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Review

The Putative Role of *TP53* Alterations and p53 Expression in Borderline Ovarian Tumors – Correlation with Clinicopathological Features and Prognosis: A Mini-Review

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Abstract

Borderline ovarian tumors (BOTs) represent an independent group among ovarian malignancies, being diagnosed at clinical stage earlier than invasive ovarian carcinomas (OCs) and characterized by a rather favorable outcome after careful surgical management. Data published worldwide showed a substantial discordance of p53 expression in BOTs. The purpose of this work was to present the current status of knowledge on the significance of *TP53* gene and p53 protein product alterations in BOTs. In general, higher p53 expression patterns were reported for ovarian malignancies compared to BOTs. Serous, mucinous, and endometrioid BOTs differ substantially in relation to p53 immunostaining, but data concerning the relationship between the protein's immunoreactivity and other clinico-pathological variables are scarce. Finally, reports published to date support the view that *TP53* alterations may not be commonly associated with the borderline phenotype of ovarian tumors but they probably occur during the development of invasive OCs. In light of these uncertainties, the impact of *TP53* alterations and p53 expression on overall survival in women affected by BOTs requires further multi-institutional studies in large cohorts of patients.

Key words: p53, *TP53*, borderline ovarian tumor, prognosis.

Introduction

Borderline ovarian tumors (BOTs) were first described by Taylor in 1929 and they were then called "semimalignant tumors of the ovary" [1, 2]. Nowadays, they are also referred to as borderline tumors of the ovary, tumors of low malignant potential, or even atypical proliferative tumors of the ovary [3-5]. BOTs were officially classified by the International Federation of Gynecology and Obstetrics (FIGO) in 1961, and re-classified by a Committee of the World Health Organization (WHO) in 1973. The terminology and diagnostic criteria for BOTs, implemented by the new 2014 WHO Classification of Tumors of the Female Genital Tract have been recently reviewed in detail by Hauptmann *et al*. [6]. One important change of this classification is the new terminology of non-invasive implants associated with serous BOT which, as any invasive foci (prior invasive implants), are now considered peritoneal low-grade serous carcinoma. Of note, even though only a small proportion of BOTs demonstrate features of microinvasion, these tumors have been classified according to consecutive FIGO classifications of ovarian carcinoma (OC), including the most current update [7].

BOTs – incidence and clinico-pathologic features

In general terms, BOTs are pathologically characterized by "hierarchical arborizing edematous papillae, focally covered by stratified epithelium with variable nuclear atypia with few mitoses and absence of destructive stromal invasion" [5]. As to histologic types, more than 96% of BOTs are either mucinous or serous subtypes, whereas other subtypes – for example, endometrioid, clear-cell, or Brenner transitional cell tumors – are uncommon [4, 8, 9]. At diagnosis, most of the cases are limited to one ovary only, representing FIGO stage I [9-11]. In relation with such an early clinical stage of the disease, the 5-year survival rate is approximately 97% [9, 12]. For more advanced stages of the disease (II-III), the 5-year survival rate approaches 87%. Unfortunately, due to late recurrences, the 10-year survival rate may be less: 70-90%. However, it should be beared in mind that a limited number of women will die early from the disease due to unfavorable prognostic factors, such as presence of invasive foci not resected completely at surgery [13-16]. Of importance, progression from BOT to invasive OC is approximately 2% and may be observed either in mucinous or serous subtypes [17]. By definition, rare cases of BOT with microinvasion, as an invasive disease, represent a higher risk for recurrence. Their monitoring with CA125 marker may have a role in the detection of recurrence [18]. Furthermore, young BOT patients with child-bearing potential are at a higher risk for recurrence, whereas older patients are at a higher risk for malignant transformation in peritoneal cavity or for distant metastases [19].

It is noteworthy that nearly 15-20% of all epithelial ovarian malignancies are finally diagnosed as BOTs. However, a number of characteristics differ BOTs from invasive epithelial OCs, for example FIGO stage at diagnosis, favorable overall outcome, or distribution of histological subtypes [9, 20]. Although there has been a decreasing incidence of OC worldwide, several studies from Scandinavia showed an increased occurrence of BOTs within ovarian malignancies during the last decades [21, 22]. Women affected by BOT are nearly 10 years younger at diagnosis compared to patients with invasive OC [9, 23]. Moreover, the increased incidence of BOTs may be associated with the application of various stimulation protocols during *in vitro* fertilization techniques [24, 25].

In contrast, BOTs are uncommonly detected in patients with *BRCA* mutations, as suggested by a nationwide study from Israel [26]. As concluded by Verbruggen *et al.* [27], "borderline ovarian tumors are neither part of the *BRCA1*- nor the *BRCA2*-related tumor spectrum".

In this context the data by Nayar *et al.* [28] is worth citing. These researchers reported that up to 10% of serous and mucinous BOTs demonstrate microinvasion, although the presence of this feature does not seem to significantly worsen prognosis in these patients. Similar observations were presented by others [29, 30]. Accordingly, Bell and Kurman [31] suggested that cytologic atypia and tumor microinvasion probably do not affect the prognosis of patients with endometrioid BOT. In a study by Roth *et al.* [32], 30 women with endometrioid BOT revealed a favorable prognosis compared to 32 patients with well-differentiated endometrioid ovarian adenocarcinoma.

In 2012, Morice *et al.* [33] looked at 80 advanced-stage serous BOT patients followed at the Gustave Roussy Institute, France for an over 30-year period (1969-1999). Invasive peritoneal foci was the only statistically established prognostic factor for evolution to invasive disease. However, another interesting finding in their analysis was that 8 (10%) patients had nodal involvement with lymph node histological features similar to those of the primary serous BOT [33]. In line, a later German study confirmed that patients with invasive foci have higher relapse rates, yet their overall prognosis is not worsened [34].

TP53 gene and p53 protein product

TP53 has been labelled "the guardian of the genome" due to the fact that it prevents the proliferation of cells with damaged nuclear DNA [35-37]. This gene consists of 11 exons and is located on the short arm of chromosome 17 at region 17p13.1 [35, 38]. Although most of *TP53* genetic alterations have been found at exons 5-9, mutations outside this region have also been reported in various human malignancies [39-41]. It is noteworthy that *TP53* is one of the most commonly mutated tumor suppressor genes in different human neoplasms, and it is involved in the development of at least a half of clinical tumors nowadays [38, 42, 43].

TP53 gene encodes p53 protein which comprises 393 amino acid residues [44]. The protein is composed of three main functional domains required for efficient binding to the recognition sites of the target genes. It is involved in a wide variety of important cellular functions, such as cell-cycle arrest at G1 and G2/M transitions, DNA repair, differentiation, senescence, and apoptosis (Fig. 1). p53 protein is present in normal cells at low levels and possesses a short half-life due to rapid turnover mediated by ubiquitination and proteolysis [45]. In cancer cells,

alterations at *TP53* result in the synthesis of a mutated protein with a prolonged half-life and increased stability and, consequently, the loss of the guardianship of the genome follows [46]. The loss of genomic stability allows for the progression of cells with damaged DNA through the cell cycle. Interestingly, not only p53 overexpression but also the absence of p53 immunostaining could be associated with *TP53* alterations [47, 48]. In cases when the *TP53* becomes altered, the half-life of p53 protein becomes substantially longer and then it is possible to detect the protein applying different analytical methods, including Western blotting or immunohistochemistry [36, 49]. In rare cases, however, when the *TP53* mutations produce truncated proteins that are not overexpressed, even highly sensitive techniques may not detect the protein [50]. Interestingly, application of two immunohistochemical labelling patterns associated with *TP53* mutations identified mutations in as many as 94% of OCs investigated [48].

TP53 alterations in BOTs

In primary human OCs, 30-80% of cases revealed *TP53* alterations, functional point mutations, deletions and/or allelic loss [42, 48, 51-54]. As pointed out by Boyd and McCluggage [55], high-grade, but not low-grade, serous OCs are associated with point mutations at *TP53* in a substantial proportion of cases. Of note, even early-stage sporadic OC patients harboring *TP53* mutations were characterized by significantly worse progression-free and disease-specific survivals [56]. In BOTs, the incidence of *TP53* alterations was found lower compared with that reported for human primary OCs, as estimated by several studies where it did not exceed 20% of cases [53, 54, 57, 58]. Yet, there are also data suggesting even the lack of *TP53* alterations in BOTs [59]. Earlier, Kupryjańczyk *et al.* [57] reported that none of pure BOTs being analyzed harbored *TP53* mutations, although gene mutations were reported in 40% of stage I OCs, including a borderline component adjacent to carcinoma in one case. In another study, only one case of BOT out of 9 had a point mutation, as evaluated with direct sequencing [53]. Not only micropapillary serous OCs but also serous borderline tumors lacked p53 mutations in the study by Katabuchi *et al.* [58]. Furthermore, Kmet *et al.* [45] reported a higher *TP53* prevalence in mucinous BOTs compared to serous tumors of low malignant potential. Finally, genetic as well as immunohistochemical analysis of *TP53*/p53 alterations confirmed the hypothesis of the dual pathways of ovarian serous carcinogenesis and reported similar *TP53* alterations in serous BOTs and low-grade serous OCs [54, 59].

The data published to date supports the view that *TP53* alterations (corresponding not in all cases to p53 overexpression, as discussed above) may not be commonly associated with the borderline phenotype of ovarian tumors, but rather occur during the development of invasive low-grade serous OCs [54, 57, 59, 60]. Even though *TP53* mutational status was correlated with p53 immunoreactivity in one study, the investigators studying the problem finally stated that "immunostaining is neither sufficiently specific nor sensitive enough to predict *TP53* mutations" [54].

An important retrospective study by Ortiz *et al.* [61] is worth citing here. These authors reported that seven of eight (88%) primary BOTs showed completely different *TP53* mutations compared with gene alterations found in subsequent invasive serous OCs. Consequently, they suggested "a nonclonal origin for the serous BOTs compared with the subsequent grade I invasive serous epithelial ovarian carcinomas" [61].

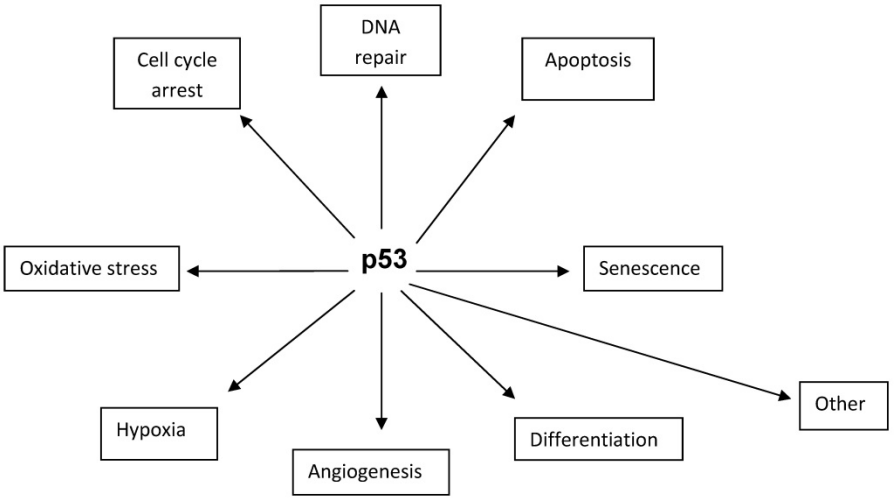

Figure 1. Some cellular functions of p53 protein.

Table 1. An overview of reports on largest patient groups studied to date, presenting p53 expression patterns in primary human BOTs. Prognostic significance of p53 expression in tumors of low malignant potential was also included.

Author(s)	No. of patients (n)	p53-positive cases (n)	Percentage of p53-positive cases (%)	Prognostic significance
Nielsen *et al.* [50]	85	17	20	p53 is not a prognosticator
Gershenson *et al.* [62]	68	13	19	decreased OS* and increased progression/ recurrence in p53-positive cases
Kuhn *et al.* [60]	54	5	9	no recurrence in p53 positive cases
Berchuck *et al.* [63]	49	2	4	NE*
Kohlberger *et al.* [65]	46	0	0	NE
Aktas *et al.* [80]	44	30	68	NE
Ciepliński *et al.* [79]	42	25	60	NE
Fauvet *et al.* [92]	34	9	26	NE
Halperin *et al.* [89]	20	0	0	NE
Lee *et al.* (1995)	17	3	18	NE
Ozer *et al.* [91]	16	not reported	not reported	NE
Gajewska *et al.* [74]	16	15	94	NE
Giurgea *et al.* [73]	15	1	7	NE
Miliaras [67]	13	0	0	NE
Kupryjańczyk *et al.* [57]	12	8	66	no recurrence in p53 positive cases

*OS, overall survival; NE, not evaluated.

p53 expression in BOTs

For BOTs, data regarding the expression pattern of p53 are still a matter of controversy since this expression pattern has been reported with a considerable inter-observer variability. Table 1 overviews the largest studies investigating p53 expression pattern in human BOTs based on the data published. Significant differences reported in the literature concerning the striking variability in p53 staining in human BOTs (from 0 to 94%) may be partly explained by different antibodies applied and various immunostaining techniques and methods of signal quantification used. A previous literature survey of clinical studies investigating p53 overexpression by stage in BOTs was conducted by Gershenson *et al.* in 1999 [62]. Examples of p53 immunostaining in primary human BOTs from our laboratory are shown in Figures 2A and 2B.

Some data reported a complete lack of p53 expression in ovarian tumors of low-malignant potential or a low (<5%) immunoreactivity [45, 63-72]. For example, none of 10 BOTs revealed p53 expression pattern by Gursan *et al.* [69]. In another report, only 6.6% of BOTs were p53-positive in contrast to benign ovarian tumors where positive reaction was not identified [73]. Interestingly, all 5 mucinous borderline tumors and 4 (57%) out of 7 serous borderline tumors expressed p53 in a recent paper from India [72]. In a work from Poland, as many as 90% of OCs and 94% of BOTs showed positive p53 immunoreactivity, and the staining pattern differed significantly between malignant tumors and BOTs [74]. This paper presented the highest incidence of p53 expression in BOTs reported so far.

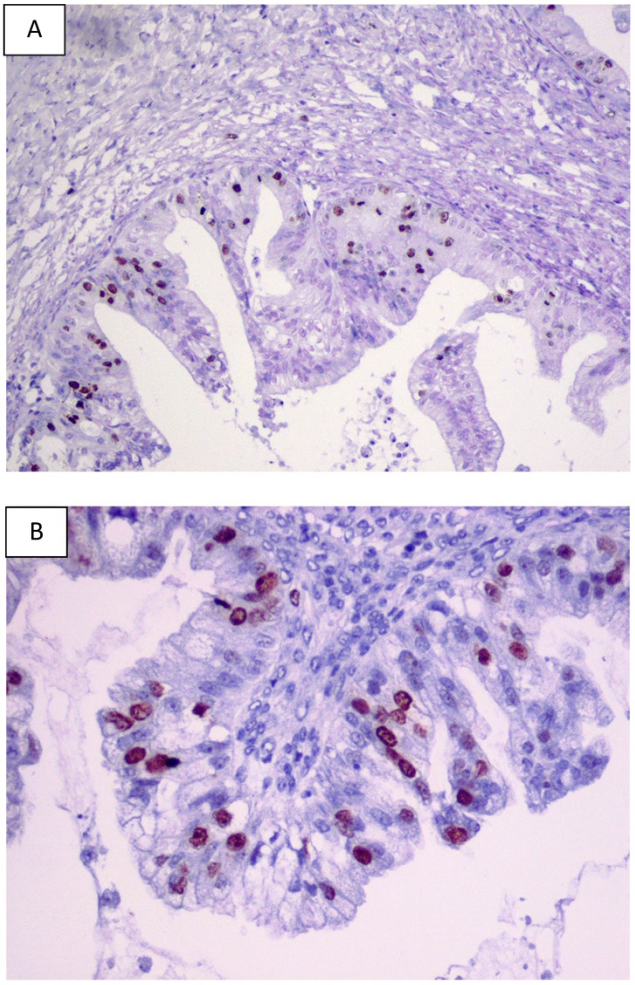

Figure 2. Examples of the p53 immunostaining in primary human BOTs (A-B). Original magnifications: 100 x and 200 x.

Another study, Kuhn *et al.* [60], investigated a cohort of 54 BOTs divided into unilateral tumors (N = 38), bilateral tumors (N = 8), and tumors with an additional component of invasive carcinoma (N = 8). p53 protein was detected in one (3%), 2 (25%) and 2 (25%) of the cases, respectively; thus, altogether 5 (9%) of 54 tumors were positive. None of the 5 patients whose tumors revealed p53 reactivity displayed disease recurrence during follow-up. Previously, a similar outcome was published by Kupryjańczyk *et al.* following a 5-year follow-up of patients with tumors expressing p53 [75].

Marcelli *et al.* [76] reported a rate of 29% of serous BOTs displaying p53 expression, and somewhat similar percentage (20%) was published by Sylvia *et al.* [71]. It has been suggested that p53 expression is significantly associated with tumor progression not only in serous [76], but also mucinous [75] BOTs. The p53 immunoexpression was observed in all BOTs at the nuclear level, yet only with mild intensity [77].

Our recent data showed that 60% of BOTs expressed p53 immunohistochemically [78, 79], similarly to results previously published by Aktas *et al*. [80]. No significant correlations between p53 expression and clinico-pathological variables of tumors were found. However, since information from 3 pregnant patients was included, we could verify and report for the first time that p53 expression in BOTs does not differ significantly between pregnant and non-pregnant subjects [79].

In the studies reviewed here, immunohistochemical analysis has been widely applied for the detection of p53 expression. Discrepancies in the percentage of reported p53-positive cases might have arisen from a potential loss of antigenic immunoreactivity during fixation process, and later in slides stored for prolonged period of time [81-83]. Some loss of histochemical reactivity between frozen and formalin-fixed slides has been underlined by reputed reviews previously [83-85]. However, both new methods of detection and monoclonal antibodies used reduce the loss of protein reactivity, even when using archival material [81, 82, 86, 87].

Interestingly, immunoreactivity of p53 protein was significantly higher in BOTs, either serous or mucinous subtypes, than in adenocarcinomas of the ovary, although the study groups were not particularly large (N = 10 for each arm) [88]. The main difference between BOTs and advanced serous papillary OCs was in regards to the increased overexpression of p53 and Ki-67 in the latter [89]. In contrast, the p53 expression was dramatically less in benign (6%) than borderline (75%) and malignant tumors (81%), the differences being highly significant [73]. Similar observations were also published by others [90, 91]. In another report, a highly significant difference in semi-quantitative p53 expression was detected between benign and borderline tumors, but not between borderline and malignant ovarian tumors [92]. Halperin *et al.* [89], the p53 immunostaining for benign ovarian serous cystadenomas did not differ significantly from that observed for BOTs, albeit the difference in staining between BOTs and advanced serous papillary OCs was of statistical significance.

Interestingly, a higher expression of p53 was associated with BOTs of serous type in a recent study by Tiwari *et al.* [93]. Similarly, p53 immunoreactivity was much more prevalent among malignant (36 of 81, or 44%) than borderline (3 of 39, or 8%) tumors and it was particularly prevalent in serous OCs (16 of 26, or 62%) [94]. On the other hand, no difference in p53 immunoreactivity between serous and mucinous BOTs was found by Fauvet *et al.* [92] and Gursan et *al.* [69], although others [91, 95] reported a significantly higher p53 expression rate in serous than mucinous tumors. Therefore, Giordano *et al.* [95] suggested that "… a significantly higher p53 expression rate observed in serous papillary cystadenocarcinomas than in mucinous cystadenocarcinomas and a higher p53 expression rate observed between borderline and malignant serous neoplasms can confirm a new model of ovarian carcinogenesis as suggested by Shih and Kurman" [96-97]. Collectively, a marked difference in p53 expression between histological subtypes of BOTs and invasive OCs has been commonly observed.

Role of p53 immunostaining as a prognosticator for BOTs

Data concerning the role of *TP53* alterations and p53 overexpression as a prognosticator in patients affected by BOTs are limited to several reports [50, 57, 60, 62] (Tab. 1). For example, none of 5 women with BOTs showing p53 immunoreactivity experienced a disease recurrence during a long follow-up [60]. Similar results were published by Kupryjańczyk *et al*. [57] where none of 8 patients affected by BOTs positive for p53 immunoreactivity, including tumors with microinvasion, revealed any evidence of recurrence during a 5-year follow-up. However, both studies included limited numbers of women and, therefore, it is not possible to draw final conclusions from them.

An interesting data was published by Gersherson *et al.* [62], showing that p53 overexpression was associated with an increased probability of advanced-stage serous BOT progression and recurrence, but also of decreased

overall survival during follow-up. Women aged 30-49 years were almost four times more likely to have progressive/recurrent disease than younger women. Finally, in the age group of ≥50 years, the presence of residual disease and p53 overexpression were found to be independent adverse risk factors for death. In contrast, neither univariate nor multivariate analysis revealed a significant prognostic effect in 85 BOTs patients during a long-term follow-up in a study by Nielsen *et al.* [50].

All in all, based on the literature review, except for Gersherson *et al.* [62], there are no studies suggesting an increased risk of recurrence and death from advanced-stage serous BOTs in patients whose tumors overexpressed p53. Relatively limited numbers of patients, lack of detailed information regarding treatment protocols and patient outcomes, as well as short durations of follow-up in some studies may have obscured the possibilities for drawing final conclusions yet.

Closing remarks

Data published worldwide demonstrates a substantial discordance of p53 expression in human BOTs. In general, patterns of higher p53 expression were reported for ovarian malignancies compared to BOTs. Serous, mucinous, and endometrioid BOTs differ markedly in regards to p53 immunostaining, however, data concerning the relationship between the protein's immunoreactivity and other clinico-pathological variables are scarce. Finally, reports published to date support the view that *TP53* alterations may not be commonly associated with the borderline phenotype of ovarian tumors, but they probably occur during the development of invasive OCs [98-100]. Considering these uncertainties, the impact of *TP53* alterations and p53 expression on overall survival in women affected by BOTs requires further multi-institutional studies in large cohorts of patients.

Abbreviations

BOT, borderline ovarian tumor; FIGO, International Federation of Gynecology and Obstetrics; OC, ovarian carcinoma; WHO, World Health Organization.

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Competing Interests

The authors have declared that no competing interest exists.

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