



The Prognostic Significance of the Signaling Proteins: PTEN, P85 α , ATM and XIAP in Primary Ovarian Cancer

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Abstract

Background: Cell signaling proteins play a crucial role in tumor behavior. ATM, PTEN, XIAP and p85 α are key cell signaling biomarkers. This study evaluates the prognostic significance of those biomarkers in primary Ovarian Cancer (OC).

Methods: ATM, PTEN, XIAP and p85 α expression was assessed immunohistochemically in a large primary OC cohort (n=525). Outcome analysis was evaluated using progression free and overall survival.

Results: High XIAP and p85 α expression was associated with features of high-risk OC, including serous histology and higher residual tumor following surgery. P85 α negative/low expression improved patients' platinum sensitivity. Although overexpression of XIAP and p85 α was associated with recurrence and poor outcome, upregulated PTEN expression improved outcome. ATM and XIAP were independent prognostic factors in predicting shorter recurrence outcome. In patients with high ATM and XIAP expression, PTEN and p85 α lost their significance on patients' outcome.

Conclusion: ATM, PTEN, XIAP and p85 α have crucial and different 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. PTEN and p85 α are more likely to behave in cell-specific contexts concerning ATM and XIAP expression status.

Keywords: DNA Repair; PTEN; p85 α ; ATM; XIAP; Ovarian cancer; Prognosis

Introduction

Platinating chemotherapeutic agents are frequently used as a standard management of Ovarian Cancer (OC) with estimated initial response rates up to 80 [1,2]. However, the prognosis of those patients is still dismal [3]. Identification of additional prognostic indicators reflecting the intrinsic tumor cell biology is essential for identifying high-risk patients. Defects in proteins that are orchestrating DNA stability and repair could provide potential prognostic factors that guide patients' management.

Ataxia-Telegiectasia Mutated (ATM) is a master regulator of DNA Damage Response (DDR) following Double Strand Breaks (DSBs) [4]. It is involved not only in DNA repair but also in cell cycle checkpoints, apoptosis, various metabolic and signaling pathways [5-7]. ATM Mutations contribute to lymphoid malignancies [8], familial breast and ovarian tumors [9,10]. Interestingly, emerging data suggests a role for ATM in different types of sporadic tumors development [11-14]. Phosphatase and Tensin (PTEN) homolog is a key tumor suppressor [15]. PTEN also plays a crucial role in cell cycle regulation, induction of cell apoptosis and transduction of multiple signaling pathways of cells [16-20]. PTEN deletion or mutation was identified in tumors [21] and recent studies have suggested a link between PTEN deletion and development of chemotherapy resistance including platinating agents commonly used to treat OC [20,22,23]. In OC, PTEN can negatively regulate PI3K/Akt pathway, hence suppress the cancer cell growth and provoke a G1 arrest of the cell cycle [24-27]. The Phosphatidylinositol-3-Kinase (PI3K) pathway has been recognized as the most commonly

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activated signaling pathway in human tumors. It is a key player in tumor progression [28-30]. Genetic mutations of components of this pathway can lead to tumor progression in numerous cancer types, including OC [31,32]. PI3K heterodimer consists of a p110 catalytic domain that phosphorylates PIP2 to PIP3, and a p85 α regulatory sub-unit [33]. The correlation between p85 isoforms and cancer development has been reported previously [34,35]. P85 α mutants promote cell survival, AKT activation, and oncogenesis [34]. Sun et al. [35] showed that mutant p85 α protein expression in chicken embryonic fibroblasts increased cell proliferation and induced oncogenic transformation. Moreover, p85 α levels were noted to be higher in ovarian tumor tissue than the normal ovaries [36]. In breast cancer, *in vivo* inhibition of p85 α activity significantly reduced tumor cells proliferation [37,38]. X-linked Inhibitor of Apoptosis Protein (XIAP) is a member of the Inhibitor of Apoptosis Protein (IAP) family [39-41]. It is involved in regulating innate immune responses [42]. XIAP is overexpressed in several cancers, including ovarian tumors [43-45] and it confers resistance to some chemotherapeutic agents [41,46-50]. Overexpression of XIAP attenuates cisplatin's ability to induce apoptosis in OC whereas its pre-clinical downregulation increases sensitivity to cisplatin in resistant serous OC cells [51-53].

A potential crosstalk between ATM, p85 α , and PTEN does exist. PI3K could activate ATM [54], which in turn can phosphorylate PTEN [55]. Similarly, p85 α can also bind to and increase PTEN lipid phosphatase activity [56]. XIAP has functional association with AKT [57] and ATM can phosphorylate AKT [58].

As ATM, PTEN, XIAP and p85 α are important signaling transduction molecules with diverse and interrelated function in OC, we hypothesized that their study could lead to the identification of subsets of high-risk invasive OC patients that may require different treatment consideration. There is limited evidence on the protein expression levels of the above biomarkers in primary OC tissue samples. To the best of our knowledge, no previous study has addressed the clinical role of this particular combination of the above four proteins in primary OC progression and prognosis. In this study, we aimed to assess the expression and co-expression of ATM, PTEN, XIAP and p85 α using Immunohistochemistry (IHC) in a large cohort of primary surgically resected OC to explore their clinicopathological and prognostic significance.

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: Tumor histology type, 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 [1]. 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, two separate areas from each case were cored. Cores were scored independently on two different occasions by one of the author (MA) and a histopathologist (IM and MT); then, the average score was used as a final score.

The primary antibody specificity for ATM, PTEN, p85 α and XIAP antibodies 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). The antibody details including concentrations used and manufacturer's information are included in the Supplementary Table 2. All the surrogate proteins supported a specific band at the expected molecular weight and as instructed by their supplier, 350 KDa for the ATM protein, 54 KDa for the PTEN protein, 85 KDa for the p85 α protein and 55 KDa for the XIAP 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 3. Then, after selecting the optimal antibody concentration, expression of ATM, PTEN, p85 α and XIAP proteins in OC was assessed by IHC using the Novocastra Novolink™ Polymer Detection Systems kit (Code: RE7280-K, Leica, Biosystems, UK). TMA sections (4 μ m) were stained with the primary antibodies diluted with Bond™ primary antibody diluent to reach the optimum working dilution: 1:100 anti-ATM rabbit monoclonal antibody, 1:50 anti-PTEN rabbit monoclonal antibody, 1:3000 of anti-PI3K p85 α mouse monoclonal antibody, and 1:500 anti-XIAP rabbit polyclonal antibody. All primary antibodies were applied and incubated with tumor specimens for 60 min at room temperature, except anti-PI3K p85 α antibody, which was incubated for 18 h at 4C. Kidney or colon tissue was used as a positive control. OC tissue was utilized as a negative control where the primary antibody step was omitted from the IHC protocol, Supplementary Figure 2.

Proteins' expression Scoring

Cores were scored independently on two different occasions by (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 subcellular 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 [59]. 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 <25 H-score for nuclear ATM, <80 H-score for cytoplasmic PTEN, \leq 140 H-score for cytoplasmic P85 α and <100 H-score for cytoplasmic XIAP. The IHC staining intensities showed negative/weak, moderate, and

strong staining for the four interrogated proteins. 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.

Results

Proteins' expression distribution

All the surrogate markers used have shown a unique localized distribution among the different cellular compartments. The protein expressions of ATM were exclusively nuclear (Figure 1). The protein expression of PTEN and XIAP were mainly cytoplasmic, (Figure 2, 3), respectively. Although, P85 α showed both cytoplasmic and membranous protein expression, the membranous expression was occasional and weak (Figure 4). After excluding uninformative 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 ATM, PTEN, XIAP and p85 α expressions were observed in 55%, 52%, 49% and 29% of cases, respectively.

Association between ATM, PTEN, XIAP and p85 α expression and clinicopathological variables

Although ATM is crucial for DDR process, clinically, apart from the significant association with serous tumor histology subtype, ($p=0.006$), the nuclear overexpression of ATM had no significant association with other parameters. Similarly, the loss of cytoplasmic PTEN protein was correlated with serous ovarian tumors ($p=0.004$). p85 α cytoplasmic low expression was strongly associated with clear cell, mucinous and endometrioid histology subtypes, ($p=0.002$) and had improved response to platinum therapy ($p=0.026$). Patients with p85 α overexpression showed high significance with residual tumor following surgery, ($p=0.002$). The upregulated expression of cytoplasmic XIAP was associated with poor prognostic parameters. Positive expression of this protein was associated with serous and clear cell histology tumor subtypes, ($p=0.023$). However, XIAP negative expression significantly improved complete tumor resection with less residual disease following surgery, ($p=0.047$). Table 1

summarizes the associations between the 4 markers and the various clinicopathological parameters.

Outcome analysis

Univariate analysis of survival: Downregulated expression of p85 α and XIAP were associated with prolonged PFS, (p85 α : $p=0.003$, XIAP: $p=0.012$). However, upregulated expression of PTEN significantly improved patients' PFS ($p=0.044$). ATM upregulation did not influence the recurrence risk of ovarian tumor cases, ($p=0.151$) (Figure 5).

At the designated cut-off points, no statistical significance was calculated for the positive expression of ATM, PTEN, p85 α and XIAP with OS, (Supplementary Figure 3).

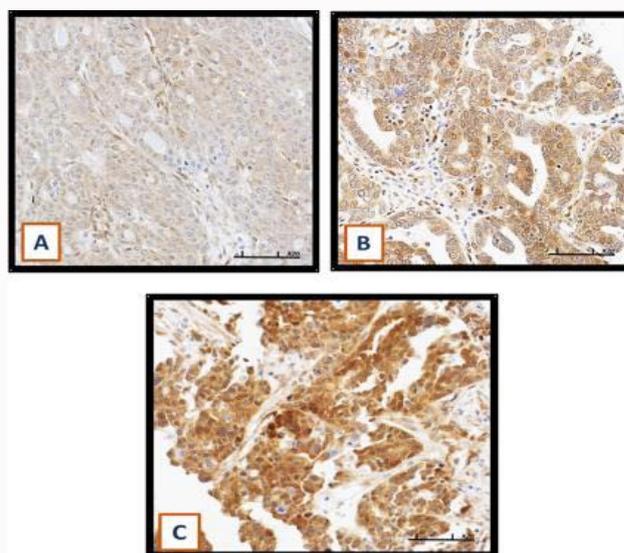


Figure 2: Immunohistochemistry expression of PTEN in tissue microarray images. Cytoplasmic expression of PTEN in cancer cells was distributed as follows; A) weak staining, B) moderate staining, and C) strong staining. Images were captured at 20-times magnifications.

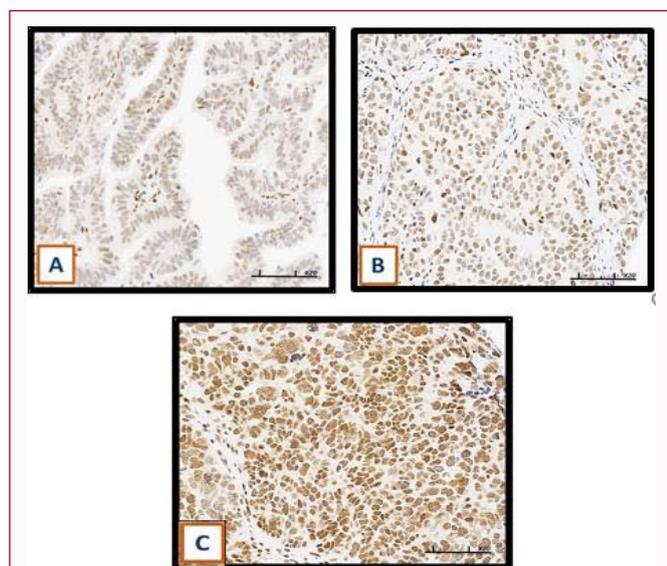


Figure 1: Immunohistochemistry expression of ATM in tissue microarray images. Nuclear expression of ATM in cancer cells was distributed as follows; A) weak staining, B) moderate staining, and C) strong staining. Images were captured at 20-times magnifications.

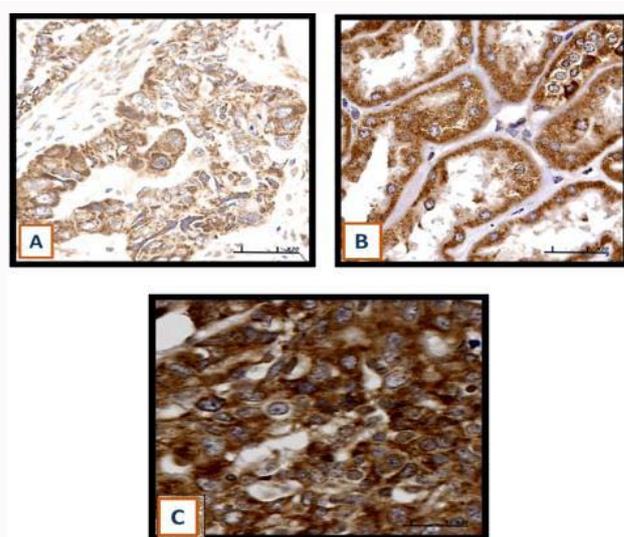


Figure 3: Immunohistochemistry expression of XIAP in tissue microarray images. Cytoplasmic expression of XIAP in cancer cells was distributed as follows; A) weak staining, B) moderate staining, and C) strong staining. Images were captured at 20-times magnifications.

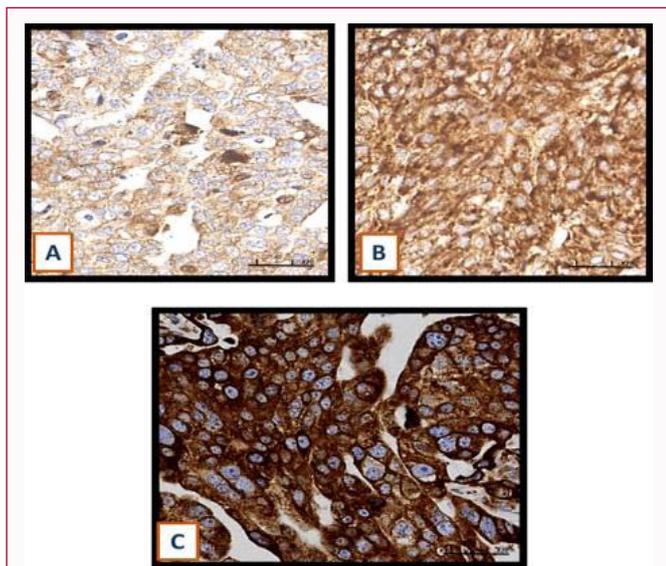


Figure 4: Immunohistochemistry expression of P85α in tissue microarray images. Cytoplasmic expression of P85α in cancer cells was distributed as follows; A) weak staining, B) moderate staining, and C) strong staining. Images were captured at 20-times magnifications.

Multivariate analysis of survival: Multivariate survival analysis showed that tumor expression of ATM and XIAP are poor independent prognostic factors for tumor recurrence in patients treated with platinum chemotherapeutic agents, (HR=1.514, 95% CI= 1.514-1.077; p=0.017) (HR=1.429, 95% CI= 1.024-1.995; p=0.036). In terms of OS, downregulated expression of ATM was an additional independent predictor of unfavorable outcome to platinum sensitivity, (HR=1.420, 95% CI= 1.038-1.944; p=0.028) (Table 2).

Given the novel role of ATM and XIAP in multivariate analysis, we investigated their altered expression influence on PTEN and

p85α in terms of cases' outcome. This was proposed to identify ATM and XIAP influence on modifying the isolated previously explained prognostic potential of both PTEN and p85α.

Outcome analysis in ATM selected cohort: Upregulated expression of PTEN was associated with favourable outcomes with prolonged PFS and OS, (p=0.002) and (p=0.031) respectively (Figure 6A). p85α overexpression was highly significantly correlated with poor PFS, (p=0.005) and a trend towards significance with worse OS (p=0.075), (Figure 6B).

Interestingly, the significant effect of both PTEN and p85α on patients' outcome in ATM negative cases was lost when ATM selection was positive (Figure 7A, 7B).

Outcome analysis in XIAP selected cohort: Similar to ATM, when cases were tested with the KM survival analyses for both PTEN and p85α revealed that upregulated expression of PTEN within the XIAP negative OC cohort was in the trend towards significance with favourable outcomes in terms of both the PFS and OS, (p=0.058) and (p=0.090) respectively (Figure 8A). P85α overexpression; however, was significantly correlated with poor PFS (p=0.019). The association with OS did not reach a statistically significant value (p-value = 0.138) (Figure 8B).

Notably, when XIAP positive cases were the selection, both PTEN and p85α lost their statistically significant influence on PFS (Figure 9A, 9B).

Outcome analysis for combined ATM and XIAP expression

After conducting spearman's correlation coefficient between both ATM and XIAP and as expected, it revealed that both proteins are highly associated with each other (p-value <0.00001). Tumors with ATM-/XIAP- had a highly significantly favourable PFS, (p=0.005). Similarly, ATM-/XIAP- patients showed improved OS, but it did not reach the statistical significance, (p=0.113) (Figure 10).

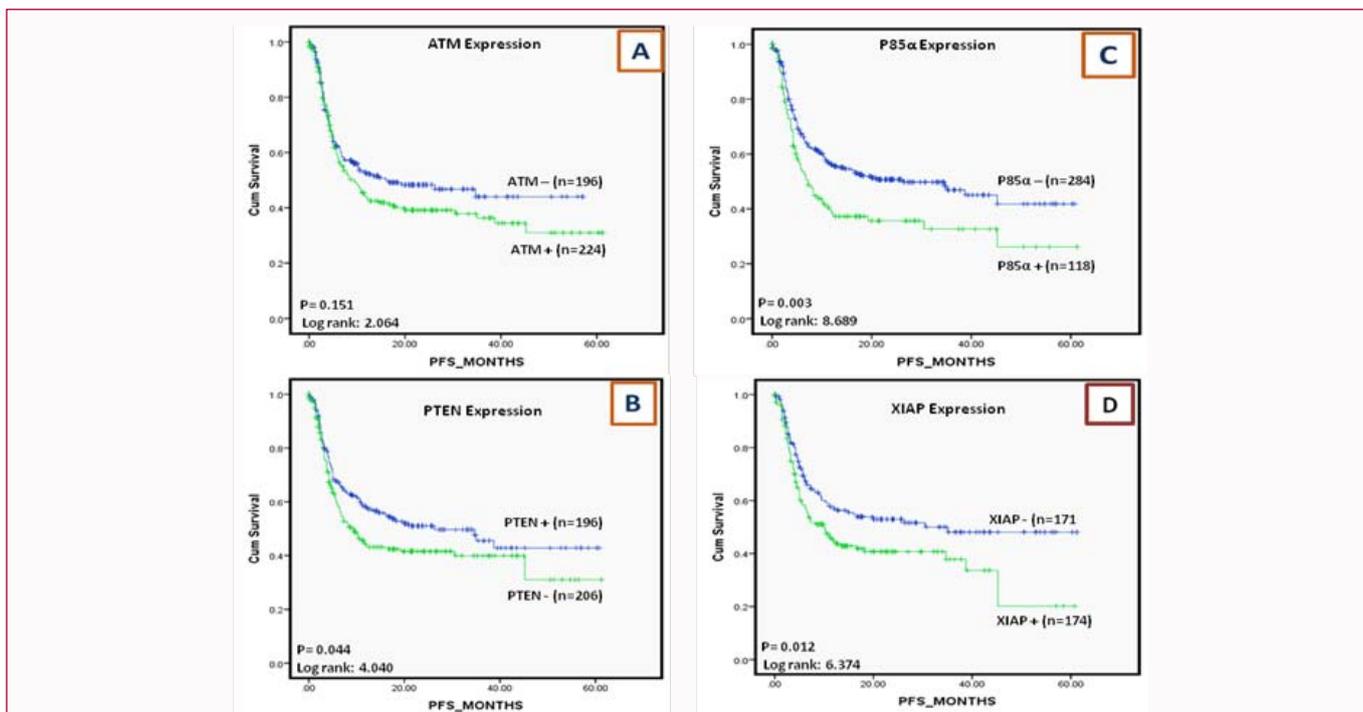


Figure 5: Kaplan Meier plots illustrating the associations between the altered expression of A) ATM, B) PTEN, C) P85α, and D) XIAP proteins with PFS in primary ovarian carcinoma.

Table 1: Clinicopathological significance of ATM, PTEN, P85α and XIAP proteins expression in the patients of primary ovarian carcinoma. Significant values are in bold.

Parameter	ATM- No. (%)	ATM+ No. (%)	P- value (X2)	PTEN- No. (%)	PTEN+ No. (%)	P- value (X2)	P85α - No. (%)	P85α+ No. (%)	P- value (X2)	XIAP - No. (%)	XIAP+ No. (%)	P- value (X2)
Pathological Type												
Serous	102 (39.4)	157 (60.6)	0.006 -16.176	136 (55.3)	110 (44.7)	0.004 -19.27	156 (63.4)	90 (36.6)	0.002 -19.284	105 (47.9%)	114 (52.1%)	0.023 -13.053
Mucinous	29 (58.0)	21 (42.0)		32 (60.4)	21 (39.6)		42 (79.2)	11 (20.8)		30 (68.2%)	14 (31.8%)	
Endometrioid	35 (50.7)	34 (49.3)		41 (64.1)	23 (35.9)		48 (75.0)	16 (25.0)		30 (51.7%)	28 (48.3%)	
CCC	26 (66.7)	13 (33.3)		27 (71.1)	11 (28.9)		33 (86.8)	5 (13.2)		9 (32.1%)	19 (67.9%)	
Mixed	6 (37.5)	10 (62.5)		7 (43.8)	9 (56.3)		11 (68.8)	5 (31.3)		10 (71.4%)	4 (28.6%)	
Other	5 (33.3)	10 (66.7)		9 (64.3)	5 (35.7)		14 (100.0)	0 (0.0)		7 (63.6%)	4 (36.4%)	
FIGO Stage												
I	70 (44.6)	70 (44.6)	0.746 -1.229	83 (54.2)	70 (45.8)	0.644 -1.192	112 (73.2)	41 (26.8)	0.356 -3.181	73 (54.5%)	61 (45.5%)	0.683 -1.498
II	27 (41.5)	38 (58.5)		35 (56.5)	27 (43.5)		47 (75.8)	15 (24.2)		26 (47.3%)	29 (52.7%)	
III	93 (48.7)	98 (51.3)		89 (48.9)	93 (51.1)		120 (65.9)	62 (34.1)		77 (48.4%)	82 (51.6%)	
IV	12 (44.4)	15 (55.6)		13 (56.5)	10 (43.5)		16 (69.6)	7 (30.4)		11 (55.0%)	9 (45.0%)	
Grade												
G1	28 (46.7)	32 (53.3)	0.681 -0.769	30 (52.6)	27 (47.4)	0.974 (0.023)	42 (73.7)	15 (26.3)	0.406 -1.802	28 (60.9%)	18 (39.1%)	0.111 (4.399)
G2	31 (41.9)	43 (58.1)		39 (53.4)	34 (46.6)		54 (74.0)	19 (26.0)		36 (58.1%)	26 (41.9%)	
G3	130 (47.6)	143 (52.4)		133 (52.0)	123 (48.0)		172 (67.2)	84 (32.8)		105 (47.1%)	118 (52.9%)	
Measurable Disease												
Non	122 (44.0)	155 (56.0)	0.188	136 (51.1)	130 (48.9)	0.113 (2.509)	203 (76.3)	63 (23.7)	0.001	125 (54.3)	105 (45.7)	0.047
Measurable	66 (49.3)	68 (50.7)	-1.133	55 (42.6)	74 (57.4)		77 (59.7)	52 (40.3)		-11.635	49 (43.0)	
Platinum sensitivity												
Sensitive	152 (46.6)	174 (53.4)	0.527	172 (54.8)	142 (45.2)	0.407 (0.302)	229 (72.9)	85 (27.1)	0.026	133 (48.5%)	141 (51.5%)	0.091 (2.852)
Resistant	38 (50.7)	37 (49.3)	-0.399	34 (49.3)	35 (50.7)		41 (59.4)	28 (40.6)		-4.964	33 (61.1%)	

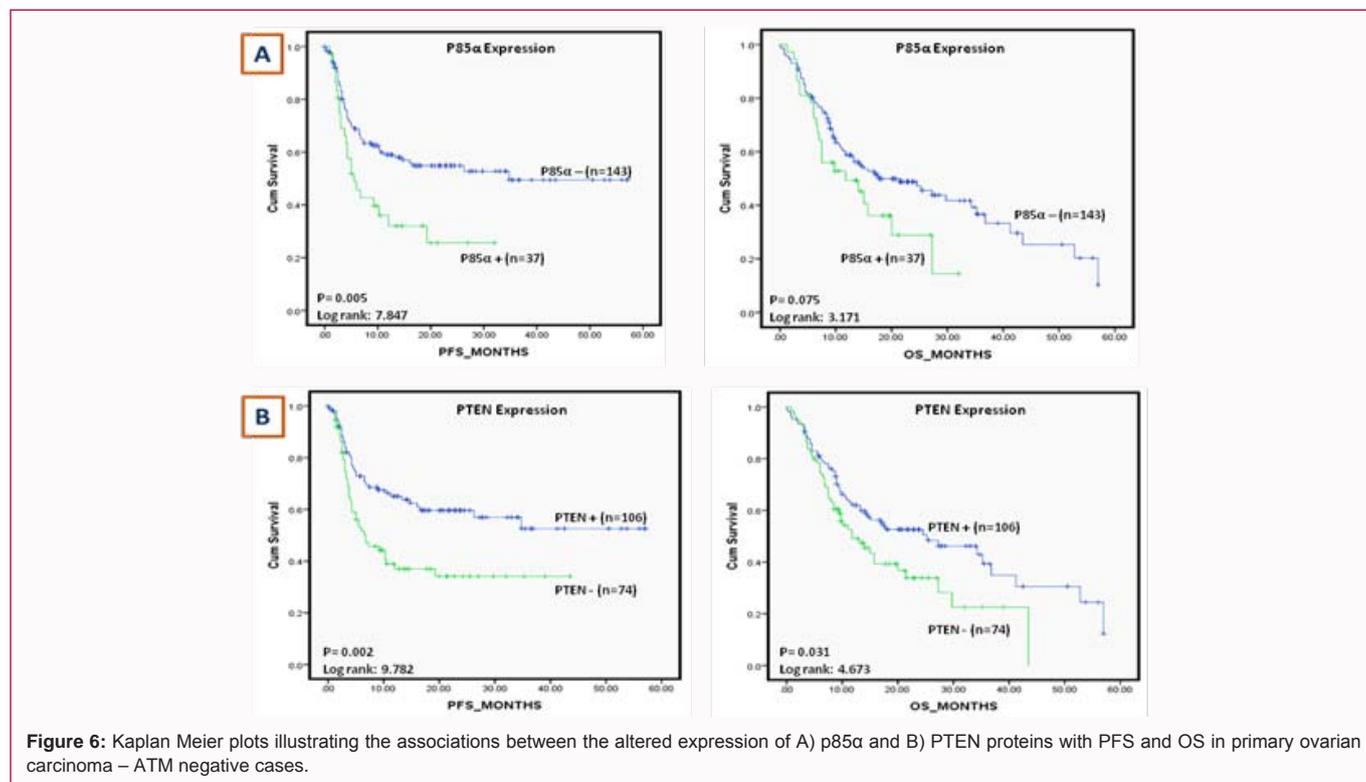


Figure 6: Kaplan Meier plots illustrating the associations between the altered expression of A) p85α and B) PTEN proteins with PFS and OS in primary ovarian carcinoma – ATM negative cases.

Table 2: 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
ATM	0.017	1.514	1.077	2.127	0.028	1.42	1.038	1.944
XIAP	0.036	1.429	1.024	1.995	0.439	1.127	0.832	1.527
PTEN	0.388	1.214	0.782	1.885	0.854	0.964	0.652	1.425
P85α	0.704	0.912	0.566	1.47	0.338	0.806	0.518	1.254
Platinum sensitivity	<0.0001	19.897	12.58	31.469	<0.0001	7.454	5.105	10.883

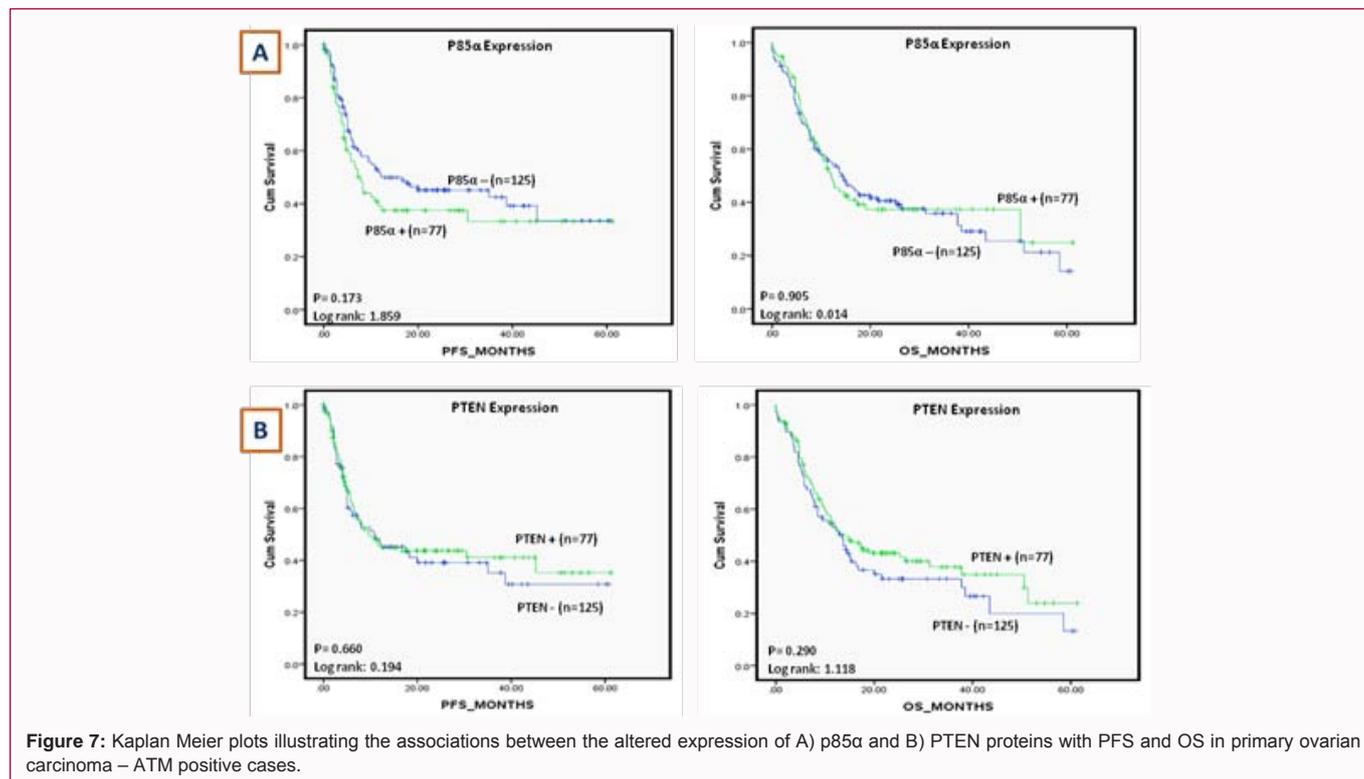


Figure 7: Kaplan Meier plots illustrating the associations between the altered expression of A) p85α and B) PTEN proteins with PFS and OS in primary ovarian carcinoma – ATM positive cases.

Discussion

Despite the previous extensive investigation of various biomarkers in both early and advanced stage OC, the differential gene/protein expression associated with disease progression and platinum sensitivity remains poorly characterized. Although the wide use of platinum-based chemotherapy has significantly improved clinical outcomes with 80% initial response rate, most ovarian tumors will develop platinum resistance, and patients eventually succumb to the disease [1,2]. Therefore, the development of biomarkers to predict the cases' survival is highly desirable. In the current study, we provide evidence that a network including the ATM, PTEN, p85α, and XIAP has a vital role in influencing cancer progression and patients' survival in ovarian tumor cases treated primarily by surgical resection. Moreover, we have shown how the differential expression status of ATM and XIAP might influence PTEN and p85α function, indicating distinctive clinically relevant groups of patients.

ATM is a crucial signaling protein that activates a complex network of DDR pathways that coordinate the cell cycle checkpoint and DNA repair functions [60]. ATM mutation can result in cell

sensitivity to DNA damage and cancer predisposition [61-63]. ATM protein is exclusively expressed in the nucleus [3,64-67]. This comes in agreement with our findings, as this protein was exclusively expressed in the nuclei of tumor cells. Multiple studies on different types of cancers have reported the prognostic effect of the ATM positive protein expression. However, different views have been described regarding the ATM function and its role in influencing tumors' behavior and patients' outcome [3,64-68]. One view by Abdel-Fatah et al. [65] in a study performed on breast cancer, ATM high expression was linked to smaller tumor size, lower histology grade, less invasiveness and improved survival. Similarly, in colonic cancer, ATM overexpression correlates with improved OS; however, no association could be established between this protein expression and tumor stage or lymph node metastasis [67]. A study performed on 97 cases of serous OC concluded that ATM overexpression had no relation with the clinical stage, tumor grade and chemotherapy status [66]. However, Hashiguchi et al. identified a significant association between the loss of ATM protein and non-serous ovarian tumors compared with the serous histology subtype. In contrast, no correlation was identified with the cases' survival [3]. Abdel-

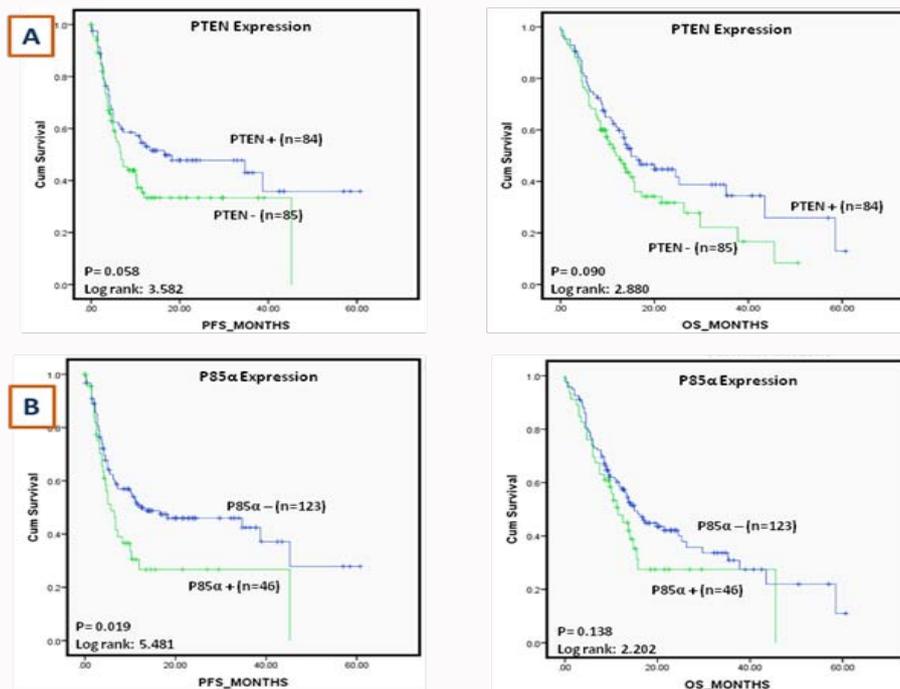


Figure 8: Kaplan Meier plots illustrating the associations between the altered expression of A) p85α and B) PTEN proteins with PFS and OS in primary ovarian carcinoma – XIAP negative cases.

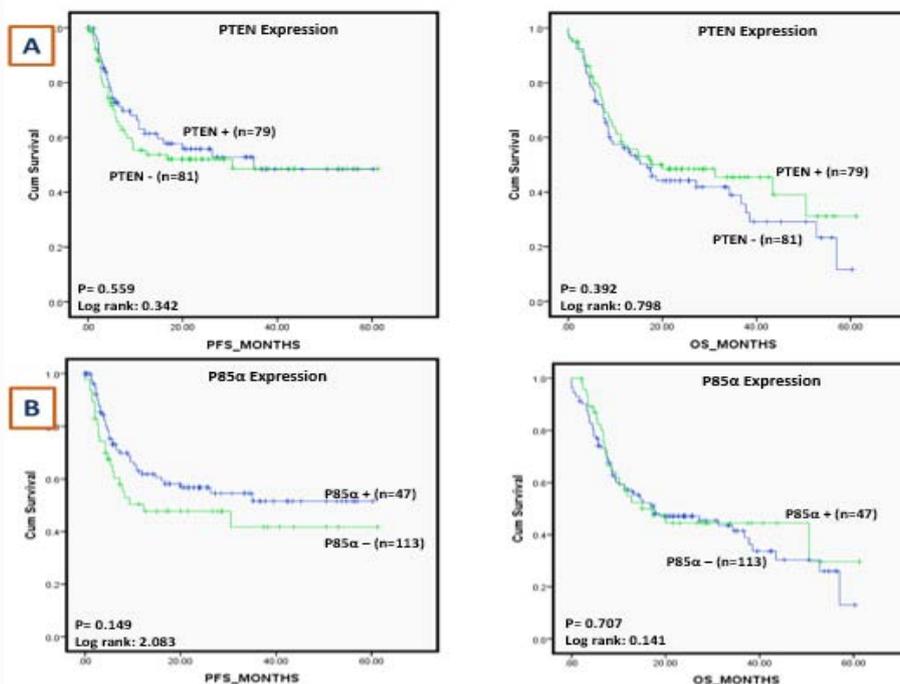


Figure 9: Kaplan Meier plots illustrating the associations between the altered expression of A) p85α and B) PTEN proteins with PFS and OS in primary ovarian carcinoma – XIAP positive cases.

Fatah et al. [65] identified a significant positive link between high ATM expression with serous carcinomas and platinum resistance. Furthermore, in univariate analysis ATM high expression in the nucleus was linked to adverse clinical outcome [64].

In our study, ATM high expression was significantly associated with serous tumor histology type. It did not show a reliable prognostic

relevance or significant association with the other clinicopathological variables. Our results come partially in line with the previously mentioned studies. There is some controversy on the role of ATM in influencing cellular fates and outcome in patients with cancer, including ovarian tumors.

PTEN is a tumor-suppressor gene [20,69]. It has been emphasized

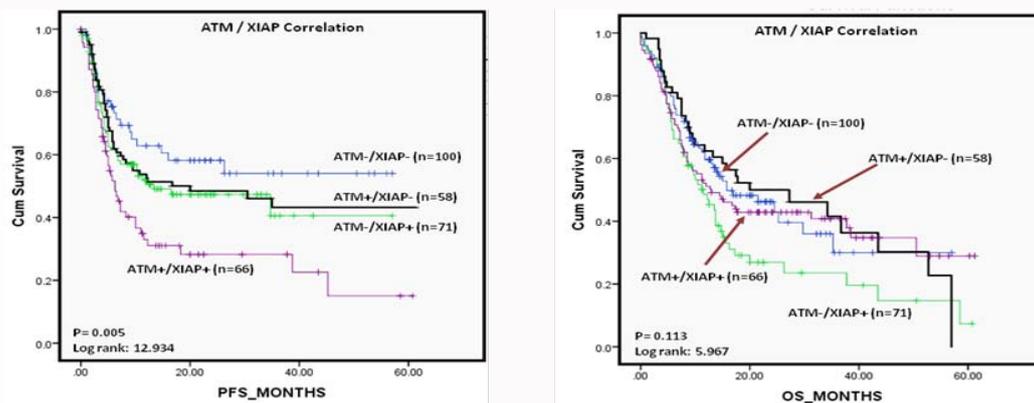


Figure 10: Kaplan Meier plots illustrating the associations between the altered expression of the combined ATM and XIAP proteins with PFS and OS in primary ovarian carcinoma.

by different studies that PTEN is involved in the conduction of multiple cellular signaling pathways, regulation of cell cycle, apoptosis, and autophagy. This protein has a vital role in cell adhesion, migration, and angiogenesis. PTEN abnormal expression and genetic mutations were observed in glioma, breast, prostatic cancer, and other tumors. Interestingly in ovarian tumors, PTEN down-regulation can predispose to the development and progression of cancer. The expression level of PTEN in normal ovarian tissue, benign, borderline tumors and ovarian cancer is gradually decreased [70,71]. Wu et al. [72] showed that, transfecting PTEN gene in platinum resistant OC cell lines significantly enhanced cisplatin sensitivity. Yan et al. [73] demonstrated that PTEN protein could make the cisplatin-resistant OC cell lines sensitive to chemotherapy. Remarkably, a previous study demonstrated that not just PTEN expression is decreased in ovarian tumors but also a more significant decrease in chemotherapy resistant cells was noticed, suggesting the existence of a correlation between PTEN low expression with OC progression and resistance to treatment [74]. Similarly, in endometrial cancer reduced PTEN protein expression was observed compared with the normal endometrial tissue; further highlighting the protective role of this protein [75]. Cai et al. [27] also linked the reduced PTEN expression detected in OC to poor survival. The aforementioned findings come in agreement with the current study. The reduced biological activity of PTEN protein in primary ovarian tumor tissue specimens was positively associated with serous histology type. PTEN overexpression significantly improved patients' outcome. The high cytoplasmic expression of PTEN did not appear to significantly affect the response to platinum treatment in the studied cohort. Considering some differences found with other studies regarding the relationship between PTEN overexpression and response to platinum therapy, the constitution of the cohort itself could have an impact on the expression and behavior of these proteins. A study conducted by Abe et al. [76] on clear cell carcinoma demonstrated no association between the aberrant PTEN expression and development of platinum resistance, an inline observation with the current research.

Plethora of studies focused on different methods to evaluate the expression of p85 α subunit in several tissues' tumor specimens to highlight its role in the regulation of the fundamental cellular processes, such as the cell cycle and cell survival [77-80]. To the best of our knowledge, there is minimal information on the IHC expression of p85 α protein in primary OC. Here, our results show that the aberrant localization of p85 α protein in the cytoplasm of

tumor cells has implications on both the clinicopathological patients' characteristics and disease outcome. Downregulated p85 α expression showed significant association with improved surgical resection, platinum sensitivity and patients' survival. These results come in agreement with a study conducted by Zhang et al. [36]. Zhang et al. [36] evaluated p85 α protein level by WB methodology, and then it was compared between normal ovarian tissue, benign, borderline, and malignant ovarian tumors. p85 α expression was significantly elevated in cancerous tissue, indirect observation of its aggressive role. Similarly, upregulated p85 α was in association with higher ovarian tumor grade [36]. Interestingly, preclinical inhibition of p85 α activity enhanced platinum sensitivity in malignant cells [36]. Zhou et al. [38] showed that p85 α was associated with aggressive breast cancer characteristics and poor prognosis. This current study revealed that the downregulated expression of p85 α protein is a significant favourable prognostic factor in primary OC. This protein may play an essential role in the development of platinum resistance and cancer progression.

A member of the IAP family, XIAP prevents apoptosis through direct inhibition of effector caspases (caspases 3, 6, 7, and 9) [41,81]. It has been well-documented that XIAP plays a crucial role in tumor maintenance in different malignancies [50,82-84]. Lack of XIAP in ovarian tumor cells promoted their sensitization to apoptosis [52]. Moreover, its high expression in OC cells can be used to determine the tumor prognosis and response to therapy [51,52,85,86].

Our investigation of the clinicopathological impact of the altered protein expression of XIAP protein in the primary OC cases unveiled a strong positive association with aggressive tumor phenotype. Zhang et al. [41] supported the findings and highlighted that high expression of cytoplasmic XIAP was associated with shorter PFS. Similarly, XIAP overexpression significantly reduced PFS in clear cell carcinoma [48]. In addition, Hussain et al. [87] linked XIAP low/negative expression to favourable tumor characteristics in breast cancer.

Clarification of the important biomarkers that can influence patients' survival is vital. We have identified that both ATM and XIAP were independent poor prognostic indicators for PFS, a novel finding in this study. This comes in line with Grabsch et al. [67] in which ATM was independent poor prognostic protein in colorectal cancer. Moreover, a trend towards significance was identified for high ATM expression in breast cancer as an independent marker of prognosis [65]. ATM inhibition has recently emerged as a promising

anti-cancer strategy [88]. Moreover, in OC ATM high expression was correlated with aggressive clinicopathological parameters and poor outcome. However, it did not show significance as an independent prognostic protein in multivariate analysis [64], which may be related to the limited number of cases included in the study. Similarly, in this study XIAP was an independent factor for poor PFS. This finding comes in agreement with Miyamoto et al. [48] who showed that XIAP was an independent factor for poor outcome in clear cell carcinoma. Moreover, XIAP knockdown not just retarded ovarian tumor cells proliferation but also it promoted carboplatin-induced cell apoptosis [41]. Another study on breast cancer revealed XIAP as an independent poor prognostic factor [87].

These above observations suggest that ATM and XIAP high expression tumors may indicate a high-risk subpopulation of invasive OC patients. Therefore and further splitting the studied cohort according to their corresponding ATM and XIAP expression, interestingly, when both biomarkers were positive, PTEN and p85 α lost their significance on patients' outcome, a cardinal observation in the current study. Importantly, patients with combined low/negative ATM and XIAP had significantly improved recurrence free outcome. Given the previously described role for ATM and XIAP in influencing patients' outcome [69], we speculate a novel role for both proteins in modulating the effect of both PTEN and p85 α . However, further detailed functional studies are required to confirm this hypothesis.

Conclusion

ATM, PTEN, p85 α and XIAP expression are predictors of patients' outcome in primary ovarian tumors. The data presented here reveal a novel network between the investigated proteins with vital clinical applications in OC. PTEN and p85 α are more likely to behave in cell-specific contexts concerning ATM and XIAP expression status. We propose that both latter biomarkers have a master role in orchestrating the clinical influence of the other network proteins. Therefore, their expression revealed a distinct clinical group with different biological features from others with a remarkable driving effect on the outcome. Future evaluation utilizing functional and preclinical studies is essential to further explore the role of this network in primary ovarian tumors.

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