Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i2.4684

IMMUNOHISTOCHEMICAL EXPRESSION PATTERNS OF P53 IN SURFACE EPITHELIAL OVARIAN TUMORS - A DESCRIPTIVE STUDY IN CENTRAL KERALA

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Abstract

Introduction: Although there have been significant advancements in the treatment of gynaecological malignancies, ovarian tumors continue to be the most deadly. The current study aims to evaluate p53 expression in different types & histological grades of epithelial ovarian tumors (EOT) by means of immunohistochemistry (IHC).

Material & Methods: In the present descriptive study, the 50 epithelial ovarian tumors specimen received at department of pathology during the period of two year were chosen for the research. Parameters such as capsule rupture, ascites, tumor size, metastasis, stage at presentation & tumor grade were correlated.

Results: Of the fifty EOT instances, twenty (40%) were malignant, five (10%) were borderline, & twenty (50%) were benign. With 24 cases (48%), serous tumors constituted the majority. With 14 instances (70%) of serous malignancies, the largest group was made up of 3 cases (15%) of mucinous malignancies, 2 cases (10%) of endometrioid carcinoma, & 1 case (5%), of clear cell carcinoma. Every benign & borderline EOT had a wild type or p53 negative result. Of the 20 malignant tumors, 14 (or 70%) were serous malignancies & p53 positive. 20% of HGSC & LGSC displayed aberrant null staining, whereas 73.4% of them displayed widespread p53 staining. There were positive relationships between P53 staining & CA 125, ascites, & capsule rupture.

Conclusion: The research emphasizes how varied p53 expression rates & staining patterns exist, & how accurate p53 IHC interpretation is essential for the diagnosis of distinct EOT.

Keywords: Epithelial Ovarian Tumors, high-Grade Serous Carcinoma, IHC, p53.

INTRODUCTION

In women, ovarian tumors account for 3% of all cancers, & they cause over 140,000 deaths annually in the world.[1,2] More than 90% of ovarian malignancies are epithelial tumors.[3] In the western

countries, the incidence is rising steadily or slowly, but it is rising quickly in the Asian subcontinent.[4]

Malignant tumors are more common in people aged 40 to 65, while over 80% of ovarian cancers are benign & occur in younger age groups, ranging from 20 to 45 years. Nulliparity,[5] high socioeconomic groups, positive family history, increasing age of reproduction, & increasing age are significant etiological risk factors.

The "World Health Organisation (WHO)" classifies primary ovarian neoplasms based on histogenetic principles, primarily pertaining to the origin of the tumors, which includes germ cells, mesenchyme (sex cord & stroma), & coelomic surface epithelial cells. Apart from both benign & malignant primary neoplasms, there exist borderline tumors that are non-invasive & may or may not have malignant potential.[6]

Ovarian surface epithelial tumors are classified as clear cell, endometrioid, transitional cell, mixed epithelial cells, serous, mucinous, & undifferentiated carcinomas based on their morphological characteristics. The prognosis, molecular profile, probable places of genesis, & therapeutic response of these subtypes vary considerably.[7]

To choose patients who may have a good or bad clinical result, it would be crucial to identify novel biological prognostic markers. This could also help to enhance therapy planning.[8] It is believed that steroid hormones like progesterone & oestrogen are crucial in the carcinogenesis of ovarian cancers. In a similar vein, Ki-67 is a proliferation marker that can be used to forecast the course of several cancers, including ovarian neoplasms.[9] Apoptosis regulators, particularly Bcl-2 & p53, have also been investigated as possible indicators of prognosis for epithelial ovarian cancer.[8]

The most commonly changed gene in human malignancies is TP53, & the majority of epithelial ovarian tumors lack functional p53 protein.[10] The relationship seen in the literature between p53 IHC positivity & histological subtype has been contentious. Thus, it is necessary to investigate p53 IHC in an Indian cohort while taking into account all the technical variables that can have an impact on the staining.

The objective of this research is to assess the p53 expression using immunohistochemistry (IHC) in various histological grades & kinds of epithelial ovarian tumors (EOT).

MATERIAL & METHODS

In the present descriptive study the 50 epithelial ovarian tumors specimen received at department of pathology during the duration period of two year were chosen for the research. The ethical permission was taken from institutional ethical committee before the commencement of study.

Every specimen that was collected underwent thorough gross & histological analyses. A standardized proforma was used to analyze the medical records & gather pertinent clinical facts. Following the examination of the slides stained with hematoxylin & eosin, the tumors were categorized using the WHO 2017 classification. The heat-induced epitope retrieval approach was used for IHC, & all 50 patients had the marker p53 (Path insitu, a mouse monoclonal antibody that is ready to use against both p53-wild & mutant staining) done.

The initial run of the PATHINSITU mouse monoclonal antibody against p53 used colon cancer & Bloom Richardson grade II breast carcinoma as positive controls, as directed in the product datasheet. As positive controls, cases that tested positive in subsequent runs were also kept. Sections with p53 antibody-free & IHC-performed controls were also kept as negative controls. The P53 expression & staining patterns of each tumor were analysed.

The study examined many clinical & histomorphological factors, including age, tumor laterality, ascites, tumor size, capsule rupture, metastasis, stage at presentation, tumor grade, & number of mitoses. When necessary, special stains like mucicarmine & PAS were used. Clinical information & serum CA-125 values were gathered from the case files. Treatment for ovarian tumors with radiotherapy or neoadjuvant chemotherapy was not allowed since it might affect the results of the IHC staining.

Interpretation of Staining: The marker's nuclear staining was taken into account & reported as a percentage. Positive: two different kinds of positive staining patterns are observed: (a) diffuse nuclear positivity owing to p53 missense mutation if more than 70% of cells are positive. (b) A non-sense mutation in p53 results in null /complete absence of staining. When one to seventy percent of the cells exhibit patchy staining, the case is deemed wild type.

The percentage represents the portion of tumors that are p53 positive. Software for statistical analysis is SPSS version 25.0. To determine if there is a statistically significant relationship between p53 expression & the parameters under investigation, the chi-square test & Fisher's exact test were employed. $P < 0.05$ is maintained as the level of significance P value, while $P < 0.001$ is the high significance P value.

RESULTS

Of the fifty EOT instances, twenty (40%) were malignant, five (10%) were borderline, & twenty (50%) were benign. Figure 1 illustrates that the majority, or 24 instances or 48%, were of serous tumors.

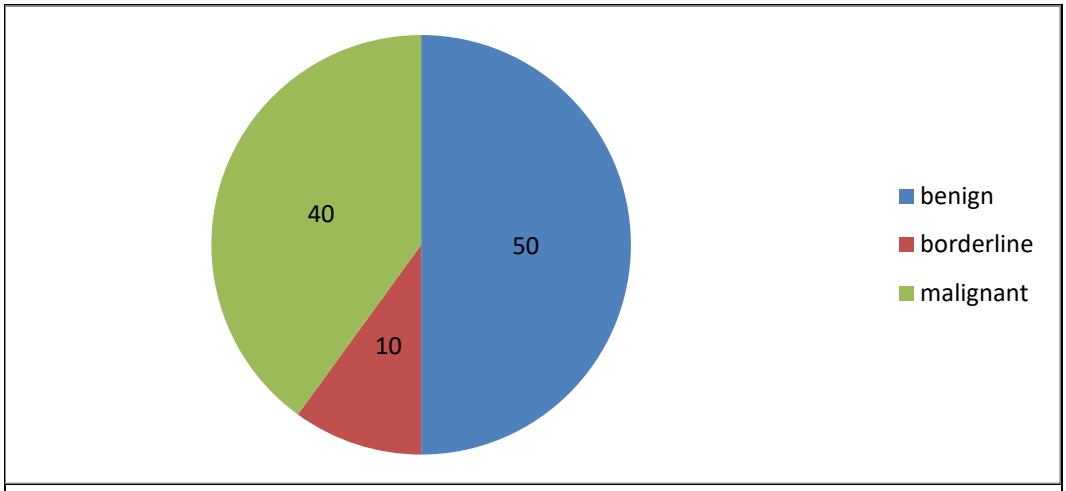

Figure 1: Distribution of tumors

The majority of instances, or 14 cases (or 70%), were serous malignancies, followed by mucinous tumors, or 4 cases (20%). Figure 2 illustrates 1 clear cell carcinoma (5%) & 2 endometrioid carcinoma patients (10%).

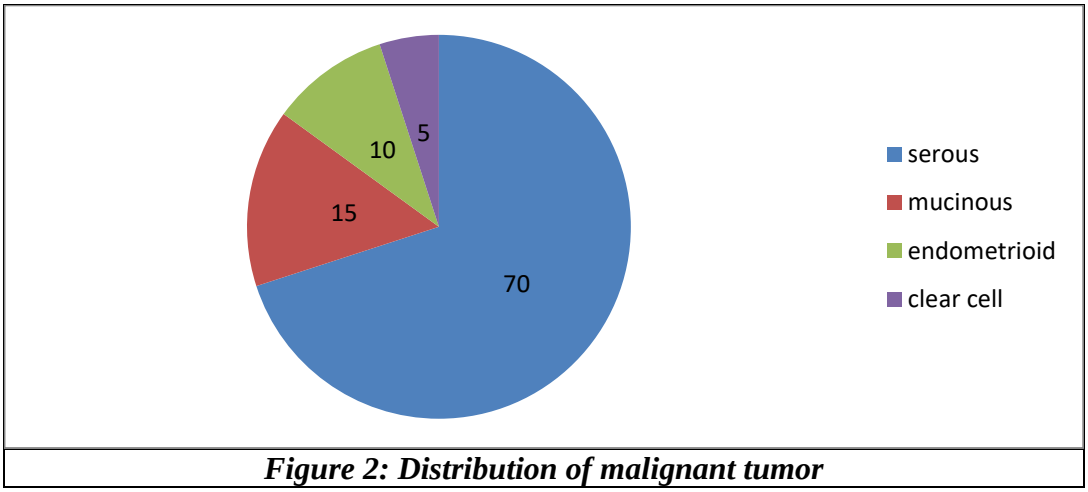

Figure 2: Distribution of malignant tumor

All EOTs that were benign or borderline were p53 negative & wild type. Of the 20 malignant tumors, 14 (or 70%) were serous malignancies & p53 positive. Every case of endometrioid

carcinoma, clear cell carcinoma, & mucinous carcinoma tested negative for p53. Figure 3 illustrates that one (7.14%) of the 14 serous carcinomas were p53 deficient.

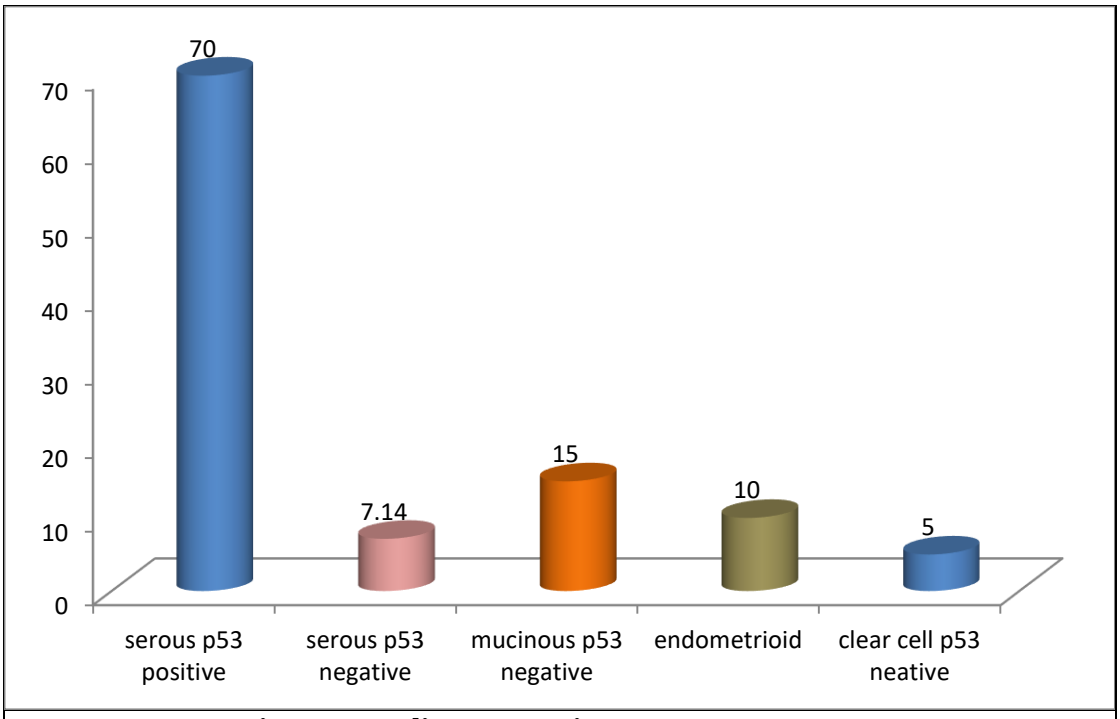

Figure 3: malignant ovarian tumors express p53.

20% of HGSC and LGSC displayed aberrant null staining, whereas 73.4% of them displayed widespread p53 staining. Table 1 displays a p53 negative example of the HGSC.

Grade	P53negative/wild type case	P53 positive –null pattern	P53 positive >70% positivity
LGSC	0	0	1 (9.1)
HGSC	1 (6.6)	3 (20)	10 (90.9)
Total	1 (6.6)	3 (20)	11 (73.4)

Table 1: The distribution of p53 positive in high- & low-grade serous cancers

In 35% of cases with stage 1 tumors, all instances with stage 2 tumors, & 86.7% of cases with stage 3 & above tumors, p53 was positive. P value of less than 0.001 indicated statistical significance for this outcome. As a result, as table 2 illustrates, p53 positivity increased with increasing stage.

Tumor stage	P 53 positivity	P value
Stage 1	35%	<0.001
Stage 2	63%	
Stage 3	86.7%	

Table 2: Association p53 staining with tumor stages

The relationship between p53 staining & independent factors such as ascites, serum CA 125 levels, & capsule rupture reveals that, out of the p53 positive serous tumor patients, 12 cases had ruptured capsules & 3 cases had intact capsules. Statistical significance was determined, yielding a P value of 0.002. Fifty percent of the ascites-related tumors were p53 positive & fifty percent were p53 negative. Ascites-related tumors had p53 positivity in 50% of cases, with 32% showing strong staining & 18% showing no staining at all. Ascites was present in every p53 positive instance. With a P value of less than 0.001, the link was determined to be statistically significant. Just 41 cases CA

125 levels were available for examination. For p53 positive tumors (aberrant >60% diffuse staining), the mean serum CA 125 was 410.05 u/ml; for p53 positive tumors (null staining), it was 1587.12 u/ml; & for p53 negative tumors, it was 56 u/ml. There was a P value of less than 0.001 & statistical significance was determined.

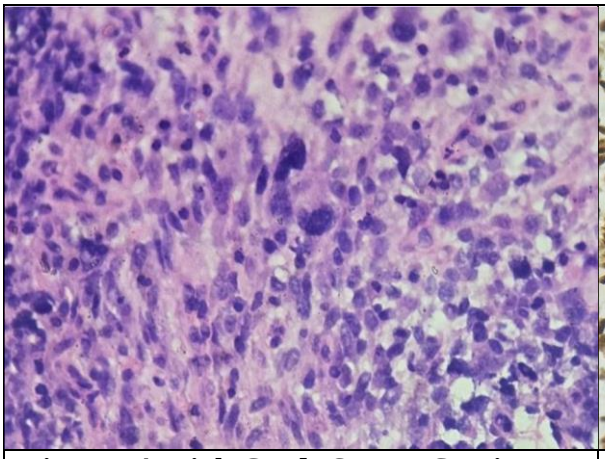

Figure 1A.High Grade Serous Carcinoma.

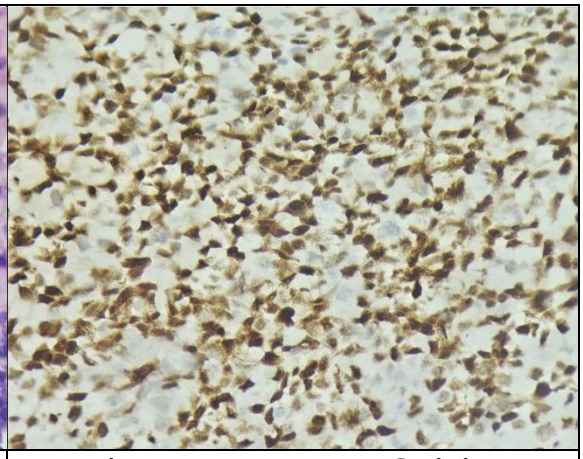

Figure 1B.Mutant p53 Staining

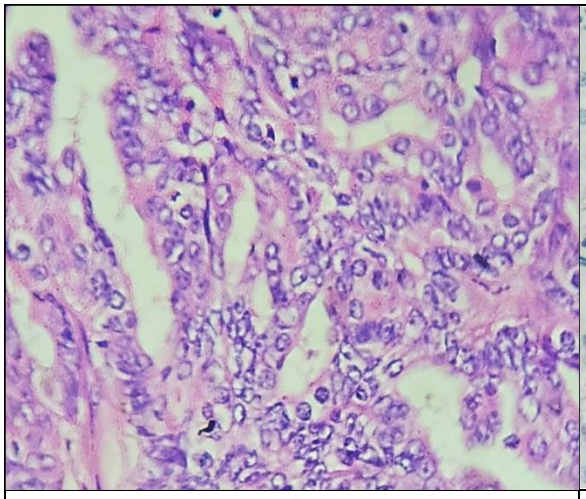

Figure 2A. Low Grade Serous Carcinoma

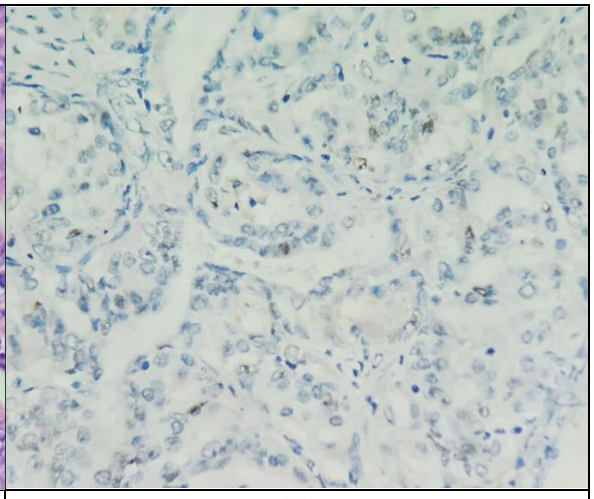

Figure 2B. p53 Wild type Staining x400

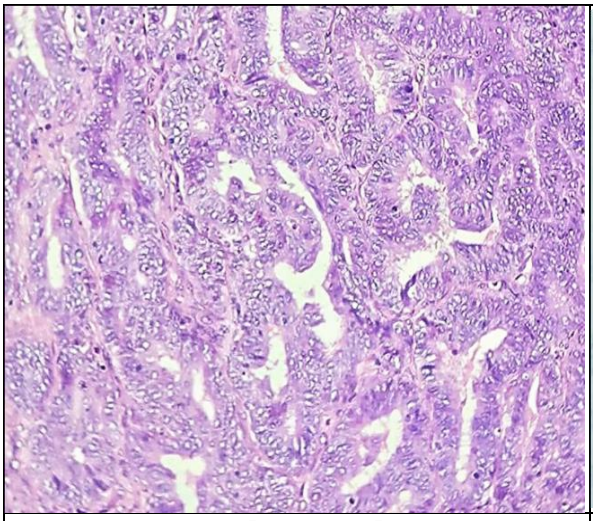

Figure 3A. Endometrioid Carcinoma,

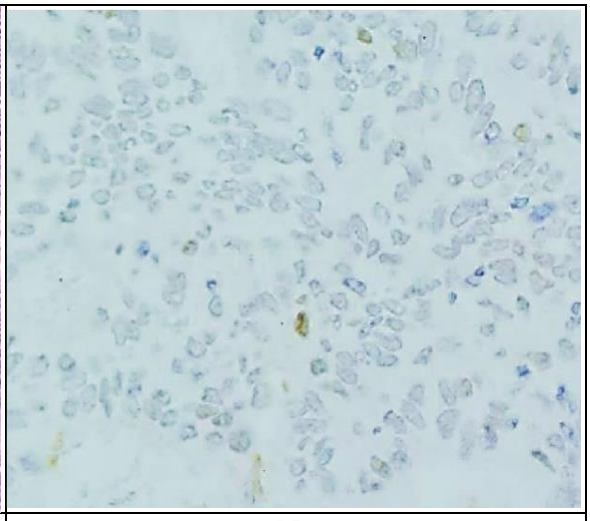

Figure 3B. p53 Wild Type Stainingx 400

DISCUSSION

The term "ovarian cancer" is broad & encompasses a variety of neoplasms that originate from the ovary; carcinomas account for 90% of ovarian cancer cases. Surface epithelial ovarian tumors (SEOTs) are categorised as clear cell, endometrioid, serous, mucous, transitional cell, & mixed epithelial neoplasms based on morphological characteristics.[11] The cause of SEOTs is not well understood, & despite the identification of a number of risk factors, nothing is known about how these factors directly contribute to the condition. One of the major risk factors for the development of ovarian cancer is somatic & germline genetic abnormalities.[12]

The majority of epithelial ovarian malignancies are associated with the loss of functional p53 protein, making p53 the most frequently changed gene in human cancers.[13] The relationship in the literature between p53 IHC positivity & histological subtype has proven contentious. Therefore, it is imperative to investigate p53 IHC in an Indian cohort while taking into account all technical parameters that may have an impact on the staining (such as the antibody clone, IHC technique, stain interpretation, etc.).[14]

Fifteen (75%) of the twenty malignant tumors were p53 positive, & they were all classified as serous malignancies. Every benign & borderline EOT tested negative for p53. This is consistent with other research that found that p53 was mutated or inactivated in roughly 50% (range 13.7–82%) of ovarian tumors that were invasive, infrequently in tumors that were borderline, & almost nonexistent in benign tumors or normal ovarian epithelium.[15-17]

All of the p53-positive tumors in our investigation were serous cancers. Clear cell tumors malignant mucinous tumors & endometrioid carcinomas were p53 negative. The results had a strong correlation with previous research.[17] Although Lassus et al. & Sylvia et al. showed somewhat lower positive rates, the results were similar to those of investigations by Havrileskyet al., Leitao et al., & Chiesa et al.[17-21] The method of counting p53 positive, the short sample size, & the variation in slide interpretation across observers could all be to blame for this.

20% of HGSC & LGSC displayed aberrant null staining, whereas 73.4% of them displayed widespread p53 staining. A HGSC instance had a p53 negative result. Eighty percent of serous ovarian carcinomas are p53 positive, according to research published in 2007 by Chiesa-Vottero et al. and in 2011 by Bilyk et al. The expression of the protein varies according to the degree of differentiation. High-grade tumors have p53 positivity throughout.[21, 22] A study by Anna Yemelyanova et al. suggests that TP53 wild-type should not be inferred from a complete lack of expression using p53 immunohistochemical scoring methods.[23] Furthermore, several of the earlier research that used nuclear sequencing did not improve the scoring system because they rated the staining intensity. Hence, in the event that p53 mutations are present in ovarian carcinomas, intensity grading & positive index calculation are irrelevant.[20]

Thirteen of the patients with p53-positive serous tumors had intact capsules, while two had burst capsules. After determining statistical significance, a P value of 0.002 was obtained. Research demonstrating p53 positive links the theory of serous tubal in situ cancer to capsule rupture in HGSC.[24,25] Ascites was present in every p53 positive instance. According to a study by Sylvia et al., 84.21% of tumors with ascites werep53 positive.[17] The mean blood CA 125 value for positive staining (diffuse & null positive) was substantially higher from the 41 available values than for benign tumors. This was consistent with the outcomes reported by Angelopoulou et al. & Sylvia et al.[17,26] According to a study by Hafner et al., p53 may be able to detect residual disease more accurately than CA 125.[27]

CONCLUSION

In clinical use, P53 IHC serves as a surrogate diagnostic for p53 gene mutation. Serous EOT is p53 positive, with increased expression in high-grade serous carcinomas & at advanced stages. It facilitates the distinction between high-grade & low-grade serous carcinomas, as well as between borderline & malignant tumors. Additionally, it can help distinguish between serous & endometrioid carcinomas. In ordinary practice, both diffuse staining & null positive patterns need to be analysed.

More research is necessary to determine the relationship between the null staining pattern & the worse prognosis. For p53 IHC, selecting an antibody clone that recognises both wild & mutant staining is therefore essential. It is necessary to comprehend p53 staining patterns in order to use it in conjunction with a panel of other antibodies for accurate categorization & additional study of morphologically puzzling EOT.

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