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Prognostic value of INPP4B protein immunohistochemistry in ovarian cancer

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Summary

Purpose of investigation: Ovarian cancer is associated with poor prognosis and altered protein expression patterns may be useful for identifying patients likely to have poor disease outcomes. The impact of altered INPP4B protein expression on prognosis is unclear. The aim of this study was to evaluate the implication of INPP4B expression changes in a large series of ovarian cancer tissue samples. *Materials and Methods:* Tissue microarrays were constructed from 599 epithelial ovarian tumors and stained with antibodies for INPP4B, p53, and PTEN. Proportional hazard models were used to estimate survival hazard ratios (HRs) associated with altered protein expression. *Results:* Seventy-nine percent of the ovarian cancers demonstrated loss of INPP4B, whereas 53% showed aberrant p53 expression (i.e., complete loss of p53 or over-expression of p53) and 8% showed loss of PTEN. INPP4B was frequently lost in serous and endometrioid cancer subtypes, aberrant p53 expression was most common among serous subtype, and loss of PTEN was most common among endometrioid tumors (p for all three proteins across histologic subtypes ≤ 0.0001). INPP4B loss or aberrant p53 expression were both associated with increased mortality (HR = 1.84; 95% CI 1.27 - 2.68 and HR = 3.10; 95% CI 2.33 - 4.11, respectively); however, in multivariate models, only the relationship with p53 achieved statistical significance (HR = 1.20; 95% CI 0.82 - 1.76 for INPP4B and HR = 1.73; 95% CI 1.28 - 2.34 for p53). *Conclusion:* The INPP4B protein is frequently lost in serous and endometrioid subtypes of ovarian cancer. A possible prognostic role of INPP4B for endometrioid ovarian tumors requires further evaluation.

Key words: Ovarian cancer; Prognosis; INPP4B; PTEN; p53; Survival.

Introduction

Ovarian cancer is the most fatal gynecologic malignancy (case fatality approaching 70%) due to typically advanced stage at clinical presentation and poor prognosis [1, 2]. Chemotherapy for ovarian cancer consists of platinum compounds in combination with taxanes [2]. Poor survival rates follow, in part, resistance to chemotherapy [2]. Currently, ovarian cancers are classified based on tumor clinical stage and histologic features, but neither individual response to chemotherapy nor prognosis relate to these features alone [1]. Identification of new molecular markers, such as altered expression of key proteins, is critical for the development of targeted treatments. The deregulation of proteins in pathways involved in cell-cycle progression, apoptosis, and angiogenesis is likely to contribute to poor prognosis and to platinum resistance [3].

The phosphoinositide 3-kinase (PI3K)/AKT signaling pathway is important for the regulation of cell growth, proliferation, differentiation, apoptosis, and intracellular trafficking [4]. Studies of ovarian cancer cell lines and animal models reveal that activation of these pathways may lead to chemotherapy resistance [3]. PI3K signaling is altered in up to 45% of ovarian cancers [5]. Somatic mutations of genes in the PI3K/AKT pathway are rare, whereas gene amplifications are more common [5]. Mutations in the class I PI3K gene (*PIK3CA)* and *PTEN*, a negative regulator of PI3K/AKT signaling have been reported in ovarian cancers, however the frequency of alterations is modest compared to in other epithelial cancers [6]. In this study, we investigated the role of a recently identified tumor suppressor gene in the PI3K pathway, inositol polyphosphate 4-phosphatase (INPP4B), in ovarian cancer prognosis in a large series of unselected 599 epithelial ovarian tumors originating from patients in Ontario, Canada.

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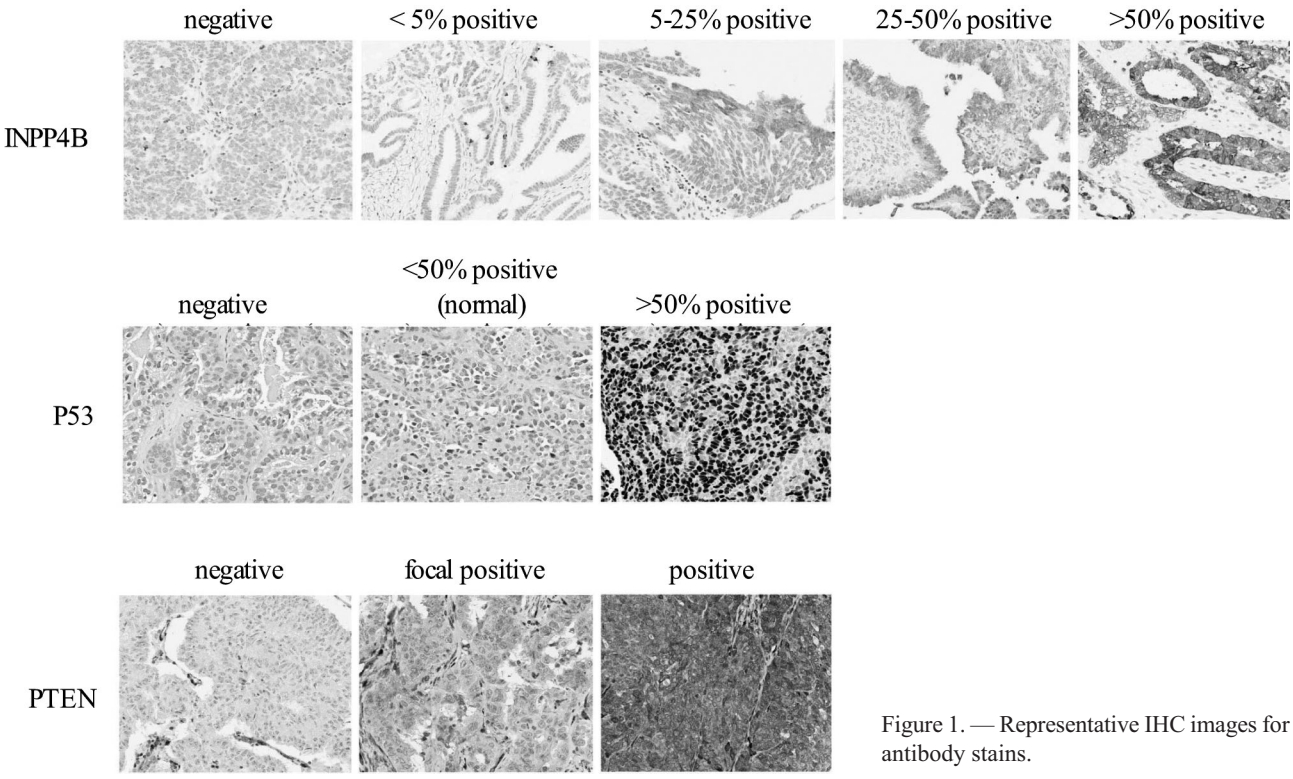

Figure 1. — Representative IHC images for antibody stains.

Materials and Methods

Study population

In the province of Ontario, Canada, all residents newly diagnosed with primary invasive epithelial ovarian cancer from January 1995 through December 1999 and from January 2002 through December 2004 were identified by checking case notifications at the Ontario Cancer Registry. Pathology reports were reviewed by the investigators for each case, to determine study eligibility, and tumor histologic type. Patients were between 20 and 79 years of age at the time of diagnosis. Through a standardized-script risk-factor questionnaire, information on known and suspected ovarian cancer risk factors and demographic information was collected by telephone. Details of clinical staging, treatments received, and response to treatment were obtained from medical record review. The study was approved by the institutional review boards of the University of Toronto and Yale University (see [7] for further detail on the study population and *BRCA* mutation analyses).

Ovarian cancer case confirmation and tumor block collection

Diagnostic confirmation and coding of tumor characteristics (invasive *vs.* borderline, histologic type, and stage) was determined through pathology reports and other medical records. Paraffin-embedded tissue blocks representative of the ovarian carcinoma were requested for each confirmed ovarian cancer case. Medical record information was requested from treating hospitals a minimum of one year after diagnoses, to ensure that primary surgery and chemotherapy had been completed.

Immunohistochemistry

Tissue microarrays (TMAs) were constructed, with duplicate 0.6 mm cores from each of the 599 patient tumors included in this study. INPP4B-specific monoclonal antibodies were produced by immunizing mice with purified recombinant human His-INPP4B and were characterized as described [8]; P53 (NCL-p53-DO7) and PTEN (M362729) protein expression were assessed by immunohistochemistry using commercial antibodies and standard techniques. All stained TMA slides were digitized using a slide scanner at 40X magnification. INPP4B expression was scored based on percent positive tumor cells within the following categories: completely negative, >0 but ≤5%, >5 but ≤25%, >25 but ≤50%, and >50% positive nuclear staining. p53 protein expression was also scored based on percent positive tumor cells: completely negative, >0 but ≤50% positive staining, and >50% positive nuclear staining. Negative and >50% positive staining patterns are indicative of p53 aberrations. PTEN staining showed both nuclear and cytoplasmic localization and was scored based on both expression patterns. Staining was quantified by intensity, with negative expression representing undetected staining in tumor cells and positive expression in stromal cells; focal positive expression representing weaker staining in tumor compared to stromal cells; and positive expression representing equal staining intensity in tumor and stromal cells. Figure 1 shows representative images for each of the stains.

Ascertainment of outcomes

Survival status was determined both by computerized linkage of subject identifying information to death certificate data maintained by the Ontario Cancer Registry, and by chart review at local hospitals. This linkage provided information on date and cause of death. The Ontario Cancer Registry, which began in 1964, compiles information on cancer incidence, mortality, and survival in Ontario. Previous evaluation of the Ontario Cancer Registry record-linkage approach for determination of vital status from death certificates has shown that it is more accurate than manual approaches.

Table 1. — *Characteristics of 599 epithelial ovarian cancer cases, Ontario, Canada.*

Characteristic	
Age at diagnosis, mean (SD[1])	57.4 (11.8)
Age at interview, mean (SD)	59.8 (11.8)
Body mass index five years prior to diagnosis (kg/m^2), mean (SD)	26.0 (8.8)
Histology, n (%)	
Serous	315 (53%)
Mucinous	40 (7%)
Endometrioid	139 (23%)
Clear cell	43 (7%)
Other[2]	62 (10%)
Stage, n (%)	
I	124 (21%)
II	106 (18%)
III	283 (47%)
IV	84 (14%)
BRCA1 mutation, n (%)	42 (7%)
BRCA2 mutation, n (%)	28 (5%)
Ten-year survival (yes), n (%)	246 (41%)

[1]SD = standard deviation.
[2]Other includes the following tumor types: clear cell, mixed histology, epithelial not otherwise specified, and adenocarcinoma not otherwise specified.

Statistical analysis

Based on *a priori* considerations, various staining scoring categories were collapsed for carrying out statistical analyses. For PTEN, staining was coded as "negative" (i.e., complete loss of expression) or "positive" (i.e., including focal positive cells). For p53, staining was coded as "normal," "positive" (i.e., over-expression), or "negative" (i.e., complete loss of expression). In a secondary analysis, the authors also created an "aberrant" expression category that combined tumors with no p53 expression (i.e., absent) with those over-expressing p53. For INPP4B, staining was coded as "negative" (i.e., ≤5% of cells stained positive) or "positive" (i.e., >5% of cells stained positive). Associations between clinicopathological characteristics and protein expression were assessed using the t-test or χ^2 test, as appropriate. The main endpoint evaluated in this study was ovarian cancer-specific survival, defined as the duration from date of diagnosis to date of death from ovarian cancer. Survival was censored at death from another cause or at September 30, 2010, the most recent limit of available death-certificate information. The Kaplan-Meier method was used to estimate unadjusted survival curves and the log-rank test was used to compare survival rates. The authors employed Cox proportional hazards models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) associated with protein expression. In multivariate analyses, they adjusted for age at diagnosis (continuous), histologic subtype—serous, mucinous, endometrioid, clear cell, and other (mixed histology, epithelial not otherwise specified, and adenocarcinoma not otherwise specified), and Stage (I, II, III, IV). Survival analyses were conducted on the entire dataset, as well as on subgroups defined by histologic subtype. All analyses were carried out using SAS Version 9.1 . All *p*-values are two-sided.

Results

In total, 599 epithelial ovarian cancer cases were available for analysis. Selected clinical characteristics are presented in Table 1. The distributions of INPP4B, PTEN and p53 expression by histologic subtype, stage and *BRCA* mutation status are summarized in Table 2. INPP4B expression was lost in 79% of tumors; the loss was most frequent in serous (84%) and endometrioid (76%) subtypes. Eight percent of the tumors showed loss of PTEN expression, which was most com-

Table 2. — *Distribution of INPP4B, p53 and PTEN staining by histologic subtype, stage, and BRCA mutation status.*

Characteristic		INPP4B			p53				PTEN		
Total, n (%)	All cases n = 566 (100%)	Positive n = 117 (21%)	Absent n = 449 (79%)	All cases n = 588 (100%)	Normal n = 277 (47%)	Over-expression = 232 (39%)	Absent n = 79 (13%)	Aberrant n = 311 (53%)	All Cases n = 585 (100%)	Positive n = 540 (92%)	Absent n = 45 (8%)
Histology, n (%)											
Serous	300 (53%)	48 (16%)	252 (84%)	308 (52%)	94 (31%)	160 (52%)	54 (18%)	214 (69%)	305 (52%)	293 (96%)	12 (4%)
Mucinous	35 (6%)	20 (57%)	15 (43%)	38 (7%)	31 (82%)	6 (16%)	1 (3%)	7 (18%)	39 (7%)	37 (95%)	2 (5%)
Endometrioid	132 (23%)	32 (24%)	100 (76%)	138 (24%)	96 (70%)	32 (23%)	10 (7%)	42 (30%)	136 (23%)	113 (83%)	23 (17%)
Clear Cell	40 (7%)	13 (33%)	27 (67%)	43 (7%)	39 (90%)	2 (5%)	2 (5%)	4 (9%)	43 (7%)	39 (91%)	4 (9%)
Other[1]	59 (10%)	4 (7%)	55 (93%)	61 (10%)	17 (28%)	32 (52%)	12 (20%)	44 (72%)	62 (11%)	58 (94%)	4 (6%)
P-value			<0.0001				<0.0001	<0.0001			0.0001
Stage, n (%)											
I	116 (20%)	39 (34%)	77 (66%)	122 (21%)	97 (79%)	18 (15%)	7 (6%)	25 (21%)	122 (21%)	108 (89%)	14 (11%)
II	101 (18%)	24 (24%)	77 (76%)	104 (18%)	58 (56%)	29 (28%)	17 (16%)	46 (44%)	102 (17%)	91 (89%)	11 (11%)
IIII	266 (47%)	42 (16%)	224 (84%)	279 (47%)	101 (36%)	135 (48%)	43 (15%)	178 (63%)	278 (48%)	265 (95%)	13 (5%)
IV	81 (14%)	12 (15%)	69 (85%)	81 (14%)	21 (26%)	49 (60%)	11 (14%)	60 (74%)	81 (14%)	74 (91%)	7 (9%)
Unknown				2		1	1	2	2	2	
P-value			0.0004				<0.0001	<0.0001			0.06
BRCA1 mutation, n (%)	41 (8%)	4 (10%)	37 (90%)	42 (7%)	4 (10%)	27 (64%)	11 (26%)	38 (90%)	41 (7%)	38 (93%)	3 (7%)
BRCA2 mutation, n (%)	25 (5%)	2 (8%)	24 (92%)	28 (5%)	5 (18%)	17 (61%)	6 (21%)	23 (82%)	27 (5%)	25 (93%)	2 (7%)

[1]Other includes the following tumor types: mixed histology, epithelial not otherwise specified, and adenocarcinoma not otherwise specified.

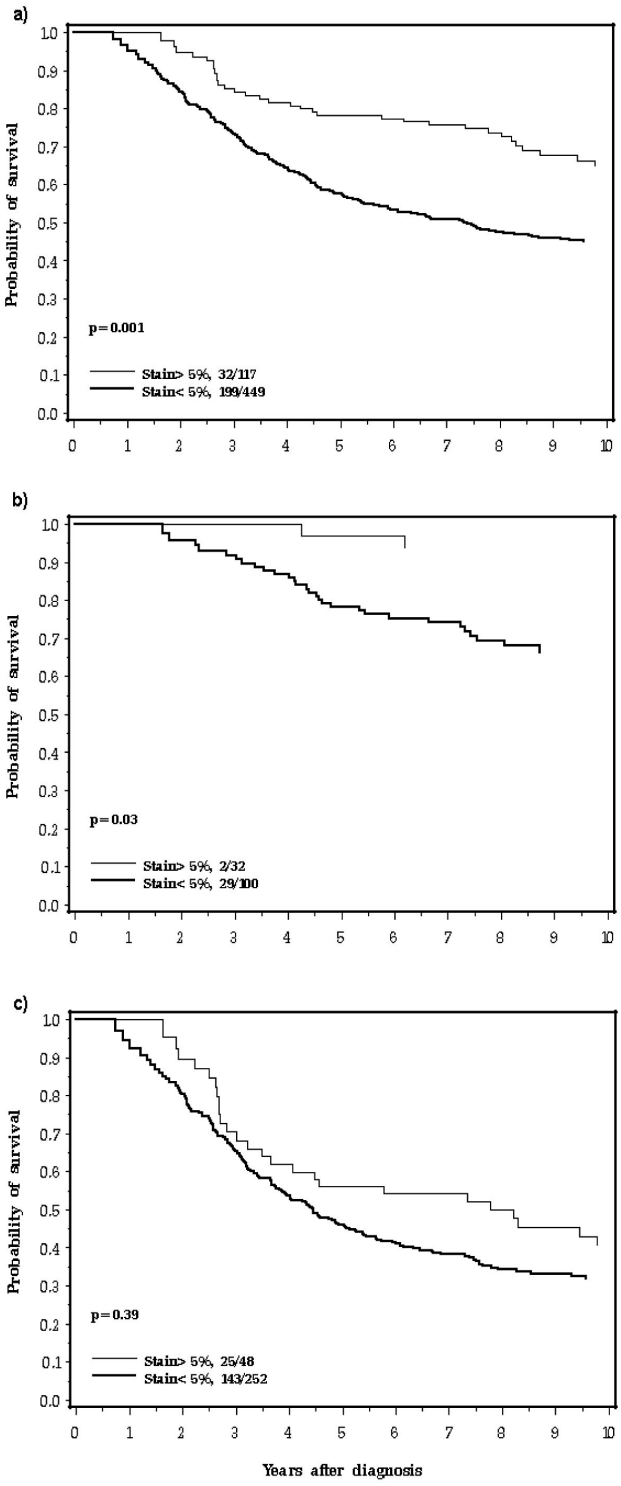

Figure 2. — Ten-year survival among ovarian caner patients by INPP4B expression among – a) all subtypes, b) endometrioid subtype only, and c) serous subtype only.

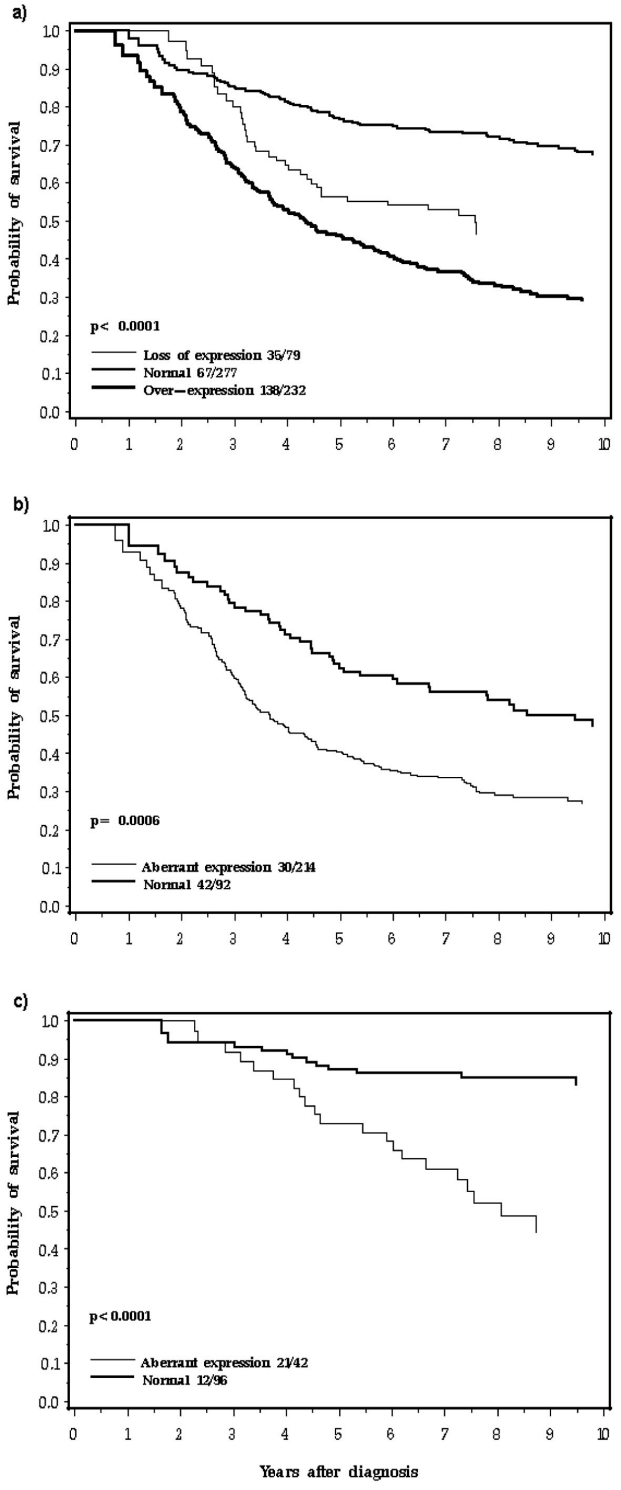

Figure 3. — Ten-year survival among ovarian caner patients by p53 expression among – a) all subtypes, b) serous subtype only, and c) endometrioid subtype only.

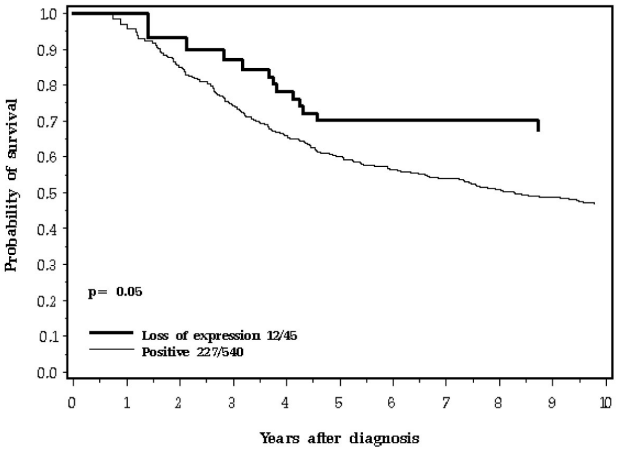

Figure 4. — Ten-year survival among ovarian caner patients by PTEN expression.

mon among the endometrioid subtype (17%). Approximately one-half of the tumors had normal p53 expression (47%), while 39% showed over-expression. and 13% had complete loss of p53 expression. Sixty-nine percent of serous tumors showed aberrant p53 expression (i.e., over-expression or complete loss of expression). Tumors from women with a *BRCA1* or *BRCA2* mutation were more likely to show loss of INPP4B and aberrant p53 expression.

Overall, loss of INPP4B expression was associated with significantly worse survival (HR = 1.84; 95% CI 1.27 - 2.68; $p = 0.0001$) (Figure 2a). Among the histological groups, the effect was strongest for women with endometrioid cancers (HR = 5.15; 95% CI 1.23-21.6; $p = 0.03$) (Figure 2b). INPP4B loss was not associated with survival among the other subtypes ($p \geq 0.70$)(Figure 2c for serous subtype). Women with tumors with normal p53 expression experienced significantly better survival than women with aberrant p53 expression (i.e., either complete loss or over-expression)

Table 3. — *Co-expression of INPP4B and p53.*

	INPP4B, n (%)		Total
p53, n (%)	Positive	Absent	
Normal	85 (33%)	176 (67%)	261
Over-expression	12 (16%)	65 (84%)	77
Absent	18 (8%)	205 (92%)	112
			561

P for Cochran-Mantel-Haenszel test = 0.003.

(HR = 3.10; 95% CI 2.33 - 4.11; $p < 0.0001$) (Figure 3a). After stratification by histologic subtype, aberrant p53 expression was associated with significantly worse prognosis for both serous (HR = 1.85; 95% CI 1.30 - 2.62; $p = 0.0006$) (Figure 3b) and endometrioid subtypes (HR = 4.81; 95% CI 2.35 - 9.84; $p < 0.00001$) (Figure 3c). Loss of PTEN expression was associated with better overall survival (HR = 0.56; 95% CI 0.31 - 1.00; $p = 0.05$); however, the sample size was too small to evaluate this relationship according to histologic subtype (Figure 4). Loss of INPP4B was strongly correlated with aberrant p53 expression: 61% of INPP4B-negative tumors also had aberrant p53 expression compared to 26% of INPP4B-expressing tumors (Cochran-Mantel-Haenszel test $p = 0.003$; Table 3).

Univariate and multivariate hazard ratios and 95% CIs for ovarian cancer-specific mortality associated with INPP4B, PTEN, and p53 expression are presented in Table 4. Loss of INPP4B expression was associated with a two-fold increased mortality risk (HR = 1.84; 95% CI 1.27 - 2.68); $p = 0.001$) in the univariate model; however, after adjusting for age, histology and stage, the relationship was weaker and did not achieve statistical significance (HR = 1.20; 95% CI 0.82 - 1.76; $p = 0.36$). This effect was limited to the endometrioid subtype (HR = 4.04; 95% CI 0.89 - 18.4) compared to 1.0; 95% CI 0.64 - 1.58 for the serous tumors)(data not shown). In the univariate analysis, both p53 over-expression and complete loss of expression were strongly associated with

Table 4. — *Ovarian cancer-specific mortality associated with p53, PTEN and INPP4B expression.*

Protein	Univariate HR[1] (95%CI)	*p*	Multivariate HR[2] (95%CI)	*p*	Multivariate HR[3] (95%CI)	*p*	Multivariate HR[4] (95%CI)	*p*
INPP4B								
Positive	1.00 (ref)		1.00 (ref)		1.00 (ref)		1.00 (ref)	
Negative	1.84 (1.27-2.68)	0.001	1.28 (0.87-1.88)	0.21	1.20 (0.82-1.76)	0.36	1.19 (0.80-1.78)	0.39
p53								
Normal	1.00 (ref)		1.00 (ref)		1.00 (ref)			
Overexpression	3.33 (2.48-4.47)	<0.00001	2.27 (1.65-3.12)	<0.00001	1.79 (1.31-2.44)	0.0003		
Absence of p53	2.44 (1.62-3.67)	<0.00001	1.63 (1.07-2.49)	0.02	1.56 (1.02-2.38)	0.04		
Aberrant[5]	3.10 (2.33-4.11)	<0.00001	2.10 (1.54-2.85)	<0.00001	1.73 (1.28-2.34)	0.0004	1.81 (1.32-2.49)	0.0002
PTEN								
Positive	1.00 (ref)		1.00 (ref)		1.00 (ref)			
Negative	0.56 (0.31-1.00)	0.05	0.80 (0.44-1.44)	0.46	0.75 (0.41-1.37)	0.35	0.76 (0.42-1.38)	0.36

[1]HR = hazard ratio; CI = confidence interval. [2]Multivariate HR adjusted for age at diagnosis (continuous) and histologic subtype (serous, mucinous, endometrioid, clear cell, other). [3]Multivariate HR adjusted as in column 2, plus stage (I, II, III, IV). [4]Multivariate HR adjusted as in column 3, and additionally for all three proteins (i.e., INPP4B, p53 and PTEN expression). [5]Aberrant includes tumors with no p53 expression or those over-expressing p53.

an increased risk of death (HR = 3.33; 95% CI 2.48 - 4.47; $p < 0.00001$ and 2.44; 95% CI 1.62 - 3.67; $p < 0.00001$, respectively); these values were reduced but remained significant in multivariate analyses (HR = 1.79; 95% CI 1.31 - 2.44; $p = 0.0003$ and HR = 1.56; 95% CI 1.02 - 2.38; $p = 0.04$, respectively). This relationship did not vary by histologic subtype (HR = 1.82; 95% CI 0.25 - 2.65 *vs.* 2.46; 95% CI 1.05 - 5.75 for serous and endometrioid subtypes, respectively)(data not shown). The association between PTEN expression and ovarian cancer-specific mortality present in univariate analysis (HR = 0.56; 95% CI 0.31 - 1.00; $p = 0.05$) became non-significant in multivariate analysis (HR = 0.75; 95% CI 0.41 -1.37; $p = 0.35$).

The relationship between loss of INPP4B or aberrant p53 expression and survival did not vary by stage (data not shown). Survival analysis by stage for PTEN expression was not possible since there were no Stage I/II tumors showing loss of PTEN.

Discussion

The goal of the current study was to evaluate whether tumor INPP4B protein expression conveys better or worse prognosis of invasive epithelial ovarian cancer. In this study of 599 women with such disease, aberrant p53 expression was associated with significantly increased ovarian cancer mortality. Loss of INPP4B expression was associated with worse survival for the endometrioid subtype; however, this association did not achieve statistical significance in the multivariate model. In contrast, loss of PTEN was weakly associated with improved survival in the univariate model; however, the number of cases in this category was small (n = 45). Expression of all three proteins varied across histologic subtypes and stage. Loss of INPP4B was common among serous and endometrioid cancers. Aberrant p53 expression was also common among serous tumors, more so than among the other histologic types. In contrast, loss of PTEN was most common among the endometrioid subtype.

INPP4B was originally identified as an enzyme that hydrolyzes the 4-position phosphate of $PI(3,4)P_2$, *in vitro* [9]. The *INPP4B* gene resides at 4q31.21, a chromosomal locus frequently disrupted in breast cancer cell lines and basal-like, high-grade breast tumors [10]. Importantly, INPP4B was identified in an RNAi-based genetic screen for genes that suppress transformation of human mammary epithelial cells [11]. Together, these data suggest that INPP4B is a tumor suppressor protein, functioning through its regulation of PI3K signaling [12]. In support of this notion, downregulation of INPP4B in malignant proerythroblasts has been shown to be associated with increased phospho-AKT levels, correctable by re-expression of INPP4B [13]. Allelic losses of INPP4B, loss of INPP4B transcript and loss of INPP4B protein expression have been reported to occur in a majority of *BRCA1* mutant [14] and basal-like breast cancer subtypes [8, 14]. Loss of INPP4B expression also correlates with shortened patient survival [14]. Therefore, like *PTEN*, INPP4B is a candidate tumor suppressor lipid-phosphatase that interferes with the PI3K/AKT pathway, and its substrate, $PI(3,4)P_2$, plays an important role in AKT activation *in vivo*. As a result, this protein is being investigated as a biomarker and as a potential therapeutic target in breast cancer. There is increasing interest in the prospect that inhibition of the PI3K/AKT signaling pathway represents a potential treatment regimen. Interestingly, the present authors found that most of the tumors from women with a germline *BRCA1* or *BRCA2* mutation showed loss of INPP4B (as well as aberrant p53 expression).

To our knowledge, only one study (n = 50) to-date has evaluated whether INPP4B expression is associated with prognosis [14]. Gewinner *et al.,* reported that absence of INPP4B detectability by IHC (26%) was associated with significantly reduced overall survival compared to patients with low (12%) or high (62%) expression ($p < 0.0001$). Loss of INPP4B expression was also associated with increased prevalence of lymph node metastases at diagnosis ($p = 0.04$). The authors did not report which histologic subtypes were included. In the present study, a high proportion of the ovarian tumors lacked INPP4B expression (79%), and low expression was associated with poor survival; however, with adjustment for clinical stage, histologic subtype or p53 status, INPP4B was not an independent marker of ovarian cancer prognosis. Similarly, when results were stratified by histologic subtype, the prognostic role of INPP4B was limited to the endometrioid subtype ($p_{\text{multivariate}} = 0.07$), although this finding requires confirmation in a larger sample of endometrioid tumors.

P53 is the most commonly mis-expressed or mutated tumor suppressor gene in human cancers [15]. Normally, p53 promotes cell-cycle arrest and initiation of repair mechanisms or shunting of cells to apoptosis [15]. This gene is also involved in the transcriptional regulation of PTEN and PI3K/AKT which are required for *p53*-mediated apoptosis [16]. The impact of p53 expression on ovarian cancer survival remains equivocal (reviewed in [17]). In a meta-analysis of 53 studies, aberrant p53 expression was associated with poor survival (pooled HR = 1.47; 95% CI 1.33 - 1.64) with significant heterogeneity between the studies and by histologic subtype [17].

Between 30-80% of high-grade invasive ovarian carcinomas carry *P53* mutations [5, 18, 19], although rates as high as 98% have also been reported [20]. p53 mutations result in aberrant protein expression: either complete loss of expression or over-expression and is a common feature of high-grade serous carcinomas (compared with borderline serous, clear cell and endometrioid cancers) [21]. In the present study, aberrant p53 expression was more common in the serous subtype than in the others (69% of serous *vs.* 9% clear cell tumors and 30% of endometrioid). We found that both complete loss of expression and over-expression of p53 were associated with increased ovarian-cancer specific mortality (HR for aberrant p53 expression = 1.73; 95% CI 1.28 - 2.34). Further, the present data suggest that loss of INPP4B is

strongly correlated with aberrant p53 expression: 61% of INPP4B-negative tumors also had aberrant p53 expression compared to 26% of INPP4B-expressing tumors with aberrant p53 expression.

After p53, PTEN is the second most frequently mutated tumor suppressor gene in human cancers [22]. PTEN mutations occur in a wide range of tumor types [23]. PTEN is a lipid phosphatase that interferes with the PI3K pathway by dephosphorylating the 3-phosphate on $PI(3,4,5)P_3$ to generate $PI(4,5)P_2$[22]. Thus, PTEN functions as a tumor suppressor through its ability to turn off the PI3K pathway. PTEN is frequently mutated in endometrioid cancers but not in other ovarian cancer subtypes [5, 24]. In the present study, only 8% of ovarian tumors showed loss of PTEN expression, though loss was more common in endometrioid subtypes (17%) in accordance with the published literature [5, 24]. Endometriosis or retrograde menstruation implants on the ovary or transformation of ovarian surface epithelium to endometrial-like cells can lead to the development of clear cell or endometrioid tumors, some of which characterized by *PTEN* or *MYC* mutations, leading to loss of or over-expression of these proteins [25, 26]. *PTEN* mutations are commonly observed in normal epithelium of the endometrium as well as in endometriosis and in endometrioid adenocarcinomas, suggesting that loss of PTEN expression may be a step in the progression from endometriosis to cancer [27-31].

Results of studies evaluating the influence of PTEN expression on ovarian cancer recurrence have been mixed. Several studies reported no relationship between loss of PTEN and survival [32-35] whereas two observed that reduced PTEN expression was associated with shortened relapse-free interval [36] or decreased disease-free survival [37]. These analyses have been limited by somewhat small sample sizes (range 20-232), by the inclusion of only a subset of histologic subtypes or of only early- or late-stage tumors, along with large variability in proportions of ovarian tumors staining negative for PTEN [33, 35-39]. Results from the largest study (n = 232) showed that lack of PTEN staining was associated with earlier stage disease, non-serous subtypes, and improved progression-free survival but not overall survival [38]. We found no significant relationship between PTEN expression and risk of ovarian cancer-specific mortality. Improved endometrial cancer survival has been seen with loss of PTEN [40], perhaps due to enhanced sensitivity to chemotherapy. Collectively, the data support a possible role for PTEN in the development of endometroid tumors, and possibly clear cell tumors as well [24, 38].

To our knowledge, this is the largest study to-date that has evaluated the prognostic role of INPP4B expression for ovarian cancer. A major strength of this study is the large sample size, allowing stratified analyses by histologic subtype. Moreover, the use of TMA allowed for simultaneous staining of many tumor samples. However, possible weaknesses of this analysis include the resultant smaller sample size despite the large number of women initially eligible for inclusion in the TMA. In addition, we cannot exclude a possible effect of neoadjuvant chemotherapy on protein expression; however, we did not have access to this information.

Conclusion

In summary, we observed here that aberrant p53 expression is a frequent event in serous ovarian cancer and that it is associated with relatively poorer survival. Although not an independent marker of prognosis, loss of PTEN was more often found in endometrioid then in serous tumors. They have also shown that loss of INPP4B expression may be a prognostic marker for ovarian cancer, in particular for those of the endometrioid subtype. This data support a possible role of multiple pathways of development for the various ovarian cancer subtypes. Integrating tailored treatment options based on tumor protein expression may ultimately lead to improved outcomes following the diagnosis of ovarian cancer.

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