up-regulation together with other DNA repair proteins may affect cancer phenotype and lead to resistance not just to the conventional anticancer therapy, but also to monotherapy using PARP inhibitors. Accurate knowledge of the PARP expression levels in cancerous tissues will provide a broad understanding of the DNA repair capacity and help guiding the treatment strategies of cancer [45]. Currently, IHC methodology is used in vivo for PARP1 protein level estimation [45-48]. Preclinical studies and clinical trials in both breast and ovarian cancers showed the efficacy of PARP inhibitors in clinical practice [11,49-52]. Schreibe et al. [53] stated that PARP1 is mostly localized in the nucleus and accounts for approximately 75% of the overall PARP enzymatic activity. Similarly, Gan et al. [54] also identified PARP1 in OC patient to be mainly expressed in the nucleus. This finding comes in agreement with the current study;

PARP1 showed predominant nuclear staining in PARP1 positive cases. Our results also complied with several publications in breast cancer, in which the nucleus was the prevalent localization of the PARP protein [55-58]. Moreover, in this current study we have shown that overexpression of PARP1 protein was in significant relation with serous tumor histology, higher-grade and residual disease post-surgery. Similarly, Resta et al. [59] concluded from his study on OC tissue specimens that, high PARP1 expression was associated with higher-grade tumors. Similarly and in another study, positive PARP expression was significantly linked to higher-grade ovarian tumors [60]. Although tumors with increased expression of PARP1 were associated with resistance to chemotherapy in a study performed by Gan et al. [54], this was not identified in our study. However, our results comes in line with Wysham et al. [61] who concluded that combined high expression of PARP1, FANCD2, and p53 proteins was associated not only with a doubled three year risk of recurrence, but also with platinum resistance; However, the isolated high PARP level had no effect on platinum sensitivity. In addition, PARP1 overexpression also impacted negatively on both PFS and OS, a finding concluded by the same study [61]. Interestingly, in this current study the effect of PARP1 overexpression on outcome was also maintained in platinum sensitive patients, a finding that would concur with the previous studies that confirmed the predictive significance of PARP1 in OC. They demonstrated that increased expression of PARP is associated with poor outcome [54,59,61].

Polβ is a key player in DNA BER and it promotes genomic stability [62]. There is emerging data on the role of polβ in human carcinogenesis as increased level of this protein was measured in cancer cells. Higher levels of polβ have been identified in colonic and prostatic tumors, when compared with the normal tissue levels [25,63]. In addition, mutations in polβ were also observed in solid tumors. These mutations can influence the response to chemotherapy and promote aggressive tumor phenotype [64]. Polβ overexpression is being particularly more frequent in uterine, breast, ovary, stomach and skin tumors [65]. In OC, mutations in polβ was also recognized, although their clinical significance remained unclear [66]. In the current study, polβ overexpression in the unselected whole cohort was associated with poor outcome. In addition, polβ high level was also in borderline significance with platinum resistance. On the other hand and in platinum sensitive sub-group, low polβ expression was linked to better outcome. Our data implies that polβ is a useful predictor of outcome in ovarian tumor cases.

To date, very limited information is available about the clinical influence of polβ high expression on OC patients. High levels of polβ protein was found in preclinical created high-grade serous ovarian carcinoma [67]. Similarly, in IHC study of primary Gastric carcinoma, Polβ overexpression showed close association with aggressive tumor characteristics [68]. This finding comes in agreement with the current study. In addition, polβ deficiency in mice is embryonically lethal [69] and embryonic fibroblasts derived from such mice are hypersensitive to alkylating agents [24,70]. On the other hand, polβ overexpression in pre-clinical models enhanced the relative resistance to DNA-damaging agents [27]. Moreover, polβ depletion in colorectal cancer cell lines promoted their platinum sensitivity [71].

As PARP1, XRCC1 and polβ interact with each other [72-76]; we performed XRCC1, PARP1 and Polβ co-expression analyses. Overall, we demonstrated that patients with tumors that have low expression of those proteins develop less aggressive tumor in terms of both clinicopathological characteristics and better survival outcome. On the other hand, high expression of both XRCC1 and Polβ was associated with significant resistance to platinum agents. Similarly and in platinum sensitive patients, PARP1, XRCC1 and Polβ high protein levels was correlated with a significant unfavorable outcome when compared with low protein expressers. This provides further evidence to the synergistic clinical influence of the surrogate biomarkers on primary OC patients. Importantly, in multivariate Cox model, XRCC1 overexpression was independent predictor of worse cancer recurrence. Therefore, this provides further evidence for the significance of positive XRCC1 in negatively influencing patients' outcome. This finding comes in line with another study, XRCC1 protein expression was assessed by IHC methodology in 138 consecutive head and neck cancer specimens. High XRCC1 expression was independently associated with increased risk of disease progression [44].

To conclude, DNA damage repair process is a key player in OC progression. In this study, we aimed to identify the driver proteins that are significantly associated and differentially expressed in OC patients. In general, all the surrogate biomarkers were linked to unfavorable tumor characteristics and poor outcome. Clinically, our study provided novel findings and useful prognostic indications. Importantly, we provided the evidence that XRCC1, PARP1 and polβ work simultaneously on primary OC patients to exhibit poor prognostic behavior. In addition, XRCC1 overexpression acted as an independent factor for poor cancer progression. In this study, we identified several promising protein candidates that were intimately associated with tumor prognosis. Therefore, these novel findings may potentiate further scientific and clinical approaches in prognostic biomarkers and therapeutics.

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