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Acquisition of a second mutation of the Tp53 alleles immediately precedes epithelial morphological transformation in ovarian tumorigenicity

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Abstract

Objective—Tp53 mutation is frequent and associates with malignant, high-grade ovarian cancer. However, the details about the progression of Tp53 mutation from heterozygous to homozygous, and association between genotypes and morphological transformation are not clear. We further investigated the expression and mutation of Tp53 and associated markers such as p21 and HDM2 in ovarian cancer.

Method—Areas of contiguous ovarian surface epithelia linking morphological normal monolayer to multilayer neoplastic cells were analyzed for the correlation of Tp53 pathway alteration in relation to morphological transformation, by immunostaining and sequencing of *Tp53* gene in cells from laser captured microdissection.

Results—Consistent with previous reports, Tp53 staining is positive in 78% of the tumors. The staining of p21 is positive in about 12%, and HMD2 is positive in only 1% of the tumors. In 9 out of 10 cases of p21-positive tumors, p53 is also positive. In the majority of cases of epithelial histological transitions, overexpression of Tp53 correlates with morphological transformation: Tp53 is negative in monolayered cells and positive in neoplastic lesions. Morphological transformation also closely correlates with cell proliferation as indicated by Ki-67 staining and loss of p21 expression. We detected heterozygous mutation of Tp53 in the monolayers adjacent to neoplastic cells.

Conclusions—p21 expression is an indicator of a wild type Tp53 and lack of p21 in the presence of Tp53 expression predicts an inactivated Tp53. Tp53 inactivation immediately precedes morphological transformation of the ovarian surface epithelium in most cases, and the histological transitional epithelia containing a heterozygous Tp53 mutation are thus pre-neoplastic lesions. We propose that the loss of a second allele of Tp53 leading to the loss of p21 expression, and subsequent

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Appendix A. Supplementary data: Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygyno.2009.03.023.

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cell proliferation, compose a sequence of events that lead to morphological transformation and instigation of ovarian epithelial tumor development.

Keywords

Ovarian cancer; Epithelium; Pre-malignant lesion; Tp53; Heterozygous; p21; HDM2/MDM2

Introduction

Epithelial ovarian cancer is generally believed to rise from the surface epithelium covering the ovarian surface [1–3]. An alternative argument is that ovarian carcinomas originate from mullerian duct derivatives, such as rete ovarii [4]. This idea is supported by recent observations that epithelial cells lining the fallopian tubes may be the source of malignant cells and some ovarian cancer may originated from fallopian tubes [5,6]. Nevertheless, early lesions can be observed in the inclusions cysts of ovaries from surgeries of ovarian tumors [7–9]. Lesions were observed to contain morphologically normal, monolayer epithelia of ovarian surface or inclusion cysts linked contiguously with multilayer malignant cells [9,10]. These observations indicate that at the least some fractions of ovarian carcinomas are derived from ovarian surface or inclusion cyst epithelial cells. These epithelia transitions linking normal and malignant cells are also valuable materials to analyze biomarkers and the steps of progression from normal to malignant cells [9,10].

High-grade ovarian cancer often associates with mutation of the tumor suppressor Tp53 [11–15]. Tp53 mutation is found in early, pre-cancer lesions, suggesting that mutation of Tp53 is an early event [16,17]. Presumably, first a base change/mutation occurs at one of the two Tp53 loci in a precursor cell. At a secondary step, the wild type copy of the *Tp53* gene is lost or replaced by the mutant allele, resulting in homozygous mutation found in malignant cells. These details have not yet been observed in ovarian tumor tissues [6,15].

In addition to mutations, Tp53 inactivation can occur because of an increased expression of HDM2, a Tp53 binding protein that mediates Tp53 degradation [18,19]. Tp53 produces its tumor suppressor function by both transcription-dependent and independent pathways [20–22]. Many Tp53 transcriptional targets have been identified, and cell cycle inhibitor p21 is a well-known Tp53 induced gene [23,24]. Upon activation, Tp53 induces p21 expression, and p21 subsequently mediates cell cycle arrest [23,24].

Although the basic and cellular mechanisms of the Tp53 pathway have been extensively studied, the prevalence of the molecular details in ovarian cancer is not yet thoroughly examined and documented in tumor tissues. In ovarian cancer cells, regulation of p21 by Tp53 was investigated and found prevalent [25]. In this study, we investigated the expression of Tp53, p21, and HMD2 and analyzed Tp53 mutation in ovarian cancer to determine the correlation with morphological features. The study enables us to identify morphological normal ovarian surface epithelial layer immediately adjacent to malignant cells as possible pre-neoplastic lesion. The cells located at this lesion exhibit a heterozygous mutant Tp53. The results enabled us to suggest a model and the sequence of events in ovarian cancer development.

Materials and methods

Ovarian tissues and tumor specimens

Epithelial ovarian tumor specimens were obtained from patients who underwent surgical resection at Fox Chase Cancer Center. The ten archived ovaries serving as normal controls were from patients with benign uterine lesions. Three ovarian tumor tissue microarrays (TMAs) containing a total of 170 cores from 85 individual ovarian tumor cases were provided by the

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Tumor Bank Facility of Fox Chase Cancer Center. We also used a collection of 38 archived ovarian tumor tissue blocks collected over the years and are not included in the TMAs. Some of these cases contain contiguous epithelia linking benign and neoplastic lesions. All these samples were obtained with informed patient consent for research and the study was approved by the Institutional Review Board of Fox Chase Cancer Center.

In the 3 ovarian cancer TMAs, the 85 ovarian tumors include 62 serous cystadenocarcinomas, 3 endometrioid adenocarcinomas, 3 mucinous cystadenocarcinomas, 3 clear-cell carcinomas, 2 mixed mesodermal tumors, 2 metastatic ovarian carcinomas, 5 undifferentiated adenocarcinomas, and 4 borderline/low malignant potential (LMP) tumors (among which two were serous and two were mucinous). One case of benign serous cystadenoma was also included. The majority of the serous carcinoma cases were high-grade (Grade 3) and the 3 mucinous carcinoma cases were low grade (Grade 1). Of the 80 malignant cases, 69 were stage III/IV, 9 were stage I/II and the stage information of the remaining 2 cases was unavailable. All the information is presented in Supplemental Table 1.

Of the 38 whole sections of ovarian tumors, 28 were malignant including 18 serous adenocarcinomas, 5 mucinous adenocarcinomas, 3 endometrioid adenocarcinomas, and 3 clear-cell carcinomas; 9 were LMP tumors including 6 serous and 2 mucinous subtypes; the remaining one case was a benign fibrous cystadenoma. Areas of contiguous epithelia with histological transition from benign to malignant epithelium were identified in 10 out of these 38 ovarian tumors.

Generally, fresh tissue specimens of approximately 1-cm cubes were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All tumors were histologically classified according to the World Heath Organization (WHO) classification and the surgical stages were determined according to the classification of International Federation of Gynecology and Obstetrics (FIGO).

Immunohistochemistry

Consecutive 5-μm sections were cut and mounted on sialinized slides (Superfrost Plus, Fisher Scientific Inc.). Sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. The sections were subjected to antigen retrieval by boiling in 0.01 M sodium citrate buffer (pH 6.0) in a steamer for 20 min and allowed to cool in the buffer for 20 min. Immunostaining was performed using the mouse DAKO LSAB System and the Peroxidase (DAB) Kit (Dako Corporation, Carpinteria, CA, USA). Endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide in absolute methanol for 15 min and washed once with distilled water and three times with phosphate-buffered saline (PBS). The antibodies used include: rabbit anti-Ki-67 (Mib-1) (1:200 dilution, Novocastra, Newcastle, United Kingdom); mouse anti-p53 (1:300 dilution, clone Do-7, Dako Corporation, Carpinteria, CA, USA), which recognizes the epitope located between the N-terminal amino acids 1 and 45 and possibly between amino acids 37 and 45 of the human p53 protein; mouse anti-$p21^{WAF1/CIP1}$ (1: 50 dilution, clone SX118, Dako Corporation, Carpinteria, CA, USA), which recognizes the epitope located to the last 20 amino acids of $p21^{WAF1/CIP1}$; mouse anti-MDM2/HMD2 (1:100 dilution, clone IF-2, Calbiochem, EMD Biosciences, San Diego, CA), which recognizes the epitope within amino acid residues 26–169 of human, HDM-2 protein, and rabbit anti-calretinin (1:300 dilution, Zymed, San Francisco, CA). After blocking the nonspecific binding with blocking reagent (Protein Block Serum-Free, DAKO) for 30 min, the sections were incubated overnight with antibodies at 4 °C in a humidified chamber. After rinsing 3 times in PBS, the sections were incubated for 30 min with Link Antibody (biotin-labeled secondary antibody, LSAB Kit, DAKO), rinsed, immersed in 1× PBS 3× 5 min, and incubated with horseradish peroxidase-labeled streptavidin–biotin complex for 30 min. The sections were then visualized with the chromagen 3, 3′-diaminobenzidine (DAB) containing

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0.05% hydrogen peroxide for 5 min and then lightly counterstained with Mayer's hematoxylin. The primary antibody was replaced with mouse non-immunized IgG as a negative control and no immunohistochemical staining was observed.

Evaluation of the staining intensity and positive cell numbers of Tp53, $p21^{WAF1/CIP1}$, and HDM2 was performed independently by two pathologists in a blinded manner. Tumors with an intermediate or strong nuclear p53 immunoreaction in more than 50% of the tumor cells were scored as p53 positive. Tumors with an intermediate or stronger nuclear HDM-2 immunoreaction in more than 20% of the tumor cells were graded as HDM-2 positive.

Laser capture microdissection (LCM), DNA extraction and Tp53 mutation analyses

Tissue sections of 5-μm thickness were obtained from formalin-fixed and paraffin-embedded blocks of ovarian cancer cases and placed on Superfrost non-plus slides (Fisher Scientific) for LCM. One section was stained with H&E for histologic classification of lesions, whereas the remaining sections were deparaffinized with xylenes and rehydrated with a graded series of ethanol–water washes and stained using the HistoGene LCM staining kit (Arcturus Engineering, Mountain View, CA). Pure cell populations of different lesions (normal ovarian surface epithelial cells, cancer cells, morphological normal epithelial cells adjacent to cancer, inclusion cysts coexisting with cancer, and dysplastic lesions) were microdissected and collected using the PixCell II LCM System (Arcturus).

After LCM, DNA from the 500 to 1000 cells collected by LCM was extracted using the PicoPure DNA extraction kit (Arcturus). PCR primers flanking exons 4–11 of the Tp53 were designed according to the genomic sequence in GenBank accession number U94788 [24]. Amplified PCR products from individual TPp3 exons and flanking exon–intron junctions were purified with QIAquick PCR purification kit (Qiagen, Valencia, CA) and submitted to sequencing using the BigDye Terminator Cycle Sequencing Kit (ABI, Foster City, CA). The sequence analysis was performed using the ABI PRISM 3100 automated sequencer (ABI, Foster City, CA).

Results

Expression of Tp53, p21, and HDM2 in ovarian tumors

Expression and mutations of Tp53 have been investigated by many laboratories and the prevalence has been well established for ovarian cancer [11–17]. Our goal was to investigate in more details of the alteration of the Tp53 pathway and its impact on epithelial cell organization, and we investigated the expression of Tp53, p21, and HDM2 in ovarian cancer tumor tissue microarrays and selective tissue sections. The cell cycle inhibitor $p21^{WAF1/CIP1}$ is induced by Tp53 and may be used as an indicator of the transcriptional activity of Tp53 [23–25]; HDM2 is a negative regulator of Tp53 protein expression and the expression of HDM2 may be a mechanism for the suppression of the Tp53 activity [18,19].

The staining protocols were first refined and both positive and negative controls were established in our laboratory. Adjacent sections were stained for Tp53, p21, and HDM2. After excluding defective cores, we obtained informative results from a total of 83 cases from the initial 170 cores derived from 85 individual ovarian tumor cases (Supplemental Table 1). Tp53 is positive in 75.9% and p21 is positive in 12.0% of the cases. Of these 83 cases, only one case, a Grade 1–2 ovarian adenocarcinoma, showed HDM2 positive staining (1.2%). We examined the relationship between the expression of Tp53 and p21 (Table 1). In 9 out of 10 cases in which p21 is positive, Tp53 is also positive. In only one case of p21 positive, Tp53 negative tumor was observed. In the majority (56/65) of Tp53 positive tumors, p21 is negative. Examples of the staining are shown in Fig. 1 for a Tp53-positive, p21-negative (Fig. 1A), and a Tp53-

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positive, p21-positive (Fig. 1B) ovarian carcinoma. The Tp53-positive, p21-negative cases indicate the inability of the Tp53 to induce p21, suggesting the presence of Tp53 mutation in these tumors. These correlations are consistent with a regulation of p21 by Tp53, and Tp53 mutations that abolish the transcriptional activation of the protein are prevalent in ovarian cancer.

There are 17 cases (20.5%) of ovarian carcinomas in which both Tp53 and p21 are negative in immunostaining (Table 1). It is possible that the Tp53 pathway is not activated in these tumors. However, these p53 and p21 negative tumors may still have p53 inactivation/mutation that precluded expression of the antigens recognized by the p53 antibody used. Expression of p21 may be also induced by a Tp53-independent mechanism as suggested by one case of Tp53 negative but p21-positive tumor (Table 1).

Further examination of Tp53 and p21 staining indicates that though positive p21 staining often correlates with positivity for p53 staining, the percentage of cells with a positive p21 staining varies (Supplemental Table 1). For example, in an ovarian tumor where 80% of the tumor cells stained positive for Tp53, only 40% of the cells were found to be p21-positive in an adjacent section. In general, the percentage of cells positive for p21 is significantly less than that positive for Tp53.

Contiguous epithelia linking benign and neoplastic cells

We reviewed our collection of ovarian cancer samples to select interesting cases that contained contiguous epithelia linking morphological normal monolayer with multilayered neoplastic cells. These types of transitional epithelia may be informative for genetic and epigenetic changes that can determine or be associated with transition from monolayer epithelial cells to neoplastic cells of the transformed epithelia. Presumably, the genetic and epigenetic states of the cells around the histological transition are similar since they are derived from a relatively recent precursor cell, and the differences in gene expression and genetic mutation(s) may be critical in the formation of the morphological changes.

Over the years, we collected both malignant and benign ovarian tumors that contain both normal and adjacent tumor areas that may be informative for insight in changes in markers associated with epithelial transformation. Of the 38 cases of ovarian carcinomas collected and studied by our lab, 28 were malignant including 18 serous adenocarcinomas, 5 mucinous adenocarcinomas, 3 endometrioid adenocarcinomas, and 3 clear-cell carcinomas; 9 LMP tumors including 6 serous and 2 mucinous subtypes; the remaining one case was benign fibrous cystadenoma. Areas of contiguous epithelia with histological transition from benign to malignant epithelium were identified in 10 out of these 38 ovarian tumors. Although most of the cases we investigated are serous carcinomas, such histological transitions were found in all histological subtypes. As shown in Fig. 2 for an example of a serous ovarian carcinoma, the lesion (Fig. 2A, indicated by a “*”) contains morphological normal monolayer epithelial cells (Fig. 2B, arrowhead), which are contiguously linked with neoplastic, multilayer epithelial cells (Fig. 2B, arrows) with some gradual transition from normal to malignant. The monolayer epithelial cells appear non-neoplastic, showing no nuclear atypia or mitotic figures. Herein, we refer to these monolayer epithelia linking contiguously with multilayered overt neoplastic cells as histological transition areas, or transition epithelia. We reason such epithelial histological transitions may be informative for studying of genetic or epigenetic changes associated with neoplastic transformation.

Epithelial proliferation correlates with transformation

In all the histological transitions, a striking correlation we found is the association of cell proliferation, as indicated by Ki-67 staining, with morphological transformation from

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monolayer to multilayers (Fig. 3A). The epithelial cells residing in the monolayer regions show no mitotic figures and lack nuclear atypia. Consistently, few cells within the monolayer of ovarian surface epithelium (arrowhead, Fig. 3A), including the epithelia of the histological transition, are positive for ki67. However, a high percentage of the neoplastic cells immediately adjacent to the histological transition are Ki-67-positive (arrow, Fig. 3A). Additionally, the monolayer epithelia that are negative for ki67 are positive for calretinin (Fig. 3B), and the calretinin negative cancer cells show obvious nuclear atypia. Calretinin was determined to be a marker for ovarian surface epithelial cells but its expression is lost upon neoplastic transformation [26]. Thus, histological transition from monolayer to neoplastic cells associates with a dysregulation of cell cycle progression and proliferation. We reasoned that cell growth contributes to the formation of multilayer epithelial neoplastic cells.

Correlation of Tp53 and p21 expression with morphological transformation of ovarian surface epithelia

We further examined the epithelial histological transitions by staining Tp53 and p21. In most of the cases, the staining of Tp53 and p21 is complementary: Tp53 is negative in the monolayer region of the epithelial histological transition, where p21 is positive; Tp53 is positive in the neoplastic cells, in which p21 is negative, as shown in an example (Fig. 3C). In this carcinoma, a Tp53 positive staining suggests the presence of Tp53 mutation, and the mutant protein lacks activity to induce p21 expression.

Using larger captured microdissection (LCM), we dissected ovarian surface epithelial, hyperplastic epithelial, and tumor cells (Fig. 4A) for analysis of Tp53 mutations by PCR-sequencing. Following gaining of experience in some failed initial cases, we were able to obtain sequence information for all 8 exons (exons 4 to 11) and to identify Tp53 mutation from LCM dissected materials including ovarian surface epithelia, hyperplastic lesions, and invasive carcinoma cells from a single slide (Fig. 4). In all 4 cases of tumors in which Tp53 is positive and p21 is negative, Tp53 point mutations were found, as shown by an example (Fig. 4B). We did not find mutations in 4 cases in which p21 is positive. Thus, the results suggest that p21 expression is a very good indicator of Tp53 transcriptional activity, and positive staining for Tp53 with negative p21 suggests a mutant Tp53, and positive staining for Tp53 with positive p21 suggests a wild type Tp53.

Progressive mutation of Tp53 in epithelial transformation

In 2 cases, we were able to obtain sufficient cells from the epithelial monolayer at the site of histological transition to produce informative mutation analysis of Tp53. In the first case, no Tp53 mutation was identified in the dissected epithelial cells of the monolayer, though a point mutation (base #14487, G to T, GenBank accession U94788) was identified in the tumor, resulting in a CGT to CTT, and Arg to Leu conversion at exon 8. In the second case, following LCM and PCR-sequencing of exons 4 to 11 of Tp53 from the dissected materials (Fig. 5A), we identified a heterozygous Tp53 mutation in the transitional epithelial cells and a homozygous mutation (GTG:Val to ATG:Met) in the tumor cells (Fig. 5B). The base change occurs at exon 6 of the *p53* gene at base 13406 from “G” to “A” (GenBank accession U94788), resulting in a conversion of Val to Met in the mutant protein. We estimated that the monolayer epithelial cells dissected contained less than 10% of stromal cells, and thus the heterozygous Tp53 mutation signal was not due to an artifact of the potential contamination of stromal cells in the dissected sample. A homozygous Tp53 mutation of the same base was identified in the adjacent tumor cells (Fig. 5). Also, materials scraped from the slides that contain largely stromal cells show a wild type Tp53 sequence (Fig. 5B), suggesting that the mutation is associated with tumorigenesis.

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Thus, we have found that the monolayer epithelial cells at the histological transition exhibited a heterozygous state of Tp53 mutation, which became homozygous state in the adjacent tumor cells. We reason that these morphological normal epithelial cells in the histological transitions can be considered as precursor lesions of ovarian cancer.

Discussion

Tp53 mutation is prevalent in high-grade ovarian cancer [11–17]. When mutated, the Tp53 protein accumulates in cells [14,27], but lacks the ability to induce cell growth arrest or apoptosis [20–22]. The positive staining of Tp53 often indicates presence of a Tp53 mutation [12,14,28]. Here, we show that the staining of p21 may be an excellent indicator for the wild type status of Tp53 despite its positive staining. Likely, p21 expression is an indicator for wild type Tp53 and lack of p21 in the presence of Tp53 expression is an indicator of Tp53 mutation/inactivation. We also conclude that in a small percentage of ovarian cancer, Tp53 pathway is inactivated because of an increased HDM2.

Mutation of Tp53 was found in ovarian inclusion cysts and microscopic carcinomas, suggesting that Tp53 mutation is an early event in ovarian cancer initiation [16,17]. We further identified heterozygous Tp53 mutation at the monolayer region of epithelial histological transition. Thus, these epithelial cells are precursor lesions, and are posed to become Tp53 homozygous mutant neoplastic cells.

The transition of the contiguous epithelium from monolayer to multilayered neoplastic cells closely correlates with Tp53 homozygous mutation, loss of p21 expression, and gain of cell proliferation (indicated by Ki-67 straining) [29]. Thus, a model can be postulated that monolayer ovarian surface epithelial cells may first become mutated in one copy of the *Tp53* gene. These Tp53 heterozygous mutant cells do not express a high level of the Tp53 protein (as shown by a weak or no immunostaining) but express a high level of p21, which suppresses cell proliferation. Upon gaining homozygous Tp53 mutation by either loss or replacement of the wild type copy of Tp53 in the cells, the inactive Tp53 mutant protein accumulates, p21 induction is lost, and the removal of the cell cycle inhibitor p21 allows cell proliferation and morphological transformation. Therefore, homozygous inactivation of Tp53 releases cell growth suppression and leads to morphological transformation of ovarian surface epithelium.

However, the loss of p53 or p21 is likely not sufficient to induce cell proliferation or epithelial transformation, since knockout either of the genes in mice does not produce significant phenotype in ovaries, and is not sufficient for ovarian epithelial transformation. We have identified other events that associate with epithelial transformation, such as the loss of Dab2 and basement membrane [9]. Knocking out mouse studies indicate a role for Dab2 in epithelial apical polarity and epithelial cell organization [30]. We propose that morphological transformation (including loss of epithelial cell polarity and basement attachment) that allows epithelial cells to escape from the constraint of tissue organization, and genetic/epigenetic alteration(s) (such as Tp53 mutation) that permits cell proliferation and survival, are two independent but necessary components in ovarian surface epithelial tumor instigation and establishment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

LCM
laser captured microdissection

LMP
borderline/low malignant potential

TMA
tissue microarray

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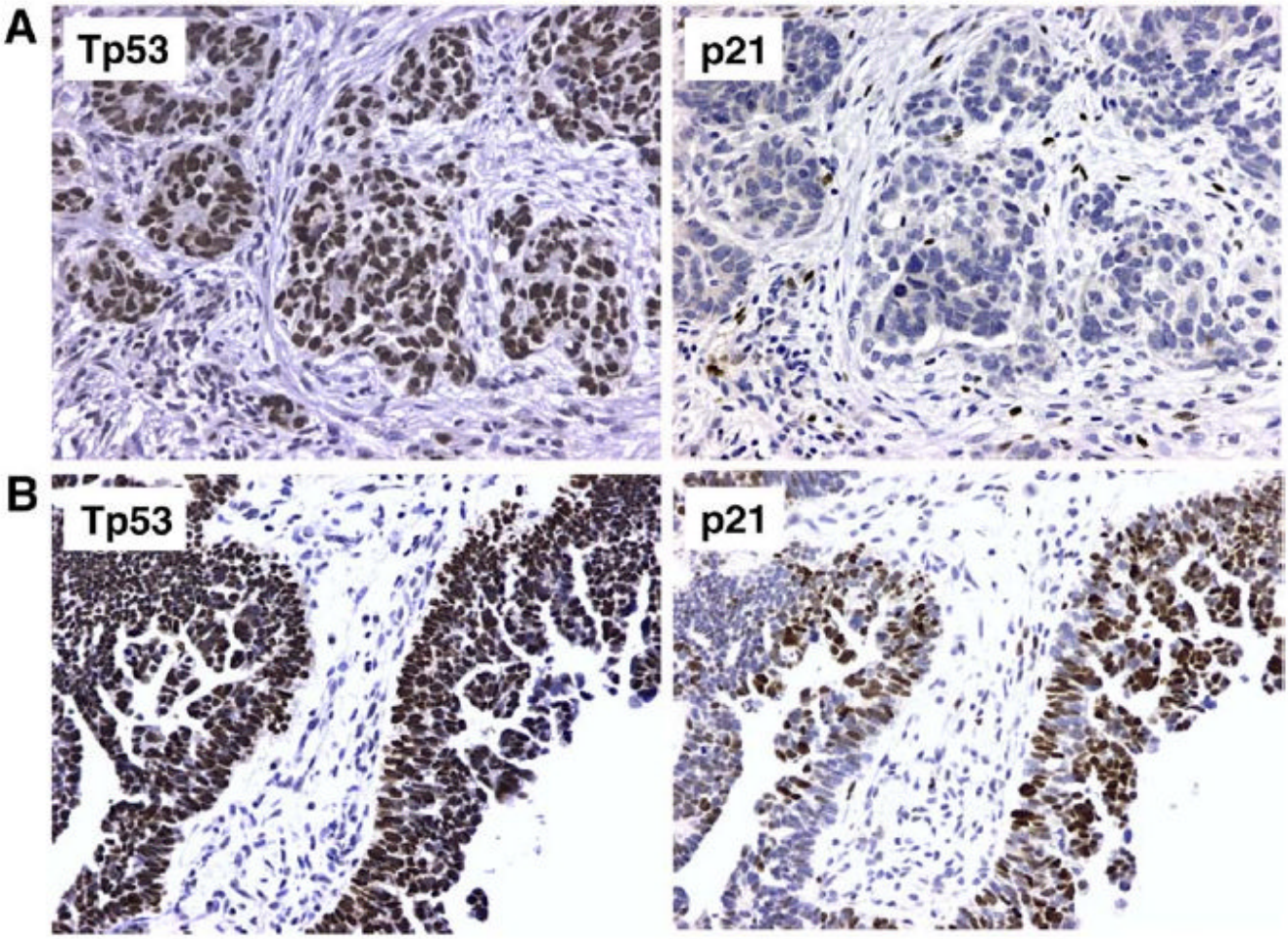

Fig. 1.

Immunostaining of ovarian cancer for Tp53 and p21. Adjacent sections of 83 ovarian carcinomas were stained with Tp53 and p21. Representative examples are shown. (A) An ovarian carcinoma stained positive for Tp53 but negative for p21. (B) An ovarian carcinoma stained positive for both Tp53 and p21.

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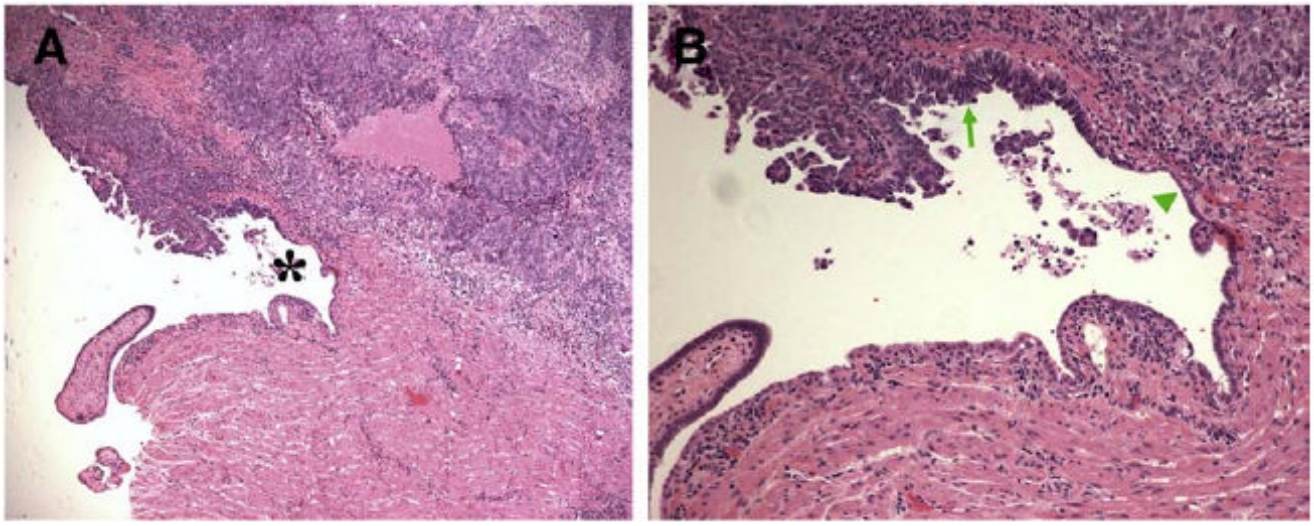

Fig. 2.

Examples of epithelial histological transitions. Reviewing a large collection of ovarian tumors identified cases containing epithelial histological transitions from benign to neoplastic. (A) An example shows an ovarian serous carcinoma containing an epithelial histological transition from benign to neoplastic. The region indicated by an "*" is shown in a higher magnification in (B). The lesion contains morphological normal monolayer epithelial cells (arrowhead), which are contiguously linked with neoplastic, multilayer epithelial cells (arrow) with some gradual transition from normal to malignant. We reason such epithelial histological transitions may be informative for studying genetic or epigenetic changes associated with neoplastic transformation.

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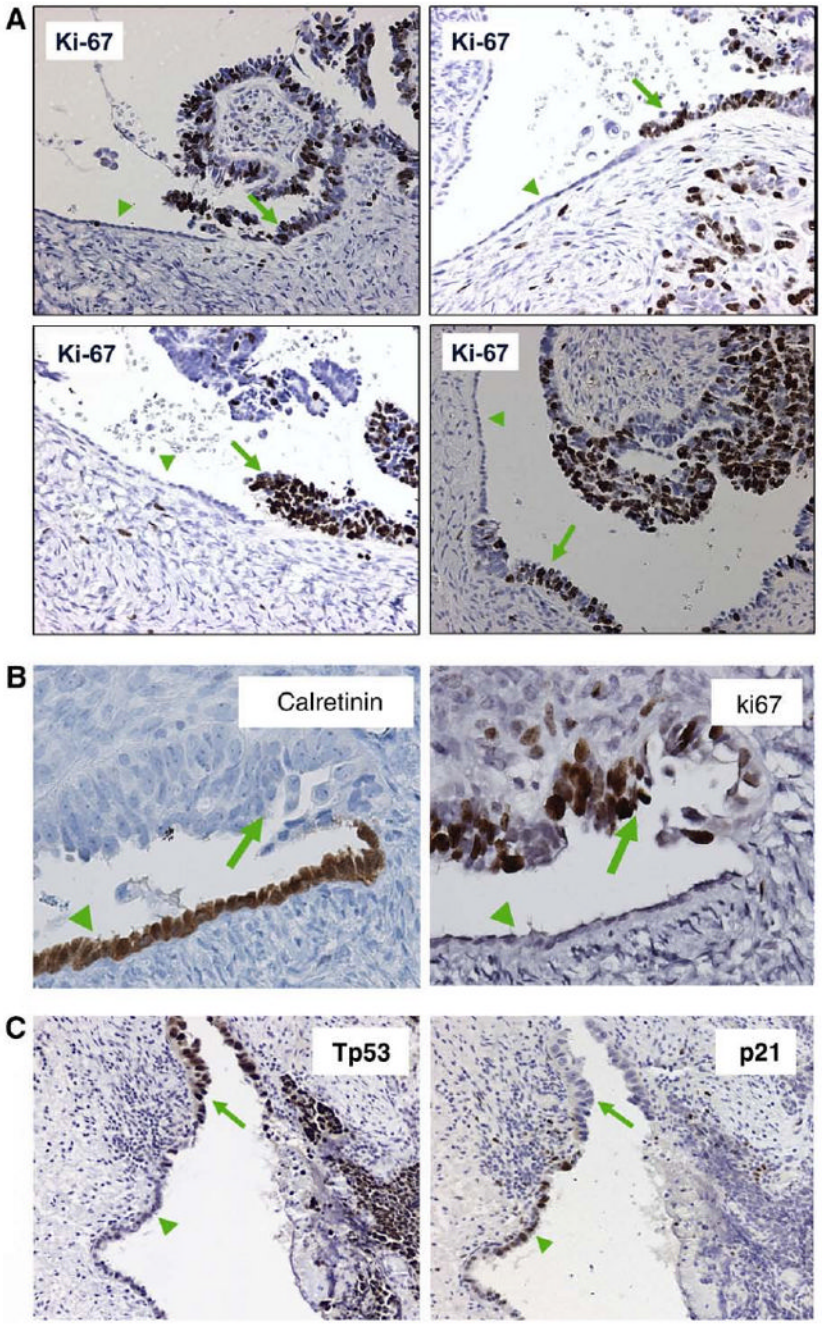

Fig. 3.

Analysis of histological transitions. (A) Four examples of Ki-67 staining as a marker for cell proliferation are presented. A striking correlation between positive Ki-67 staining and morphological transformation from monolayer (arrowhead) to multilayers (arrow) was observed, as shown in epithelial transition lesions found in all ovarian carcinomas. (B) An example shows that monolayer epithelial cells are positive for calretinin staining while the overt cancer cells are negative. (C) Staining patterns of Tp53 and p21 at the histological transitions. We examined the epithelial histological transitions by staining Tp53 and p21. In most of the cases, the staining of Tp53 and p21 is complementary: Tp53 is negative in the

monolayer region (arrowhead) of the epithelial histological transition, where p21 is positive; p53 is positive in the neoplastic cells (arrow), in which p21 is negative, as shown in an example.

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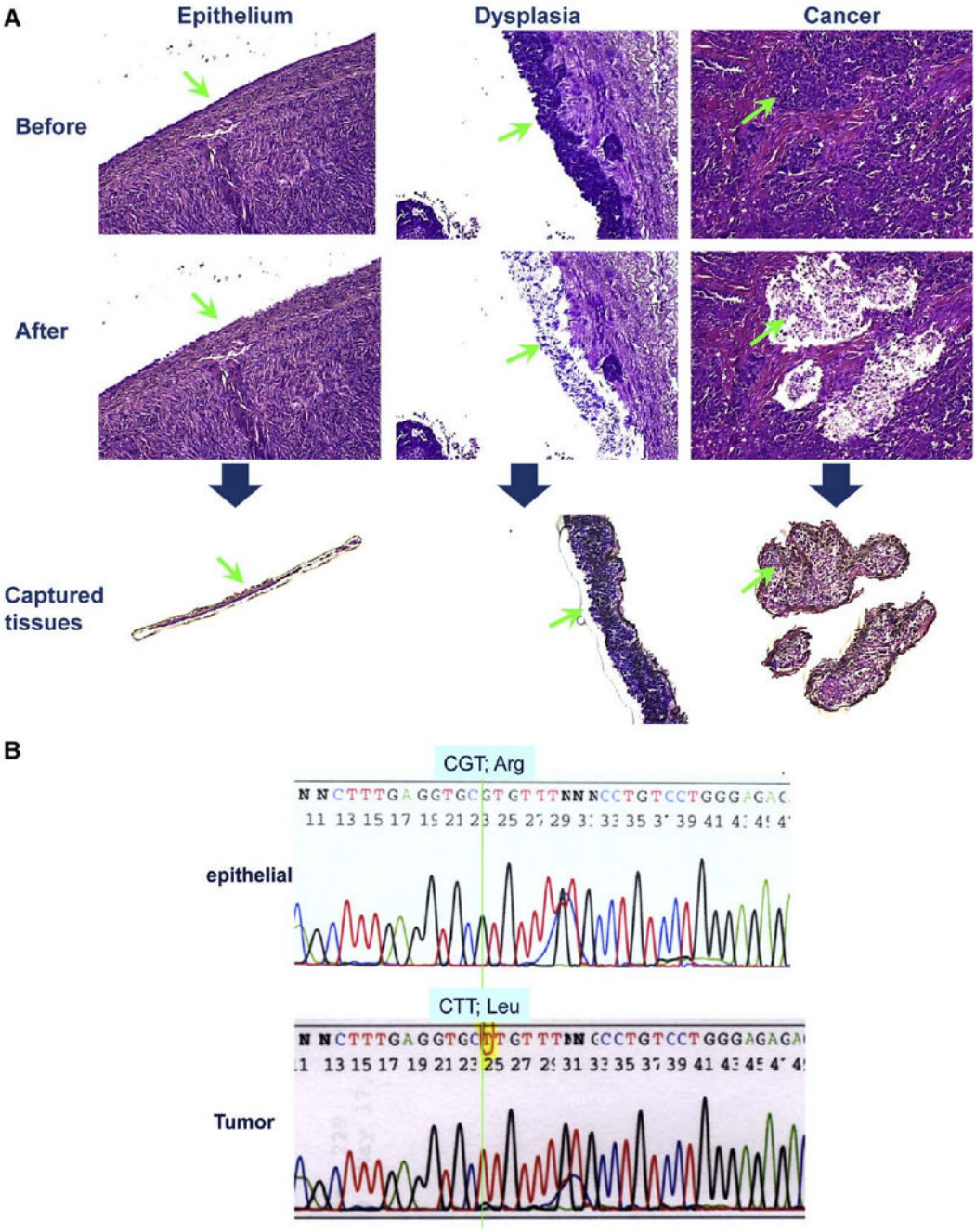

Fig. 4.

Examples showing laser capture microdissection (LCM) to dissect cells for mutation analysis. (A) Using LCM, normal ovarian surface epithelial, hyperplastic epithelial, and tumor cells were dissected for PCR and sequencing analysis. H&E stainings of slides before and after dissection are shown. Areas captured are indicated by arrows. (B) An example of sequencing to identify a Tp53 mutation from “CGT” found in normal epithelial cells to “CTT” in cancer cells.

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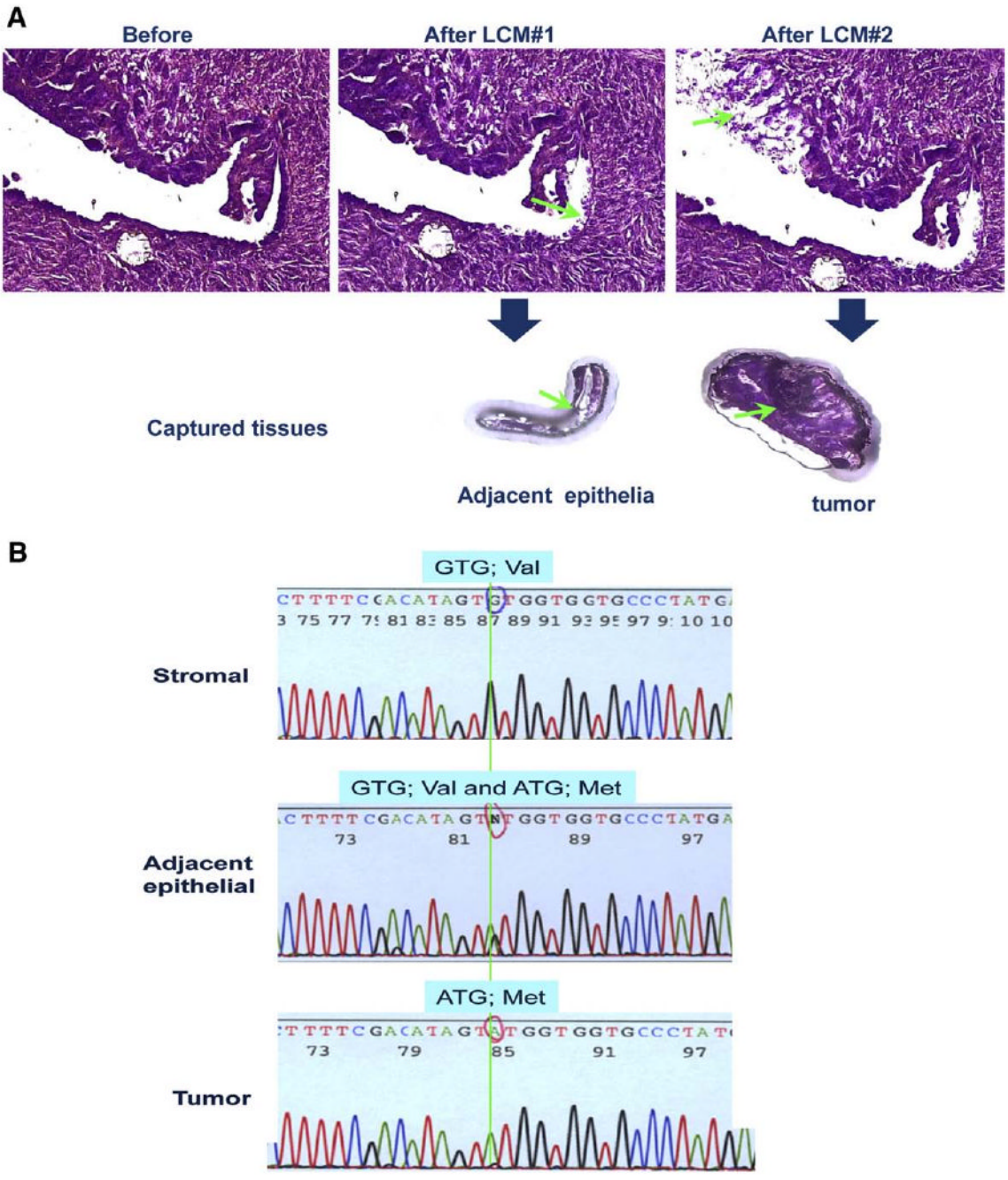

Fig. 5.

Laser capture microdissection (LCM) and mutation analysis of cells at the histological transitions. (A) Ovarian carcinomas containing histological transitions were subjected to LCM to isolate surface epithelial cells and adjacent tumor cells on a contiguous epithelium. Areas dissected are indicated by arrows. (B) The collected cells were used for PCR and sequencing to identify Tp53 mutations. Sequencing data are shown, identifying a wild type Tp53 “GTG” in stroma, heterozygous mutation “GTG and ATG” in morphological normal epithelial cells, and homozygous mutation “ATG” in tumor cells.

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Table 1

Correlation between immunostaining of Tp53 and p21.

Staining	Number	Positive rate (%)
Tp53+; p21−	56	67.5
Tp53+; p21+	9	10.8
Tp53−; p21+	1	1.2
Tp53−; p21−	17	20.5

Distribution of 83 ovarian tumors based on the combination of staining for Tp53 and p21.