

Immunohistochemical staining patterns of p53 can serve as a surrogate marker for *TP53* mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis

Anna Yemelyanova¹, Russell Vang^{1,2}, Malti Kshirsagar^{1,5}, Dan Lu¹, Morgan A Marks⁴, Ie Ming Shih^{1,2,3} and Robert J Kurman^{1,2,3}

¹Department of Pathology, Johns Hopkins University, School of Medicine, Baltimore, MD, USA; ²Department of Gynecology and Obstetrics, Johns Hopkins University, School of Medicine, Baltimore, MD, USA;

³Department of Oncology, Johns Hopkins University, School of Medicine, Baltimore, MD, USA and

⁴Department of Epidemiology, Johns Hopkins University, School of Public Health, Baltimore, MD, USA

Immunohistochemical staining for p53 is used as a surrogate for mutational analysis in the diagnostic workup of carcinomas of multiple sites including ovarian cancers. Strong and diffuse immunoexpression of p53 is generally interpreted as likely indicating a *TP53* gene mutation. The immunoprofile that correlates with wild-type *TP53*, however, is not as clear. In particular, the significance of completely negative immunostaining is controversial. The aim of this study was to clarify the relationship of the immunohistochemical expression of p53 with the mutational status of the *TP53* gene in ovarian cancer. A total of 57 ovarian carcinomas (43 high-grade serous ovarian/peritoneal carcinomas, 2 malignant mesodermal mixed tumors (carcinosarcomas), 2 low-grade serous carcinomas, 4 clear cell carcinomas, 1 well-differentiated endometrioid carcinoma, and 5 carcinomas with mixed epithelial differentiation) were analyzed for *TP53* mutations by nucleotide sequencing (exons 4–9), and subjected to immunohistochemical analysis of p53 expression. Thirty six tumors contained functional mutations and 13 had wild type *TP53*. Five tumors were found to harbor known *TP53* polymorphism and changes in the intron region were detected in three. Tumors with wild-type *TP53* displayed a wide range of immunolabeling patterns, with the most common pattern showing $\leq 10\%$ of positive cells in 6 cases (46%). Mutant *TP53* was associated with 60–100% positive cells in 23 cases (64% of cases). This pattern of staining was also seen in three cases with wild-type *TP53*. Tumors that were completely negative (0% cells staining) had a mutation of *TP53* in 65% of cases and wild-type *TP53* in 11%. Combining two immunohistochemical labeling patterns associated with *TP53* mutations (0% and 60–100% positive cells), correctly identified a mutation in 94% of cases ($P < 0.001$). Immunohistochemical analysis can be used as a robust method for inferring the presence of a *TP53* mutation in ovarian carcinomas. In addition to a strong and diffuse pattern of p53 expression (in greater than 60% of cells), complete absence of p53 immunoexpression is commonly associated with a *TP53* mutation. Accordingly, this latter pattern, unlike low-level expression (10–50% cells), should not be construed as indicative of wild-type *TP53*.

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Correspondence: Dr A Yemelyanova, MD, Division of Gynecologic Pathology, Department of Pathology, The Johns Hopkins Hospital, Weinberg Building, Room 2242, 401 N. Broadway, Baltimore, MD 21231, USA.

E-mail: ayemely1@jhmi.edu

⁵Current address: Clinical Pathology Associates, Austin, TX, USA
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Mutations of the *TP53* gene are the most common and most frequently studied molecular alterations in human cancer.^{1–3} Many studies have investigated their significance in diagnosis, prognosis, and treatment in tumors of various sites. For example, the presence of a *TP53* mutation in colorectal, lung, prostate, and breast carcinomas has been shown to be a poor prognostic factor, and for the tumors of

other sites a *TP53* mutation has been associated with chemoresistance in some studies.^{4–9} Accordingly, detection of a *TP53* mutation has taken on considerable importance in clinical practice.

While nucleotide sequencing is the most reliable technique to detect gene mutation, it is labor intensive, time consuming, and therefore, currently has limited application in clinical pathology practice. Immunohistochemical analysis of p53 expression is therefore commonly used as a surrogate for mutational analysis.^{10–13} It has been generally accepted that wild-type p53 protein is relatively unstable and has a short half-life, which makes it undetectable by immunohistochemistry.¹⁴ In contrast, mutant p53 has a much longer half-life, and therefore, accumulates in the nucleus creating a stable target for immunohistochemical detection.^{15,16} Although some studies have described immunohistochemical overexpression of wild-type p53, suggesting abnormal stability of non-mutant protein due to impaired degradation under cellular stress, strong and diffuse immunolabeling is generally regarded as indicative of a missense mutation of *TP53*.^{1,7,17} It has been proposed, but not as well appreciated that complete lack of immunohistochemical expression may be a result of a nonsense mutation leading to formation of a truncated, non-immunoreactive protein.^{18–20} In view of these different patterns of immunoexpression associated with mutant *TP53*, it is therefore not surprising that studies correlating *TP53* gene status and/or p53 overexpression with clinical outcome and response to chemotherapy in ovarian/pelvic serous carcinoma have reported conflicting results.^{10,12,19,21–27} Some of the studies that focused on ovarian high-grade serous carcinomas failed to demonstrate correlation between *TP53* mutation and survival that to some extent have been related to the fact that *TP53* mutation is an early event in high-grade serous carcinogenesis with near ubiquitous occurrence.^{21,25,28,29} In addition, accurate pathological classification of ovarian carcinoma is frequently lacking in many reports as evidenced by the use of the all encompassing diagnosis ‘ovarian cancer’, without segregating tumors by histological type. Particularly pertinent to the current study has been a failure to use a consistent definition of what constitutes p53 overexpression. Some studies have simply used a cuff-off of as little as 10% of positive cells staining, whereas other have employed complicated scoring systems, which take into account both the quantity of positive cells, as well as the staining intensity.^{22,25,27,30,31} In addition to the lack of uniform scoring systems, variations in immunohistochemical protocols make data from different studies difficult to compare and interpret. The aim of this study was to correlate immunohistochemical staining patterns of p53 expression in ovarian carcinomas with mutational analysis in order to establish practical immunohistochemical cut-points, which can be used to infer the presence of a *TP53* mutation.

Materials and methods

Case Selection and Mutational Analysis

A total of 57 cases of ovarian carcinoma, which included 43 high-grade serous ovarian/peritoneal carcinomas, 2 malignant mesodermal mixed tumors (MMMTs), 2 low-grade serous carcinomas, 4 clear cell carcinomas, 1 well-differentiated endometrioid carcinoma, and 5 carcinomas with mixed epithelial differentiation (4 mixed serous-endometrioid and 1 serous-clear cell-endometrioid carcinomas) were studied. Tumors that were previously analyzed for *TP53* mutation by nucleotide sequencing (exons 4–9) were subjected to immunohistochemical analysis of p53 expression. The details of the mutational analysis have been previously reported.^{28,29} Tumors with no identified mutations in exons 4–9 were considered to have wild-type *TP53*.

Immunohistochemistry

One tumor-rich section per case was selected for immunohistochemical analysis. Immunoperoxidase labeling was performed with the automated XT iVIEW DAB V.1 procedure on the BenchMark XT IHC/ISH Staining Module, Ventana with anti-p53 (clone Bp53–11, prediluted, Ventana). Antigen retrieval was carried out with CC1 (Ventana). Sections were incubated with primary antibodies for 16 min at 37°C. Staining was detected with I-View DAB detection system. All slides were reviewed by two pathologists (AY and MK). Cases with discordant results underwent a consensus review at a multi-headed microscope with a third pathologist (RV). Nuclear staining was considered a positive reaction. The extent of staining was estimated to the nearest 10% level of positive tumor cells. The intensity of staining was recorded as weak, moderate, or strong.

Statistical Analysis

Statistical analysis was carried out using Fisher's exact test (STATA 11.0, STATA CORP, College Station, Texas, CA, USA). *P*-values less than 0.05 were considered statistically significant.

Results

Of the 57 tumors, 36 contained functional mutations and 13 had wild-type *TP53*. Because of the limited number of cases with known p53 polymorphism ($n = 5$) and changes in the intron region ($n = 3$), these were not included in further analysis. Only functional mutations (ie, changes in nucleotide sequence that led to structural changes of the encoded p53 protein) were considered for statistical analysis.

Of 43 high-grade serous carcinomas, 30 (70%) tumors contained functional mutations (nucleotide deletion –7, nucleotide insertion –2, nucleotide

substitution -18, and nonsense mutation in 3), known polymorphisms, and changes in the intron region were found in seven tumors; no mutations were identified in six tumors.

Five tumors with mixed epithelial differentiation (4 mixed serous-endometrioid and 1 serous-clear cell-endometrioid carcinomas) all contained functional mutations (nucleotide deletion -one, nucleotide substitution -four). All five tumors displayed greater than 60% positive cells pattern of immunostaining (90% positive cells pattern was seen in four carcinomas and 60% positive cells pattern in one).

In two MMMTs, one had nucleotide deletion with complete absence of immunoeexpression; the other contained known polymorphism with 80% positive cells immunostaining pattern.

One low-grade endometrioid adenocarcinoma arising in a background of an atypical proliferative (borderline) endometrioid tumor had wild-type TP53 and less than 10% positive cells immunostaining pattern.

No mutations were identified in four clear cell carcinomas and two low-grade serous carcinomas, all of which had less than 10% positive cells.

Immunohistochemical and mutational analysis data are summarized in Table 1. All tumors with 10% of positive cells demonstrated predominately

weak staining with occasional nuclei displaying moderate intensity of staining.

Among carcinomas with wild-type TP53, one of two tumors with 20–50% of positive cells displayed strong to moderate staining intensity, the other case in this group showed weak staining. All tumors with 60–100% of positive cells demonstrated strong staining, with a few cases displaying areas of moderate intensity in addition to strongly staining areas.

In 23 cases (63%) with mutant TP53 ≥60% of tumor cells were positive for p53 (Figure 1). Nineteen of these cases contained missense mutations. Additionally, 11 cases (31%) with mutations were completely negative for p53 (Figure 2). This immunolabeling pattern was associated with nucleotide deletion and nonsense mutations in 9 of 11 cases (82%). By combining two immunohistochemical staining patterns (strong and diffuse expression and complete lack of expression), the immunohistochemical analysis correlated with the mutational analysis in 94.4% of cases (P<0.001, Fisher's exact test). Tumors with wild-type TP53 (n=13) displayed a wide range of immunolabeling patterns, with the most common pattern being ≤10% of positive cells present in six cases (46 %) (Figure 3).

Table 1 Immunohistochemical staining patterns and mutational analysis of p53 in ovarian carcinomas

Immunohistochemical staining pattern (% positive cells)	Mutational analysis						
	Wild type	Nucleotide deletion	Nucleotide insertion	Missense mutation	Nonsense mutation	Known TP53 polymorphism	Changes in intron region
Negative (n = 17)	2	7	1	1	2	2	2
≤10% (n = 8)	6	—	—	2	—	—	—
20–50% (n = 2)	2	—	—	—	—	—	—
60–80% (n = 11)	2	1	—	5	—	2	1
90–100% (n = 19)	1	1	1	14	1	1	—
Total (n = 57)	13	9	2	22	3	5	3

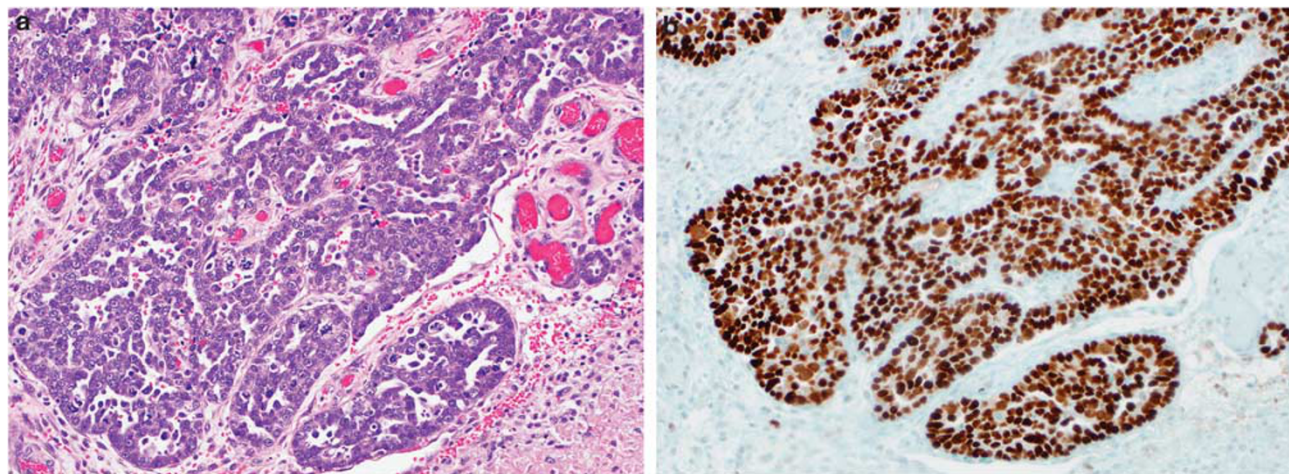


Figure 1 High-grade serous carcinoma (missense mutation, exon 6) (a); strong and diffuse immunoeexpression of p53 (100% of positive cells) (b).

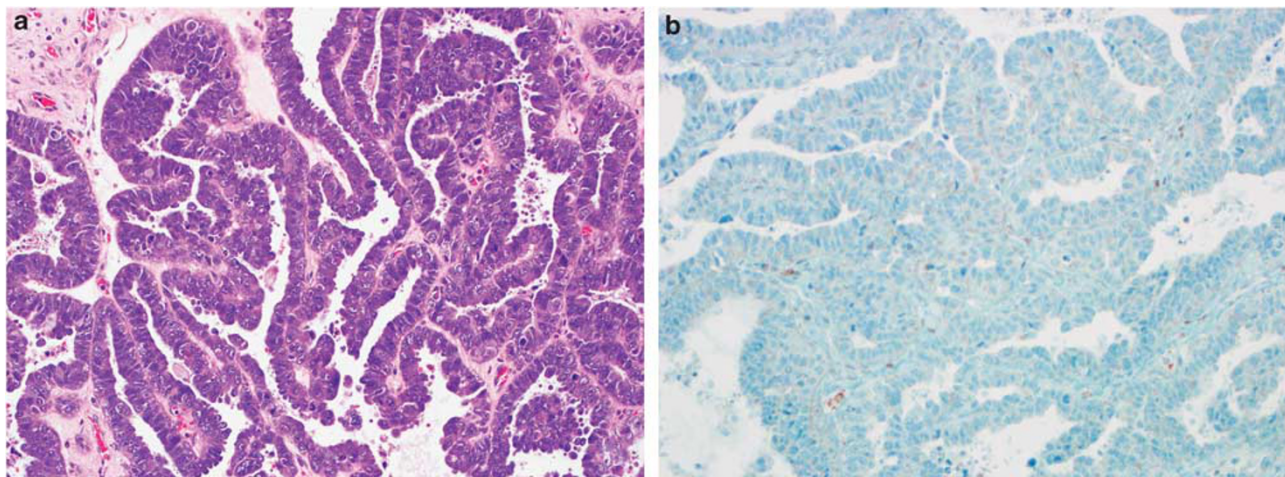


Figure 2 High-grade serous carcinoma (nucleotide insertion, exon 4) (a); completely absent immunoeexpression of p53 (0% of positive cells) with weakly positive stromal cells serving as an internal positive control (b).

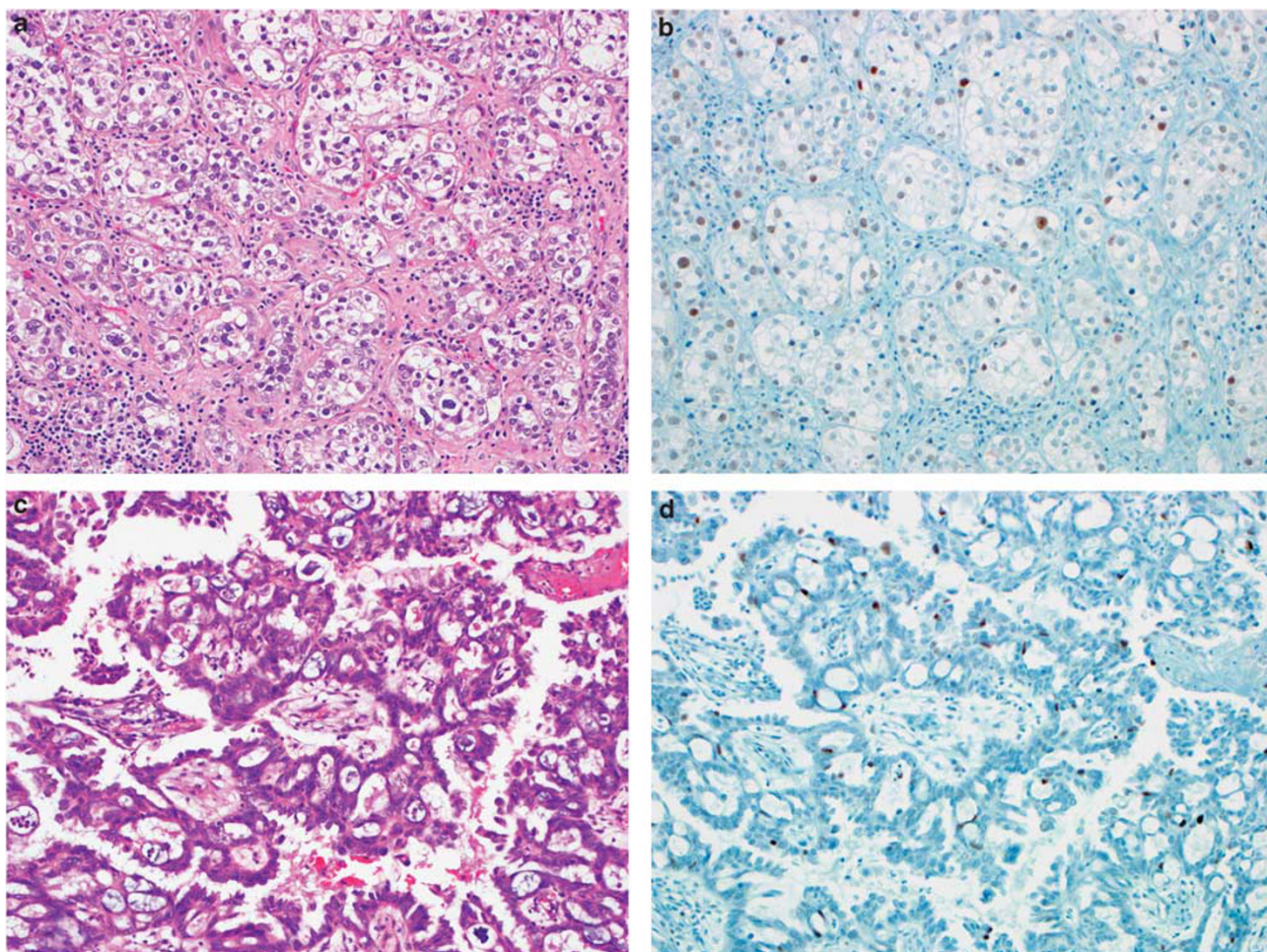


Figure 3 Clear cell carcinoma (a), and low-grade serous carcinoma (c) with wild-type *TP53*. Rare p53-positive cells (<10% cells positive) (b, d).

Discussion

In this study, p53 expression was scored as the percent of positive tumor cells. This simple method

was easy to apply compared to complex scoring schemes that take into account combination of staining distribution (percent of positive cells), as well as staining intensity. Parenthetically, all cases

with 60–100% positive tumor cells demonstrated strong-to-moderate staining intensity, therefore, inclusion of staining intensity into immunohistochemical scoring scheme would not improve performance of the test at the chosen cut-off. In addition, evaluating intensity of staining is problematic as it is difficult to reproduce and it can vary with different protocols.

Known *TP53* polymorphisms do not represent gene mutation and most of them are expected to be phenotypically silent.^{32,33} Moreover, it is not entirely clear whether *TP53* polymorphisms may or may not result in conformational changes leading to altered immunoreactivity of p53.³⁴ Because of these issues and due to the small number of such cases in this study, we chose not to analyze them. We also excluded tumors with changes in the intron region, as it was not possible to determine whether these changes could have caused a frameshift and resulted in a protein with aberrant immunorepression.

The findings in this study confirm that immunohistochemical overexpression of p53 ($\geq 60\%$ positive cells) closely correlates with a *TP53* mutation. Even more importantly, it was found that complete absence of immunolabeling was also indicative of a mutation. Our results therefore support previously published data reporting that missense mutations in the *TP53* gene lead to formation of a stable protein resulting in immunohistochemical overexpression, whereas nucleotide deletions and non-sense mutations result in protein truncation and complete lack of immunolabeling.^{18,20}

The lack of p53 immunorepression associated with nonsense (or null) mutation is very important to recognize because some studies demonstrated worse prognosis for patients with this finding compared with patients whose tumor had a missense mutation.^{19,35} If less than 50% positive tumor cells (not including cases completely lacking immunorepression) are used as a cut-off level, it would correctly identify 61.5% of cases with wild-type *TP53*. More importantly, if two patterns of immunolabeling commonly associated with *TP53* mutation (60–100% of tumor cells positive and tumors completely negative for p53) were combined, immunohistochemical analysis would correlate with mutations in almost 95% of cases.

Accordingly, p53 immunohistochemical scoring systems should not interpret complete absence of expression as consistent with wild-type *TP53*. It should also be emphasized that evaluation of cases with complete lack of immunorepression should be carried out with strict attention to external and internal controls in order to ensure that the negative result is not due to a technically suboptimal immunohistochemical preparation. The presence of rare weakly positive nuclei (a pattern commonly associated with wild type *TP53*) in stroma or adjacent non-neoplastic tissue present in the same section can serve as a useful internal positive

control. Evaluation of the complete absence of immunorepression should be made exclusively in tumor cells, as lack of immunorepression can be observed in normal non-neoplastic epithelium where it does not indicate *TP53* mutation.

There are several possible explanations for the lack of perfect correlation between *TP53* mutation and immunohistochemical expression. First, the mutational analysis in this study evaluated codons 4 through 9, which would identify most, but not all possible mutated loci.^{21,36} Second, it has been suggested that cellular stress may result in delayed degradation of wild-type p53 making it detectable by immunohistochemistry.³⁷ Third, since the sample on which mutational analysis was carried out was not the same as the sample that was immunostained, it is conceivable that tumor heterogeneity could account for the discordant findings in tumors acquiring *TP53* mutation as a late event in carcinogenesis. In conclusion, immunohistochemical staining for p53 can be used as a surrogate for mutational analysis in ovarian carcinomas, if tumors that are diffusely positive ($\geq 60\%$ of cells) and those that are completely negative are interpreted as indicative of a mutation. Additional studies of tumors from other sites are necessary in order to determine whether the findings in ovarian carcinoma can be generalized to other tumor types.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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