OVARIAN LOW-GRADE AND HIGH-GRADE SEROUS CARCINOMA: Pathogenesis, Clinicopathologic and Molecular Biologic Features, and Diagnostic Problems

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Abstract

Ovarian serous carcinomas have been graded using various systems. Recently, a 2-tier system in which tumors are subdivided into low-grade and high-grade has been proposed. This approach is simplistic, reproducible, and based on biologic evidence indicating that both tumors develop via different pathways. Low-grade serous carcinomas exhibit low-grade nuclei with infrequent mitotic figures. They evolve from adenofibromas or borderline tumors, have frequent mutations of the *KRAS*, *BRAF*, or *ERBB2* genes, and lack *TP53* mutations (Type I pathway). The progression to invasive carcinoma is a slow step-wise process. Low-grade tumors are indolent and have better outcome than high-grade tumors. In contrast, high-grade serous carcinomas have high-grade nuclei and numerous mitotic figures. Identification of a precursor lesion in the ovary has been elusive and therefore the origin of ovarian carcinoma has been described as *de novo*. More recently, studies have suggested that a proportion appear to originate from intraepithelial carcinoma in the fallopian tube. The development of these tumors is rapid (Type II pathway). The vast majority are characterized by *TP53* mutations and lack mutations of *KRAS*, *BRAF*, or *ERBB2*. Although both types of serous carcinomas evolve along different pathways, rare high-grade serous carcinomas seem to arise through the Type I pathway. Immunohistochemical stains for p53, p16, and Ki-67 for distinction of low- from high-grade tumors are of limited value but can be helpful in selected instances. This review provides an update on the pathogenesis and clinicopathologic features of these two types of serous carcinomas and addresses some of the diagnostic problems that are encountered in routine practice.

Keywords

Ovary; serous carcinoma; grading; low-grade; high-grade

INTRODUCTION

Ovarian serous carcinoma has traditionally been graded as well-, moderately, and poorly differentiated, suggesting that it is a homogeneous disease from the standpoint of pathogenesis. Multiple different grading systems have been used with variable results (reviewed in Silverberg[1]), including the FIGO system based on percentage of solid architecture the WHO system based on an impression of architecture and cytologic features, the Gynecologic Oncology Group (GOG) system based on histologic type, a system based on a combination of mitotic index and volume percentage of epithelium, a system based on presence/amount of

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hyperchromatic giant nuclei and solid or cribriform architecture, and a grading index based on a mean of the individual scores for architectural pattern, nuclear pleomorphism, nucleoli, nuclear-to-cytoplasmic ratio, mitotic index, pattern of invasion, capsule penetration, and vascular invasion. A 3-tier grading scheme that has gained much attention over the past several years is the universal grading system, which is also referred to as the Silverberg grade.[2] In this system, points are assigned for each of 3 components: architecture (glandular, papillary, or solid), degree of nuclear atypia, and mitotic index. The points for each component are added, resulting in a total score which determines the grade, analogous to that used for breast carcinoma.

More recently, a 2-tier grading system specifically for serous carcinoma, in which tumors are subdivided into low-grade and high-grade, has been proposed. Seminal clinicopathologic and molecular genetic studies from The Johns Hopkins Hospital and M.D. Anderson Cancer Center have shown that a 2-tier grading system is easy to apply, reproducible, and based on underlying molecular biologic differences between low-grade and high-grade tumors.[3–24] The purpose of this review is to provide an update on the pathogenesis and clinicopathologic and immunohistochemical features of ovarian low-grade and high-grade serous carcinomas, as well as to demonstrate why the fundamental differences between both categories of tumors support a 2-tier classification system. In addition, selected diagnostic problems are addressed.

PATHOGENESIS

Low-grade Serous Carcinoma (Table 1)

Low-grade serous carcinoma (invasive micropapillary serous carcinoma [MPSC]), has been hypothesized to arise from a serous cystadenoma (Fig. 1A) or adenofibroma which progresses to an atypical proliferative serous tumor (APST) [typical serous borderline tumor] (Fig. 1B), to non-invasive MPSC (micropapillary serous borderline tumor) [Fig. 1C], and then to invasive MPSC in a slow step-wise fashion. This has been described as the Type I pathway and is supported by several morphologic observations. First, invasive low grade serous carcinoma is associated with non-invasive MPSCs in over three fourths of cases (Fig. 1D).[10] Second, in occasional tumors, the level of differentiation of the non-invasive tumor is intermediate between APST and non-invasive MPSC, suggesting a morphologically intermediate step. Third, true early invasion in an APST or non-invasive MPSC resembles low-grade serous carcinoma (Fig. 1E).[4,23,25,26] Fourth, in several studies, non-invasive MPSCs have a higher frequency of invasive implants (Fig. 1F) compared with APST, and these implants are histologically identical to low-grade serous carcinoma.[27,28]

Both low-grade serous carcinoma and APST/non-invasive MPSC are characterized by mutations of the *KRAS*, *BRAF*, or *ERBB2* genes, in which approximately two thirds of tumors have a mutation of 1 of these genes.[19–21,29–32] However, *KRAS* and *BRAF* are much more commonly mutated than *ERBB2*. Mutations of each of these 3 genes are mutually exclusive; thus, a tumor with a *KRAS* mutation will not have a mutation of the other 2 genes, and *vice versa*.

KRAS, *BRAF*, and *ERBB2* are upstream regulators of mitogen-activated protein kinase (MAPK). Mutations of any of these genes result in constitutive activation of the MAPK signal transduction pathway, which in turn leads to uncontrolled proliferation.[33] In contrast to high grade serous carcinoma, *TP53* mutations are uncommon (8%) in low-grade serous carcinoma.[22] Identical mutations of either *KRAS* or *BRAF* have been observed in the epithelium of cystadenomas adjacent to the APSTs indicating a shared lineage and suggesting that mutation of *KRAS* or *BRAF* is an early event in the transition from a cystadenoma to an APST.[34] Serous cystadenomas that do not contain APSTs do not harbor *KRAS* or *BRAF* mutations.

Both APST and non-invasive MPSC share several allelic imbalances on multiple chromosomal arms (1p, 5q, 8p, 18q, 22q, and Xp); however, many of these are slightly more frequent in non-invasive MPSC, and some (gain of 16p) are unique to non-invasive MPSC.[19,35] Similarly, the multiple shared allelic imbalances are more frequent in low-grade serous carcinoma.[19] Thus, the number of allelic imbalances progressively increase from APST to non-invasive MPSC to low-grade serous carcinoma. This gradual chromosomal instability associated with the Type I pathway is in contrast with the high-level of chromosomal instability seen in high-grade serous carcinomas.[19,35] Gene expression profiling has also shown that invasive MPSC is more closely related to APST/non-invasive MPSC than high-grade serous carcinoma.[36]

In a recent study, Kuo *et al* assessed DNA copy number changes among affinity-purified tumor cells from ovarian serous neoplasms, including serous borderline tumors, low-grade serous carcinomas, and high-grade serous carcinomas, using high-density 250K single nucleotide polymorphism arrays.[9] The chromosomal instability indices as measured by changes in DNA copy number were significantly higher in high-grade than in low-grade carcinomas. Hemizygous ch1p36 deletion was common in low-grade serous carcinomas but rarely seen in serous borderline tumors. This region contains several candidate tumor suppressors, including *miR-34a*

The morphologic and molecular observations link each of these lesions and provide compelling evidence for the step-wise progression from cystadenomas to low-grade serous carcinoma, and this process is distinctly different from that of high-grade serous carcinoma. This pathway is akin to the adenoma-carcinoma sequence seen in the model of pathogenesis of colorectal carcinoma or hyperplasia-carcinoma sequence in endometrioid carcinoma of the endometrium.

High-grade Serous Carcinoma (Table 1)

Much less is known about the pathogenesis of ovarian high-grade serous carcinoma compared with low-grade serous carcinoma. Unlike low-grade serous carcinoma, mutations of *KRAS*, *BRAF*, or *ERBB2* occur very infrequently in high-grade carcinoma.[19–21,30–32,37,38] In contrast, *TP53* mutation occurs in 80% of high-grade tumors, and up-regulation and down-regulation of numerous other genes and various DNA copy number changes have been described.[9,13,33,36,39,40]

Genome-wide analysis of DNA copy number alterations has demonstrated significant numbers of amplifications and deletions, including homozygous deletions.[9] Among homozygous deletions, loci containing *Rb1*, *CDKN2A/B*, *CSMD1*, and *DOCK4* were most common, being present in 10.6%, 6.4%, 6.4%, and 4.3%, respectively, of 47 affinity-purified high-grade serous carcinomas. Except for the *CDKN2A/B* region, these homozygous deletions were not present in either serous borderline tumors or low-grade serous carcinomas.

The identification of the precursor lesion of high-grade serous carcinoma has puzzled investigators for decades. Since high-grade serous carcinoma nearly always presents with high-stage disease, the development of this tumor is thought to be rapid, and its origin has traditionally been presumed to be from surface epithelium or epithelial inclusions in the ovary. In an effort to detect putative precursors, investigators have focused on ovaries of women with a family history of ovarian cancer and women with *BRCA* mutations. Increased *p53* immunopositivity has been noted in the epithelium of ovaries from these women compared with controls, but these findings have not been confirmed in other studies. Mutations and/or loss of heterozygosity of *TP53* have been identified in early carcinomas and epithelial inclusions of the ovary, including identical mutations in the epithelium and adjacent carcinoma in the same cases.[41,42] These molecular findings support to the role of *TP53* mutation as an early event in the pathogenesis of high-grade serous carcinoma and that the origin for some tumors is the surface epithelium or epithelial inclusions of the ovary. Parenthetically, 10% of

ovarian carcinomas are hereditary. Of the hereditary carcinomas, most are related to *BRCA* mutations, which appear to play a role in the pathogenesis of ovarian carcinoma in this subset of tumors. Twelve to 15% of women with ovarian carcinoma, and ~15% of serous carcinomas, in large population-based series from North America have germline mutations of *BRCA*, either *BRCA1* or *BRCA2*.[43,44] The vast majority of *BRCA*-related hereditary ovarian tumors are high-grade serous carcinoma. Low-grade serous carcinoma and APST/non-invasive MPSC do not seem to be related to germline mutations of *BRCA*.

In a report by Werness *et al*, an incidental ovarian carcinoma *in situ* from a woman with a germline mutation of *BRCA1* exhibited loss of heterozygosity of this gene.[42] Also, loss of heterozygosity of *BRCA* has been demonstrated in epithelial inclusions/surface epithelium in ovaries from prophylactic oophorectomy specimens. Loss of heterozygosity has also been reported in invasive carcinoma and adjacent epithelium in stage I ovarian carcinomas from women with *BRCA* germline mutations.[41] These studies suggest that loss of heterozygosity of *BRCA* is an early event in high-grade serous carcinoma for tumors with germline mutations. Similar to *TP53*, *BRCA* appears to function as a tumor suppressor gene. Thus, patients inherit a germline mutation of *BRCA*, and with somatic loss of the wild-type allele, carcinoma develops. The exact interaction between mutations of *BRCA* and *TP53* in ovarian carcinoma is unclear. In addition to germline mutations, other molecular alterations leading to inactivation of *BRCA* include somatic mutation, promoter hypermethylation, and isolated loss of hetrerozygosity.[45,46] These putative precursor lesions are detected in inclusions in the ovary or ovarian surface epithelium and are characterized by tubal-type epithelium with varying degrees of cytologic atypia that have been termed dysplasia/carcinoma *in situ* (reviewed in Bell[47]). These findings, although they suggest that a morphologically identifiable precursor of high-grade serous carcinoma may exist in the ovary, are very rarely detected, and, therefore, it has been suggested that these tumors arise *de novo*.[48]

Recently, attention has been drawn to a lesion in the fallopian tube that has the cytologic appearance of high-grade serous carcinoma of the ovary and has been designated tubal intraepithelial carcinoma (TIC) [Fig. 2]. These lesions are almost always detected in the fimbriated end of the fallopian tube. The fimbriated end is in close proximity to the ovarian surface, and it has been suggested that the tube is the origin of a subset of "ovarian" high-grade serous carcinomas. This is supported by the following: (1) early serous carcinomas in prophylactic bilateral salpingo-oophorectomy specimens from women with *BRCA* mutations (i.e., women who are at an increased risk for "ovarian" carcinoma) can be detected in the tube, especially the fimbriated end, in the absence of an ovarian tumor, (2) identical *TP53* mutations have been reported in TIC and synchronous ovarian high-grade serous carcinomas, and (3) identical *TP53* mutations have been reported in TICs and in small foci of histologically normal tubal epithelium that diffusely expresses p53, which has been termed "p53 signature". It has been suggested that p53 signatures are precursors of TICs which in turn precede the development of high grade serous carcinoma[49–55] Moreover, it has been proposed that when there is a synchronous TIC and ovarian high-grade serous carcinoma that the fallopian tube is the primary site of origin for the "ovarian" tumor. In one study, all fallopian tube tissue from consecutively accessioned pelvic serous carcinomas was submitted for histologic examination, and 48% of tumors initially interpreted as ovarian in origin contained a TIC.[52] In an analysis of ovarian high-grade serous carcinomas at The Johns Hopkins Hospital in which all tubal tissue was submitted for histologic examination, 45% of cases contained TIC (*unpublished data*). It has, therefore, been hypothesized that neoplastic cells of TIC, or a small invasive high-grade serous carcinoma in the fallopian tube which developed from TIC, implant on the ovary, developing into a high-grade serous carcinoma that clinically and grossly appears to be an ovarian primary tumor.

Thus, the morphologic and molecular observations detailed above suggest that possibly half of "ovarian" high-grade serous carcinomas may be of tubal origin. In the other half of tumors, primary origin may have been ovarian or peritoneal. It should be noted that the criteria for distinction of primary ovarian vs. peritoneal origin are quite arbitrary. *Bona fide* well-defined precursor lesions in the ovary are rare and have not been identified in the peritoneum.

In summary, the pathogenesis of high-grade serous carcinoma (Type II pathway) is characterized by: (1) rapid development from what are now believed to be intraepithelial carcinomas very likely of tubal origin, (2) *TP53* mutations, (3) a high level of chromosomal instability, (4) in hereditary tumors, *BRCA* germline mutations, and (5) absence of mutations of *KRAS*, *BRAF*, or *ERBB2*.

Development of High-grade (Type II) from Low-grade (Type I) Serous Carcinoma

The Type I and Type II pathways of development of low-grade and high-grade serous carcinomas are generally independent. Furthermore, when low-grade serous carcinomas recur, they typically maintain their low-grade appearance throughout multiple recurrences, supporting the view that high-grade serous carcinoma does not progress from low-grade serous carcinoma. However, infrequent high-grade serous carcinomas appear to have evolved from a low-grade tumor (Fig. 3). Two percent of high-grade serous carcinomas in the series by Malpica *et al* were associated with a serous borderline tumor.[10] Another case of high-grade serous carcinoma arising within a serous borderline tumor has been reported elsewhere.[56] In a report by Parker *et al*, 2 women with serous borderline tumors recurred as high-grade serous carcinoma.[57]

At The Johns Hopkins Hospital (both in-house and consultation cases), 3% of serous carcinomas contained both high-grade and low-grade components. The latter were either APST (3 cases) or invasive carcinoma (3 cases).[5] In 4 of the 6 cases, a morphologic continuum could be identified between the low- and high-grade components. Two of the 6 cases contained identical *KRAS* mutations in both the low- and high-grade components. The other 4 cases lacked *KRAS* mutations in both components, and all 6 cases did not have *BRAF* or *TP53* mutations in either component. The finding of identical *KRAS* mutations in both components establishes a clonal relationship between the low- and high-grade tumors in those 2 cases. The remaining 4 cases were molecularly uninformative since they did not have mutations for comparison in either component. However, the lack of *TP53* mutations in all 6 cases contrasts with the high frequency of mutations typically seen in high-grade serous carcinoma (80%). These observations suggest that a very small minority of high-grade serous carcinomas may evolve from an APST, a non-invasive MPSC, or a low-grade serous carcinoma.

It should be noted that the high-grade serous carcinomas that appear to have evolved from low-grade serous tumors (Type I tumors) do not differ morphologically from those that developed along the Type II pathway. In particular, a high-grade serous carcinoma with a micropapillary pattern does not necessarily indicate origin from a low-grade tumor, as such tumors have been shown to lack *KRAS* mutations and exhibit *TP53* mutations.[3,21] In future studies, it will be important to evaluate whether both types of high-grade serous carcinomas (those with mutated *KRAS* or *BRAF* and wild-type *TP53* vs. those with wild-type *KRAS* and *BRAF* and mutated *TP53*) have a different clinical outcome and/or response to chemotherapy.

CLINICAL ASPECTS, HISTOLOGIC FEATURES, AND SELECTED DIAGNOSTIC PROBLEMS

Low-grade Serous Carcinoma (Table 2)

Patients with low-grade serous carcinoma are younger than those with high-grade tumors, with mean ages of 45–57 years and 55–65 years, respectively.[6,10,58] Almost all patients in both groups present with advanced stage disease, and the majority of low- and high-grade tumors are bilateral.

Low-grade serous carcinoma (invasive MPSC) accounts for a small proportion of all ovarian serous carcinomas. In the series by Seidman *et al*, in which the 2-tier system was used, 9% of serous carcinomas were low-grade.[58] In other large series using the 3-tier universal grading system, 6–22% of serous carcinomas were grade 1.[2,59]

Low- and high-grade serous carcinomas in the 2-tier system correspond to grade 1 and grade 2 or 3, respectively, in the universal grading system. In the series by Malpica *et al*, 94% of low-grade serous carcinomas were grade 1 in the universal grading system, and the remaining 6% were grade 2.[10] Likewise, all high-grade serous carcinomas were grade 2 or 3. Conversely, all grade 1 tumors in that study were low-grade, and 94% of all grade 2 or 3 tumors were high-grade.

Low-grade serous carcinoma is frequently associated with a non-invasive component-either serous adenofibroma, APST, or non-invasive MPSC (Fig. 1D). The non-invasive component may be absent, but when present, it can vary from focal to extensive. The invasive component is characterized by micropapillae and small round nests of cells that infiltrate the stroma in a haphazard pattern (Figs. 4A and B). The micropapillae are small and either lack fibrovascular cores or have very thin, delicate fibrovascular cores. The micropapillae and small solid nests of cells are frequently surrounded by a clear space or cleft (Fig. 4B). In tumors with marked architectural complexity, the micropapillae can anastomose with one another, forming thin, elongated, and branching structures (Fig. 4C). The surface of the micropapillae may be smooth or irregular due to hobnail cells. Occasionally, papillae are larger and contain broad fibrous cores (Fig. 4D). Rarely, other non-micropapillary invasive components include small- to medium-sized simple glands. The medium- and large-sized papillae and glands, if present, typically account for only a small proportion of the tumor and are intimately admixed within the micropapillary-rich component. Psammoma bodies are common and may be numerous (Fig. 4D). Necrosis or multinucleated tumor giant cells are not features of low-grade serous carcinoma.

The neoplastic cells can have either scant cytoplasm or a moderate amount of eosinophilic cytoplasm. However, the nuclear-to-cytoplasmic ratio may be high, and the nuclei are uniform, small, and round to oval. The chromatin is even and does not exhibit hyperchromasia. Small nucleoli may be present. Only mild variation in size and shape of nuclei are tolerated for a designation of low-grade serous carcinoma (Fig. 4E). Per the criteria in the study by Malpica *et al*, tumors with cells showing ≥3:1 variation in nuclear size and shape are classified as high-grade serous carcinoma.[10] In that study, most low-grade serous carcinomas had a mean mitotic index of 4 mitotic figures per 10 high-power fields (range, 1–12 MFs/10 HPFs). In our practice, we do not employ mitotic counts for the distinction of low-grade from high-grade serous carcinoma. Rather, we consider low-grade serous carcinoma as having only infrequent mitotic figures while high-grade serous carcinoma has readily identifiable mitotic activity.

Excellent inter- and intra-observer reproducibility of the 2-tier low-grade/high-grade system has been demonstrated. In a study by Malpica *et al*, 80 serous carcinomas, including 40 low-grade and 40 high-grade tumors per the original diagnosis, were evaluated among 9 pathologists

with 2 rounds of review.[11] In the 1st and 2nd rounds of review, all pathologists agreed on grade in 75% and 84% of tumors, respectively. In the 1st round of review, the kappa values for agreement between different reviewers ranged from 0.717 to 1.000, and the range in the 2nd round was 0.701 to 1.000. The intra-observer reproducibility yielded kappa values ranging from 0.725 to 1.000. All of the kappa values in this study were in the good to excellent range. These findings confirm the ease and reproducibility of using the 2-grade system in routine practice.

A potential pitfall in the evaluation of low-grade serous tumors of the ovary is that some low-grade serous carcinomas exhibit an inverted "macropapillary" form of invasion which can be misdiagnosed as serous adenofibroma (Fig. 5). Such variants of low-grade serous carcinoma are characterized by a haphazard infiltration of medium-sized round papillae surrounded by a clear space. These types of papillae, in contrast to micropapillae of conventional low-grade serous carcinoma, contain a fibrous stromal core; however, they are lined by the same type of neoplastic epithelium as seen in the classic micropapillae. We previously reported our experience with 14 cases of this macropapillary variant of low-grade serous carcinoma.[60] The ovarian tumor in such cases contained a background of either APST, non-invasive MPSC, and/ or conventional low-grade serous carcinoma. The macropapillary component accounted for 20% to 100% of the entire invasive component in the ovarian tumors. Three of the 9 cases with extra-ovarian disease had macropapillae in extra-ovarian sites. Molecular analysis was performed on 7 tumors, and in 4, both the macropapillary and micropapillary components contained identical *KRAS* mutations. In one of these tumors, the same *KRAS* mutation was also present in macropapillae in a lymph node metastasis. In another tumor, identical *BRAF* mutations were present in the macropapillary and micropapillary components. Two tumors contained wild-type *KRAS* and *BRAF* and were, therefore, molecularly uninformative. These morphologic and molecular findings support the interpretation that the macropapillary component is a form of low-grade invasive carcinoma.

High-grade Serous Carcinoma (Table 2)

High-grade serous carcinomas may exhibit mixtures of papillary, glandular, nested, and diffuse/solid growth patterns although any component may predominate in a given tumor (Figs. 6A-C). The papillae tend to be large and complex. The epithelium lining the papillae is usually stratified with an irregular slit-like configuration (Fig. 6A). Although a micropapillary growth pattern is typical of low-grade serous carcinoma, it should be emphasized that occasional high-grade carcinomas can also exhibit this architecture (Fig. 6D); however, they have high-grade nuclei and typically have an admixed solid growth pattern. The latter would be unusual for low-grade tumors. The glands in high-grade serous carcinoma may be round and simple or complex with irregular slit-like spaces (Fig. 6B). Some tumors may have such extensive solid architecture with diffuse sheets of neoplastic epithelium that a careful search for a glandular or papillary component may be necessary for distinction from undifferentiated carcinoma. Obvious destructive stromal invasion is generally present, but some neoplasms may be predominantly intracystic and, therefore, misdiagnosed as APST/non-invasive MPSC. The presence of high-grade nuclei excludes that possibility. Necrosis is common in high-grade serous carcinoma. Psammoma bodies can be seen but are typically less frequent compared with low-grade serous carcinoma.

The neoplastic epithelial cells are heterogeneous and may be a mixture of low-cuboidal, columnar, and hobnail shapes. Typically, there is marked variation in size and shape. The nuclear-to-cytoplasmic ratios are generally high, but at times, abundant eosinophilic cytoplasm is present. Most tumors have variable combinations of enlarged round or oval nuclei, irregular nuclear membranes, irregular chromatin distribution, hyperchromasia, large nucleoli, and abundant mitotic figures, including atypical forms (Fig. 6E). In the study by Malpica *et al*, the

median mitotic index was 38 mitotic figures per 10 high-power fields (range, 14–137 MFs/10 HPFs).[10] Bizarre mononuclear or multinucleated tumor giant cells are common (Figs. 3C and D).

At times high-grade carcinomas can display an appearance, mimicking endometrioid carcinoma (Fig. 6F). When the glands have irregular serrated luminal contours, large complex papillae lined by stratified epithelium with irregular slit-like patterns, hobnail cells, bizarre tumor giant cells, and psammoma bodies, a serous carcinoma is favored. In contrast, tumors with peripheral palisading of solid islands and nests, squamous metaplasia, or a background of atypical proliferative (borderline) endometrioid tumor or endometriosis favor high-grade endometrioid carcinoma. Immunohistochemical staining for WT-1 has been advocated as useful for this differential diagnosis, but in our experience it is not reliable. At times, distinction of high-grade serous carcinoma from high-grade endometrioid carcinoma (FIGO grade 2 or 3) is not possible, and classification as "high-grade adenocarcinoma, not otherwise specified" with a descriptive comment is necessary.

Some tumors may contain cells with clear cytoplasm. If the tubulocystic and papillary patterns characteristic of clear cell carcinoma are not present, these tumors should not be interpreted as clear cell carcinoma (Fig. 6G). When the epithelium lining the surface of large rounded papillae is smooth, a transitional cell carcinoma-like appearance can be produced and may be mistaken for ovarian transitional cell carcinoma (TCC) (Fig. 6H). Opinions vary among gynecologic pathologists as to whether pure TCC of the ovary is a distinctive entity or simply a TCC-like pattern of high-grade serous carcinoma. At present, the "jury is still out." Glandular differentiation has been described in transitional cell carcinoma; however, a diagnosis of serous carcinoma is favored when the glands merge with complex, branching papillae exhibiting epithelial tufting and solid nests surrounded by a space and irregular slit-like spaces are present. Also, psammoma bodies are more typical of serous carcinoma. Immunohistochemistry is not helpful as WT-1 expression has been described in both serous and transitional cell carcinomas.

Rarely, microcystic or signet ring cell-like change can be seen in high-grade and also in low-grade serous carcinoma(Fig. 6I).[61] Microcystic change in such tumors is produced by the presence of back-to-back cells with signet ring change and can simulate the reticular pattern of yolk sac tumor. The combination of older age, bilaterality, large papillae lined by complex and stratified epithelium, glands with irregular slit-like spaces, and psammoma bodies favors serous carcinoma. On the other hand, the combination of younger age, unilaterality, microcystic patterns which blend with other classic patterns of yolk sac tumor, such as Schiller-Duval bodies, polyvesicular-vitelline, intestinal, and myxoid patterns, and hyaline globules favors yolk sac tumor. An elevated serum AFP level is characteristic of yolk sac tumor. Immunohistochemistry may be helpful in that expression of WT-1, ER, PR, CK7, and EMA are more frequent in serous carcinoma while expression of AFP and absence of the other markers listed above are more typical of yolk sac tumor.

The distinction of low-grade from high-grade serous carcinoma is based on nuclear features, as described by Malpica *et al*, and detailed above.[10] In most tumors, the nuclei of low-grade and high-grade serous carcinomas are typically grade 1 and grade 3, respectively, in a 3-tier system; thus, the diagnosis in the vast majority of tumors is straightforward. Some tumors (approximately 4% of serous carcinomas[3]), however, exhibit nuclear features that are intermediate between low-grade and high-grade. These "grade 2" nuclei are larger and have coarser chromatin, more mitotic activity, and larger nucleoli than grade 1 nuclei. They are also relatively uniform and are smaller and less pleomorphic and have less coarse chromatin than grade 3 nuclei (Fig. 7). Thus, classification of these tumors with intermediate grade nuclei as low-grade vs. high-grade serous carcinoma will be difficult.

In a previous study, we analyzed 11 serous carcinomas with "grade 2" nuclei.[3] All 10 with staging data were FIGO stage IIIC. A significant micropapillary pattern was present in 6 (55%). Five (45%) had multiple small foci of necrosis. The mean mitotic index was 11 mitotic figures per 10 high-power fields (range, 4–18 MFs/10 HPFs). Molecular analysis was performed in all 11 tumors. None contained *KRAS*, *BRAF*, or *ERBB2* mutations, but *TP53* mutations were identified in 10 (91%). Of these 10 with follow-up, 7 patients died of disease at 6–30 months, 2 died of other causes, and 1 had no evidence of disease at 6 months. The clinicopathologic and molecular findings support grading tumors with intermediate grade nuclei as high-grade serous carcinoma. It should be noted that these tumors with intermediate grade (grade 2) nuclei are not synonymous with the rare high-grade serous carcinomas that truly have an admixed component of low-grade serous carcinoma (Fig. 3). See *Development of High-grade (Type II) from Low-grade (Type I) Serous Carcinoma* in *Pathogenesis* above for additional details.

High-grade serous carcinomas are architecturally heterogeneous, in that they correspond to moderately differentiated and poorly differentiated grades in 3-tier grading systems because some are predominantly papillary or glandular while others are mostly solid. However, they do not appear to be different from a molecular and *in vitro* drug resistance standpoint. In a study of high-grade serous carcinomas in which moderately differentiated and poorly differentiated were compared, there were no significant differences in the frequency of *TP53* mutation or extreme drug resistance for each of 10 chemotherapeutic agents.[24] In addition, the survival for patients with grades 2 and 3 serous carcinomas using the universal grading system is closer to each other compared with survival for patients with grade 1 and 2 tumors.[10,62] These biologic and clinical findings suggest that moderately and poorly differentiated tumors can be combined into a single category, justifying the use of a 2-tier rather than a 3-tier grading system.

Behavior

The few studies that have compared outcome between both types of serous carcinomas using the 2-tier system have shown that patients with low-grade tumors have better survival. In the study by Malpica *et al*, the 2-tier grading system was found to be of independent prognostic significance upon multivariate analysis, and the survival of patients with low-grade tumors was significantly higher than with high-grade tumors.[10] In that study, death due to disease was more rapid with high-grade carcinoma. The median survival was 1.7 years for patients with high-grade tumors compared to 4.2 years for women with low-grade tumors. Furthermore, in a large clinical study of only low-grade serous carcinoma, the median overall survival with stage III or IV disease was 6.8 years. Persistent disease after primary chemotherapy was the only variable associated with shorter survival time.[6] With high-grade serous carcinoma, survival beyond 5 years is unusual, but survival over 10 years can be seen in a subset of low-grade serous carcinomas. The 5-year survival rates for low- and high-grade tumors in the study by Malpica *et al* were 40% and 9%, respectively.[10] In the study by Seidman *et al*[58] in which the criteria for the 2-tier system of Malpica *et al*[10] were used, the 5-year survival rates for low- and high-grade serous carcinomas were 56% and 34%, respectively; however, this difference was not statistically significant. These 5-year survival rates for low-grade serous carcinoma in the studies by Malpica *et al*[10] and Seidman *et al*[58] are similar to our experience.[23]

Few studies have compared survival using the 2-tier vs. 3-tier grading systems. In the study by Malpica *et al*, serous carcinomas were graded using the 2-tier low-grade/high-grade, 3-tier universal, and 3-tier FIGO systems.[10] All 3 grading systems showed statistically significant prediction of survival. In view of its simplicity in application and excellent reproducibility, we advocate using the 2-tier system in routine practice.

Primary Site of Origin

Diagnostic problems may arise in the distinction of high-grade serous carcinoma of primary ovarian vs. peritoneal or tubal origin. The GOG has proposed criteria for determining peritoneal origin for tumors synchronously involving the peritoneum and ovary. They are: both ovaries must not be enlarged because of tumor involvement; the extent of non-ovarian disease must be greater than that involving the ovaries; microscopically, the ovary must show either no tumor or involvement of ovarian surface and ovarian parenchyma/stroma by tumor measuring <5 mm in greatest dimension; and the extra-ovarian tumor must display a histologic appearance consistent with that seen in ovarian serous carcinomas.[63] The criteria are arbitrary and have not been scientifically validated. We do not strictly adhere to the 5 mm criterion. Although a tumor with ovarian surface involvement and without ovarian parenchymal/stromal involvement would qualify as a peritoneal primary per the GOG criteria, we designate a tumor ≥5 mm in size involving the ovarian surface and without ovarian parenchymal/stromal involvement as an ovarian primary, unless the growth pattern on the surface of the ovary is characterized by tumor imbedded in a desmoplastic plaque typical of secondary ovarian involvement, since some primary ovarian carcinomas without significant peritoneal involvement may be predominantly located on the surface of the ovary.

The traditional criteria for determining fallopian tube origin for tumors synchronously involving the tube and ovary by Hu *et al* and subsequently modified by Sedlis are: (1) tumor arises from the fallopian tube mucosa, (2) histology of the tumor resembles tubal mucosa, (3) there is a transition from benign to malignant epithelium, and (4) the size of the fallopian tube tumor is larger than the ovarian tumor.[64,65] Recently, the presence of TIC has been proposed as indicating fallopian tube origin for cases with a synchronous ovarian mass.[50,52,55] See *High-grade Serous Carcinoma* section for *Pathogenesis* above for additional details.

In cases in which the primary site cannot be determined, this distinction usually will not be critical since most cases will typically be high-stage, and the treatment and prognosis for a high-grade serous carcinoma simultaneously involving ovary and peritoneum/fallopian tube is similar regardless of which site is designated the primary site of origin.[63,66–75]

IMMUNOHISTOCHEMICAL FEATURES

p53 (Table 3)

Although *TP53* is frequently mutated in ovarian high-grade serous carcinoma, numerous studies have erroneously made the assumption that p53 "immunopositivity" equates with a mutation. Rather, it is the pattern of immunohistochemical staining that correlates with mutation as opposed to simply a positive or negative stain. In the endometrium, a diffuse pattern of expression, which is typically 90–100% positive cells, generally correlates with a *TP53* mutation. In the ovary, that correlation is not as strong.

In a prior study, we correlated immunohistochemical expression of p53 with mutational status in ovarian serous tumors.[22] Among tumors with a *TP53* mutation, 27% were negative by immunohistochemistry (<1% positive cells), 18% had 51–90% positive cells, and 55% had >90% positive cells. Among those with wild-type *TP53*, 35% of tumors were negative by immunohistochemistry, and the remainder expressed p53, ranging from 1% to >90% positive cells. Of note, 26% of tumors with wild-type *TP53* had 51–90% positive cells, and 13% had >90% positive cells. Importantly, 33% of all tumors in that study with >90% positive cells had wild-type *TP53*. These findings demonstrate that although diffuse expression of p53 (>90% positive cells) indicates that the tumor is more likely to have a *TP53* mutation, immunohistochemistry cannot accurately predict mutation status.

While the correlation between the extent of immunohistochemical expression of p53 and mutational status is suboptimal in ovarian serous tumors, differences in the extent of expression in low- and high-grade carcinoma, however, can be diagnostically useful. In a study by O'Neill *et al*, the extent of expression was significantly different between low- and high-grade tumors. [76] In that study, the immunohistochemical extent of expression was semi-quantitatively scored using a 0 to 5+ scale. Eighteen percent of low-grade serous carcinomas showed 5+ staining (>75% positive cells) while the same pattern was seen in 64% of high-grade serous carcinomas. In contrast, a 0 to 2+ pattern was encountered in 64% and 28% of low-grade and high-grade serous carcinomas, respectively. This study shows that high-grade serous carcinoma tends to exhibit diffuse expression of p53 while expression in low-grade carcinoma is much less (Fig. 8), but some degree of overlap limits its utility in the differential diagnosis of low- vs. high-grade serous carcinoma.

p16 (Table 3)

p16 has not been studied extensively in the ovary, but this marker is of potential value in the diagnosis of ovarian serous tumors. p16 is most well known for its role as a surrogate marker for high-risk HPV associated lesions in the lower genital tract. In that setting, p16 overexpression is indirectly due to the cascade of molecular alterations occurring in cells infected by high-risk HPV. In serous carcinomas of the endometrium and ovary, this marker has also been shown to be overexpressed although the molecular mechanism is unrelated to HPV. In the endometrium, serous carcinoma exhibits diffuse expression of p16.[77,78] In this scenario, the extent of expression is diffuse (typically 90–100% positive cells), and other histologic types, such as endometrioid carcinoma, show an extent of expression that is generally much less. Similar results have been observed in ovarian carcinomas, making this a possibly useful marker for high-grade serous carcinoma of the ovary. In one study using the 3-tier universal grading system, 33%, 53%, and 79% of grade 1, 2, and 3 serous carcinomas, respectively, strongly expressed p16 in >80% cells.[79] In another study using the 2-tier low-grade/high-grade system, the extent of expression was significantly different between ovarian low- and high-grade carcinomas.[80] In that study, the immunohistochemical extent of expression was semi-quantitatively scored using a 0 to 5+ scale. Twenty-seven percent and 83% of low-grade and high-grade serous carcinomas, respectively, showed 5+ staining (>75% positive cells). On the other hand, a 0 to 2+ pattern was encountered in 50% of low-grade serous carcinomas and 8% of high-grade serous carcinomas. Although there is some overlap in the extent of staining in low- versus high-grade serous carcinoma, high-grade serous carcinoma tends to exhibit diffuse expression of p16 compared to low-grade carcinoma (Fig. 8). In contrast to high-grade serous carcinoma, expression of p16 in primary ovarian endometrioid and mucinous carcinomas is negative, focal, or patchy.[81]

Ki-67 (Table 3)

The Ki-67 proliferation index is higher in high-grade serous carcinoma compared with low-grade carcinoma (Fig. 8). In one study, the proliferation indices were significantly different between these two tumors (23% for low-grade and 55% for high-grade).[76] Fourteen percent of low-grade serous carcinomas showed a proliferation index >50% while the same index was seen in 64% of high-grade serous carcinomas. These findings indicate that a morphologically problematic serous carcinoma with a markedly elevated Ki-67 proliferation index is unlikely to be low-grade.

ER, PR, WT-1, and PAX8 are expressed in both low- and high-grade serous carcinomas. Therefore, these markers will not distinguish these tumors. However, for the differential diagnosis with non-gynecologic tumors, these markers can be selectively used with a larger panel. For example, if there is concern for malignant mesothelioma, then including ER/PR, which is commonly expressed diffusely in serous carcinoma, in a mesothelioma-carcinoma

panel is diagnostically helpful. In tumors in which a metastatic breast carcinoma is a consideration, a panel should include GCDFP-15 and mammaglobin, which are expressed in breast carcinoma, and WT-1 and PAX8, which are expressed in serous carcinoma.[82-84] Specifically, bladder, breast, and lung carcinomas with a micropapillary pattern can be distinguished from serous carcinoma with a panel including PAX8, WT-1, TTF-1, uroplakin, mammaglobin, and ER.[85]

Besides the immunohistochemical markers that are frequently used in routine pathology practice, several new biomarkers including Rsf-1 (HBXAP) and Nac1 have emerged, and their differential expression in high-grade versus low-grade serous carcinoma suggests they play roles in the development of Type II tumors.[17,86]

In summary, immunohistochemistry is not necessary for distinguishing low-grade from high-grade serous carcinoma. Generally, low-grade serous carcinoma shows negative, focal, or patchy staining for p53 and p16 and a low Ki-67 proliferation index. In contrast, high-grade serous carcinoma shows a diffuse pattern of p53 and p16 expression and a high Ki-67 proliferation index (Fig. 8).

TREATMENT ISSUES

One of the reasons why elucidation of the pathogenesis of serous carcinoma is important is that it may clarify an important aspect of response to chemotherapy. Given the differences in proliferative activity and molecular profiles of low- and high-grade serous carcinoma, it stands to reason that chemotherapy may not have the same effect in both tumors. Clinical evidence has suggested that low-grade serous carcinoma is not as responsive to conventional (taxane- and platinum-based) chemotherapy as high-grade serous carcinoma.[15] This is also supported by *in vitro* drug resistance data, in which low-grade serous carcinomas more frequently showed extreme drug resistance to paclitaxel and carboplatin compared with high-grade carcinomas. [14] Thus, identification of newer agents that are more effective against low-grade serous carcinoma are needed. CI-1040 is an inhibitor of MAPK kinase, which up-regulates MAPK. CI-1040 prevents MAPK activation, which plays a critical role in the development of low-grade serous carcinoma. *In vitro* and *in vivo* laboratory evidence has shown that CI-1040 inhibits growth of ovarian cancer cells containing a *KRAS* or *BRAF* mutation.[12,38] Therefore, MAPK inhibitors could be used as potential therapeutic agents for low-grade serous carcinomas containing a *KRAS* or *BRAF* mutation and possibly the uncommon high-grade serous carcinomas with *KRAS* or *BRAF* mutations. The GOG is currently performing a study of AZD6244, which is an inhibitor of the MAPK signaling pathway, in ovarian low-grade serous carcinoma,[87] and it is hoped that the results will lead to more optimal treatment of patients with these tumors in the future.

CONCLUSIONS

The distinctly different clinicopathologic, immunohistochemical, and molecular profiles of ovarian serous carcinomas, when classified as low-grade or high-grade, justify the use of a 2-tier grading scheme for serous carcinoma in routine practice. Most serous carcinomas can be readily categorized as either low-grade or high-grade; however, in a minority of tumors, the histologic distinction can be challenging. The use of immunohistochemical stains for p53, p16, Ki-67 in this setting may be helpful in a subset of these tumors.

Low-grade serous carcinomas are indolent tumors that exhibit low-grade nuclei with infrequent mitotic figures; micropapillary architecture is typical. They evolve from adenofibromas and APST/non-invasive MPSCs and have frequent mutations of the *KRAS*, *BRAF*, or *ERBB2* genes. They rarely harbor *TP53* mutations and are genetically stable. Their progression is slow and step-wise. In contrast, high-grade serous carcinomas are rapidly growing, highly aggressive

tumors that exhibit high-grade nuclei with readily identifiable mitotic figures. High-grade serous carcinomas are characterized by *TP53* mutations and genetic instability. Mutations of the *KRAS*, *BRAF*, or *ERBB2* genes are not usually present. Recent studies suggest that a significant number originate from intraepithelial carcinoma in the fallopian tube. Their development is rapid, and they have a better response to conventional chemotherapy compared with low-grade tumors.

Thus, it is clear that low- and high-grade serous carcinomas are distinctly different neoplasms with different pathogenesis, behavior, and response to treatment. By taking into account the pathogenesis of ovarian cancer as outlined in this dualistic model, a more rational approach to early detection can be undertaken, and novel types of treatment can be developed.

Acknowledgments

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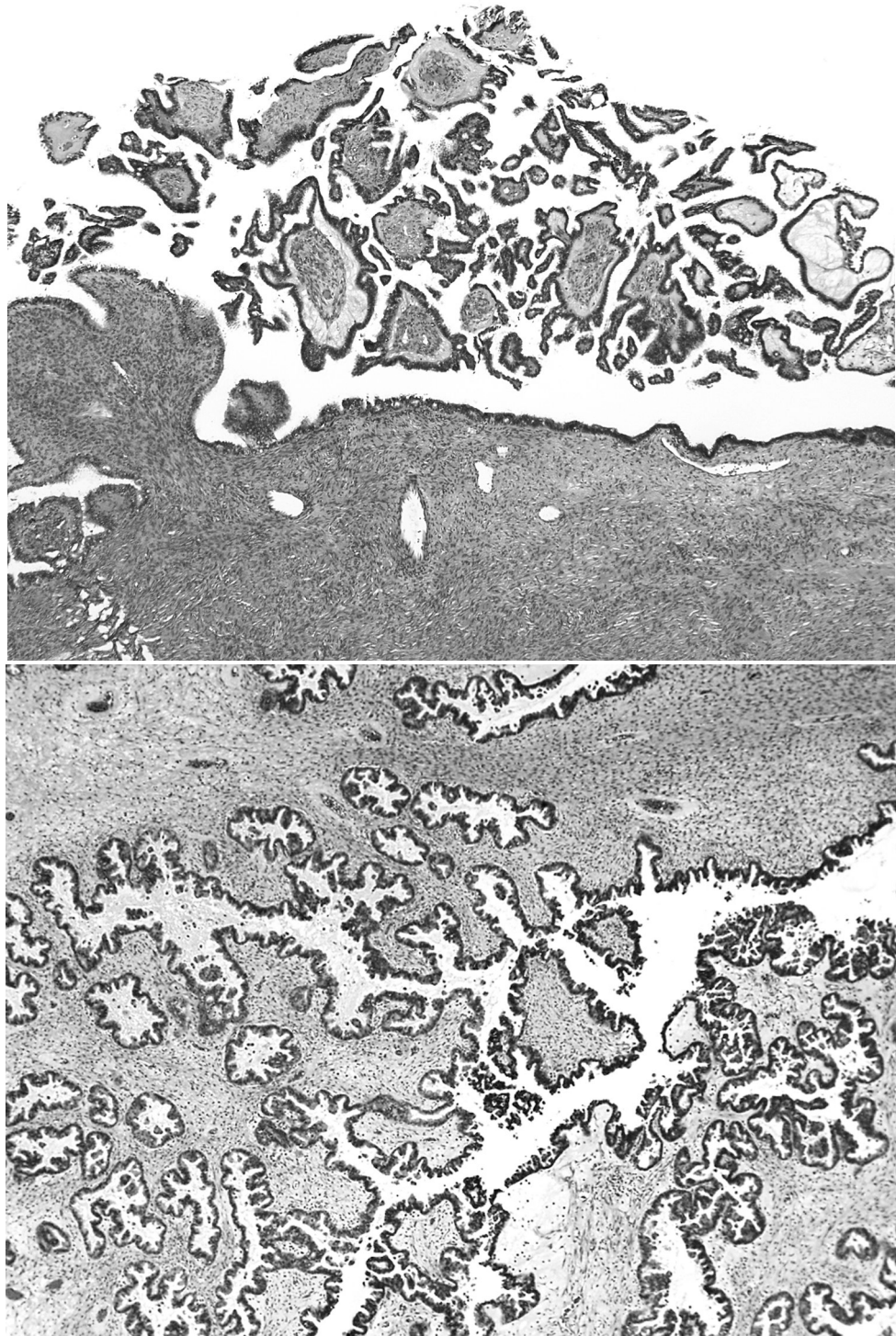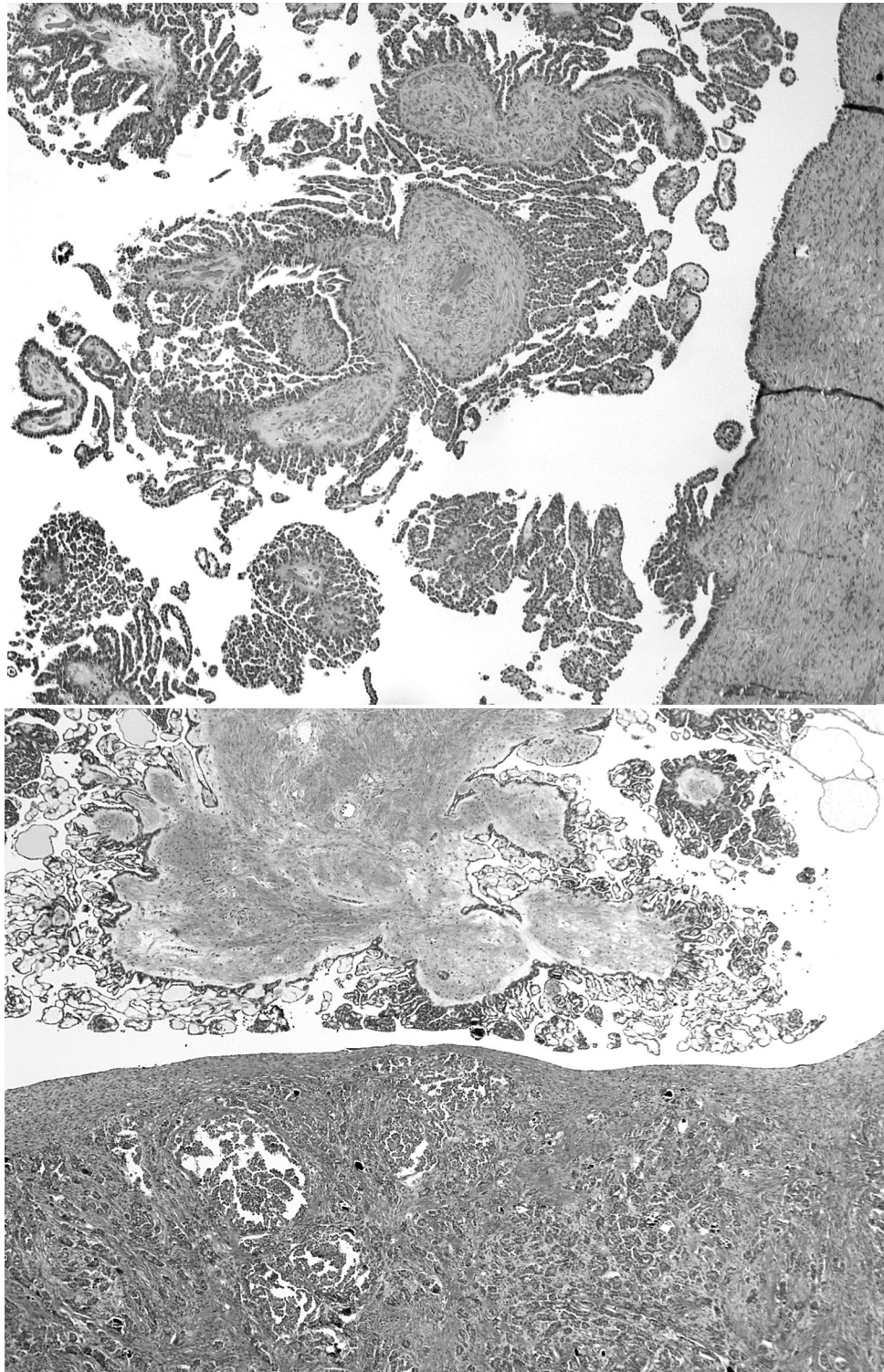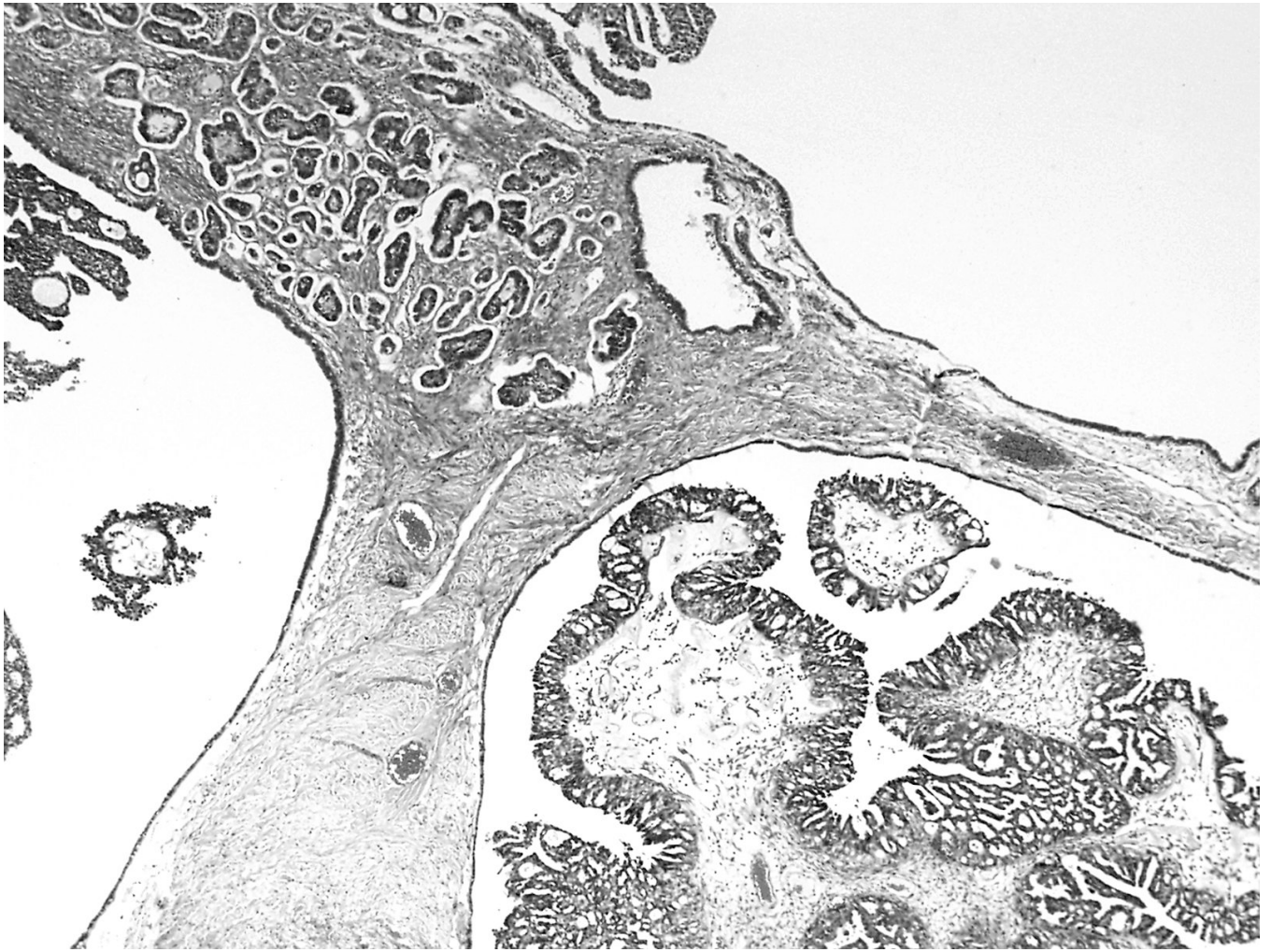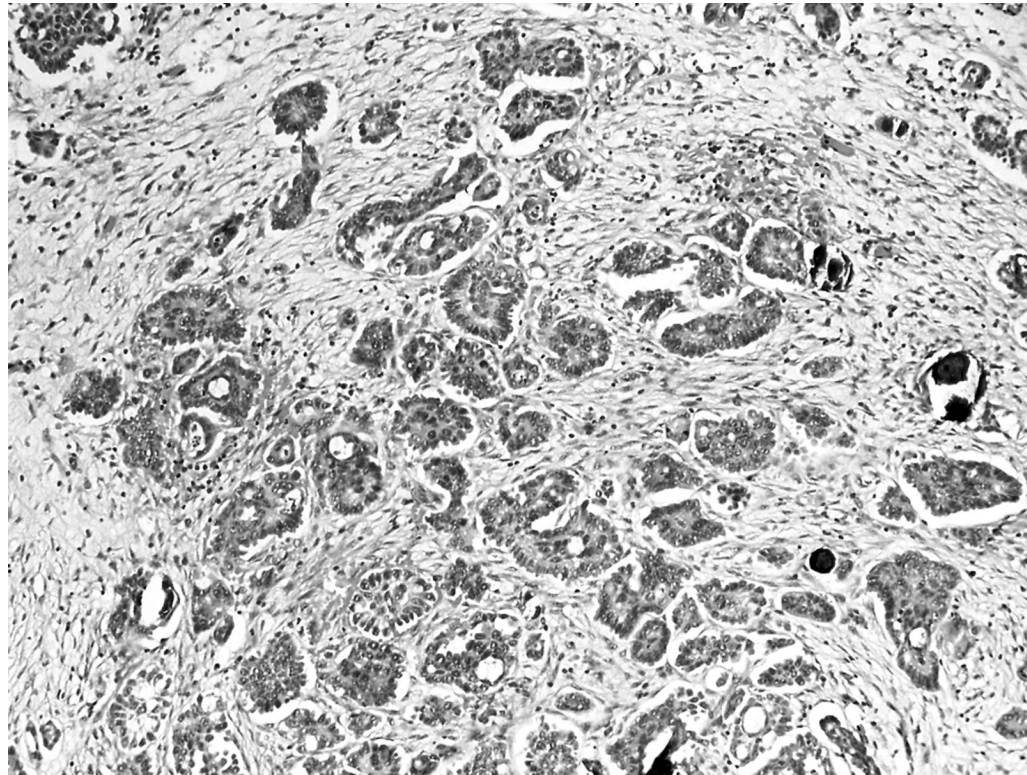
Figure 1.
Proposed model of pathogenesis of low-grade serous carcinoma. (A) Small atypical proliferative serous tumor (APST) arising in a serous cystadenoma. (B) APST. (C) Non-invasive micropapillary serous carcinoma (MPSC). (D) Tumor progression in the ovary results in invasive MPSC (*lower*); background of non-invasive MPSC in upper half of figure. (E) Microinvasion (*upper left*), which is qualitatively identical to low-grade serous carcinoma, arising within a non-invasive MPSC (*lower right*). (F) Invasive implants in the peritoneum are histologically identical to low-grade serous carcinoma.
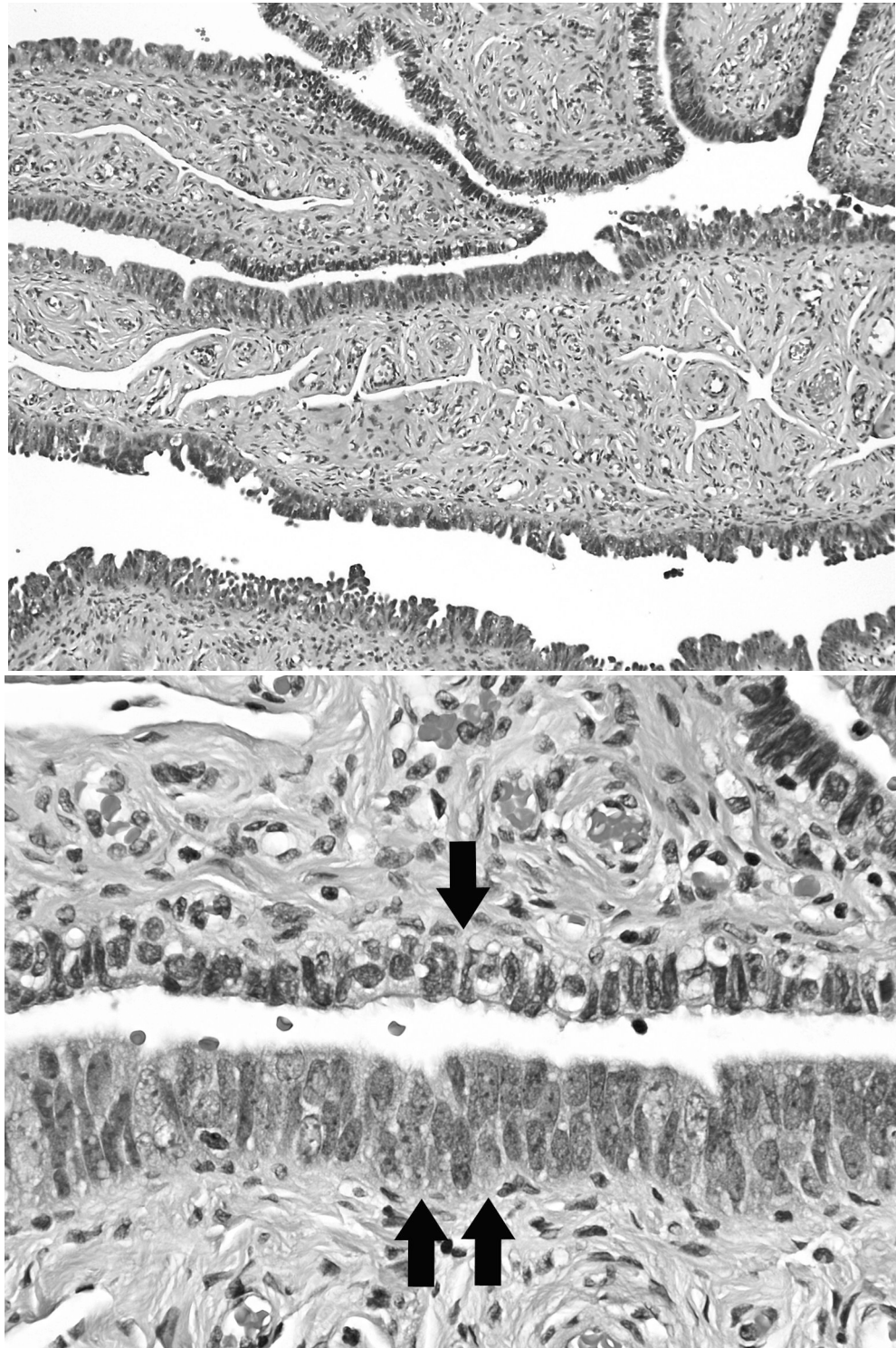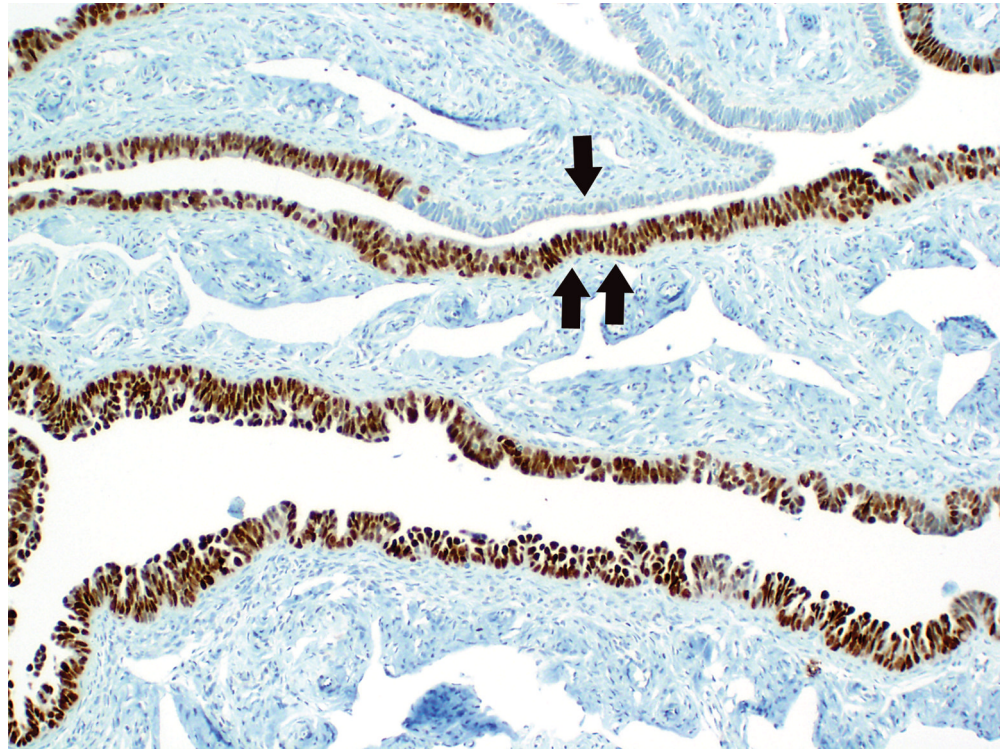
Figure 2.
Tubal intraepithelial carcinoma (TIC). (A) The epithelium of the fallopian tube mucosa with TIC is thicker compared with normal mucosa (*upper center*). (B) The neoplastic cells of TIC show enlarged nuclei, increased nuclear-to-cytoplasmic ratios, and coarse chromatin. (C) Immunohistochemical stain for p53 showing diffuse strong expression in TIC while the normal mucosa is negative. In (B) and (C), *single arrow* represents normal epithelium, and *double arrows* represent TIC.
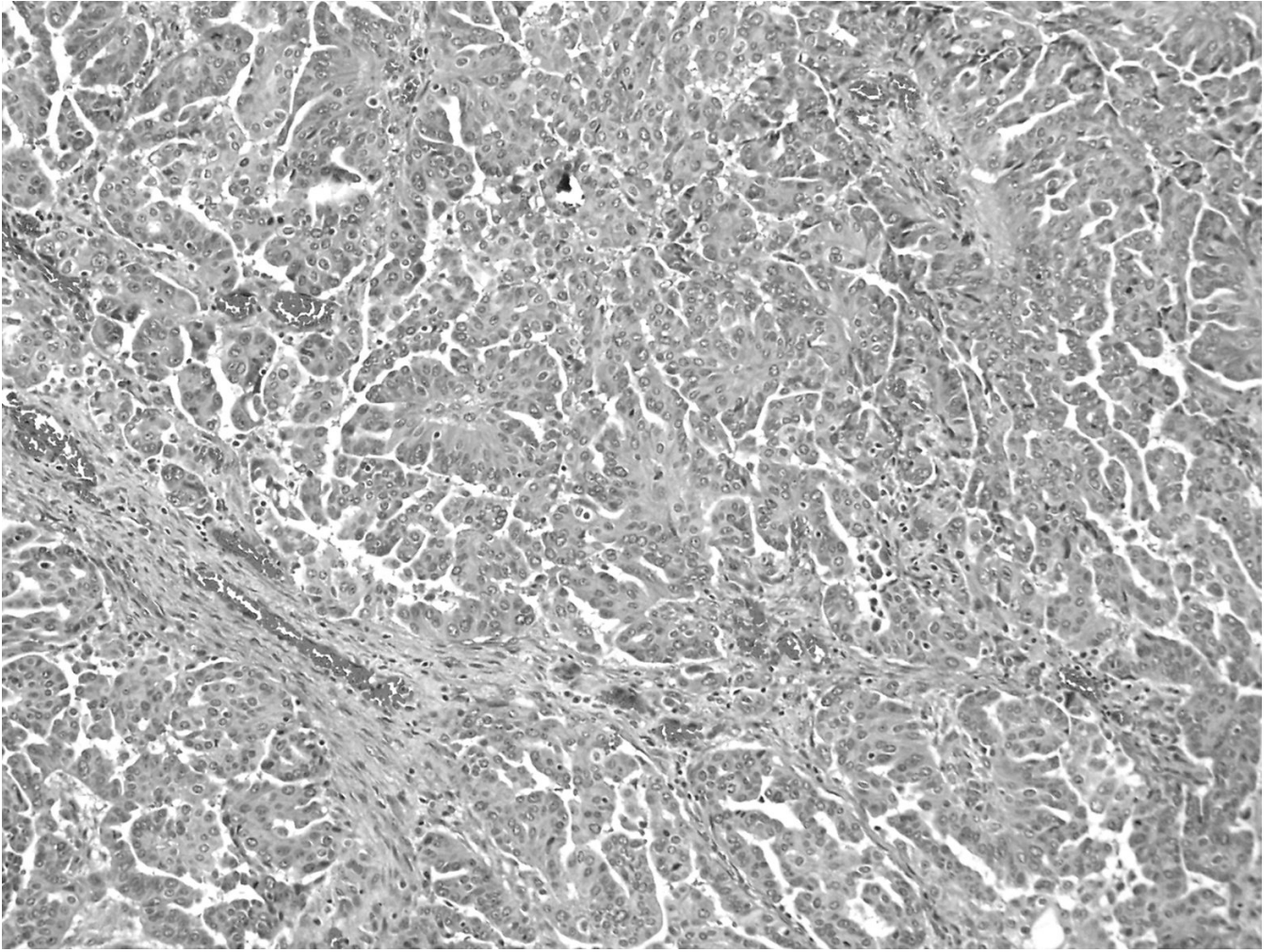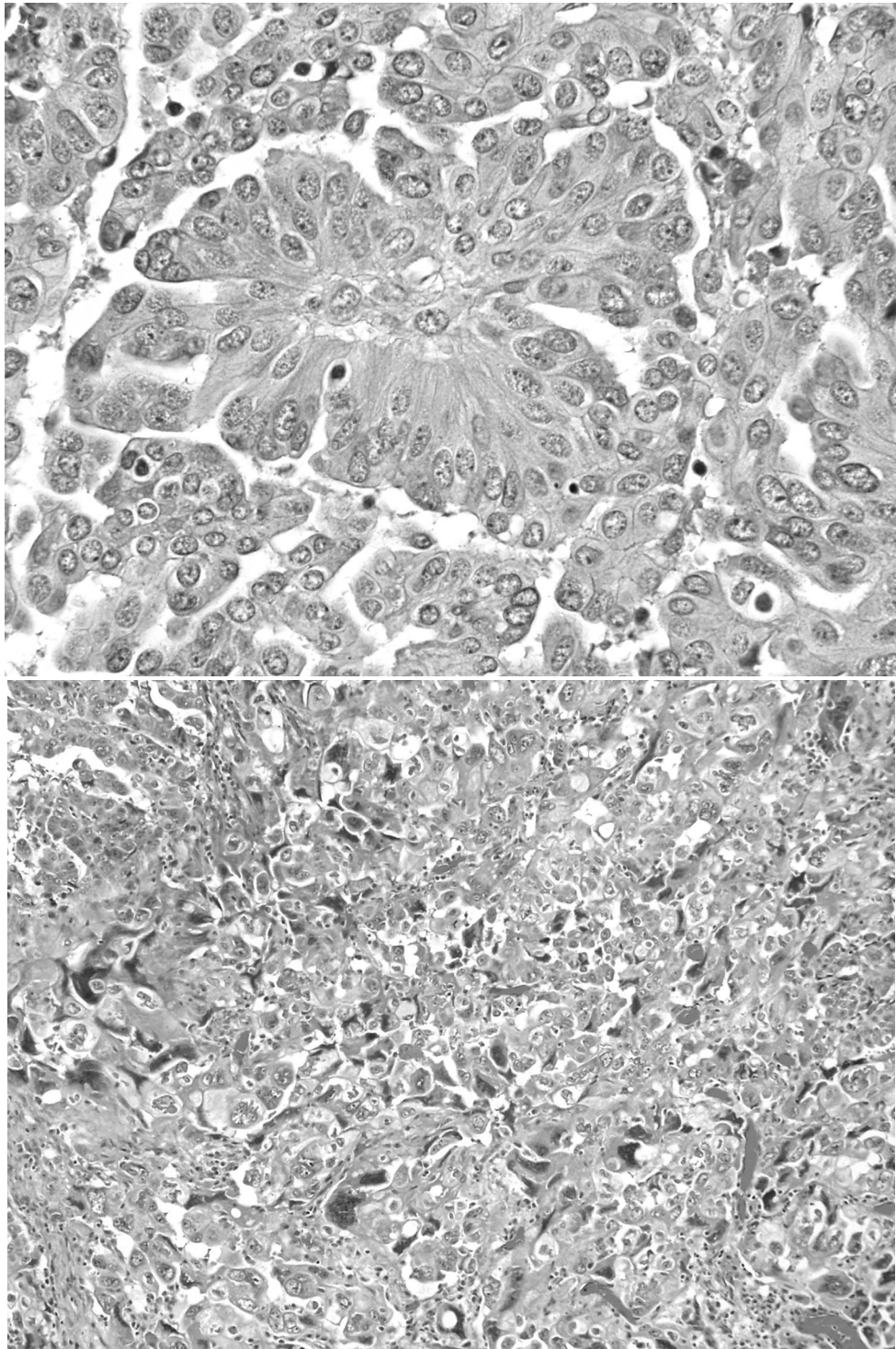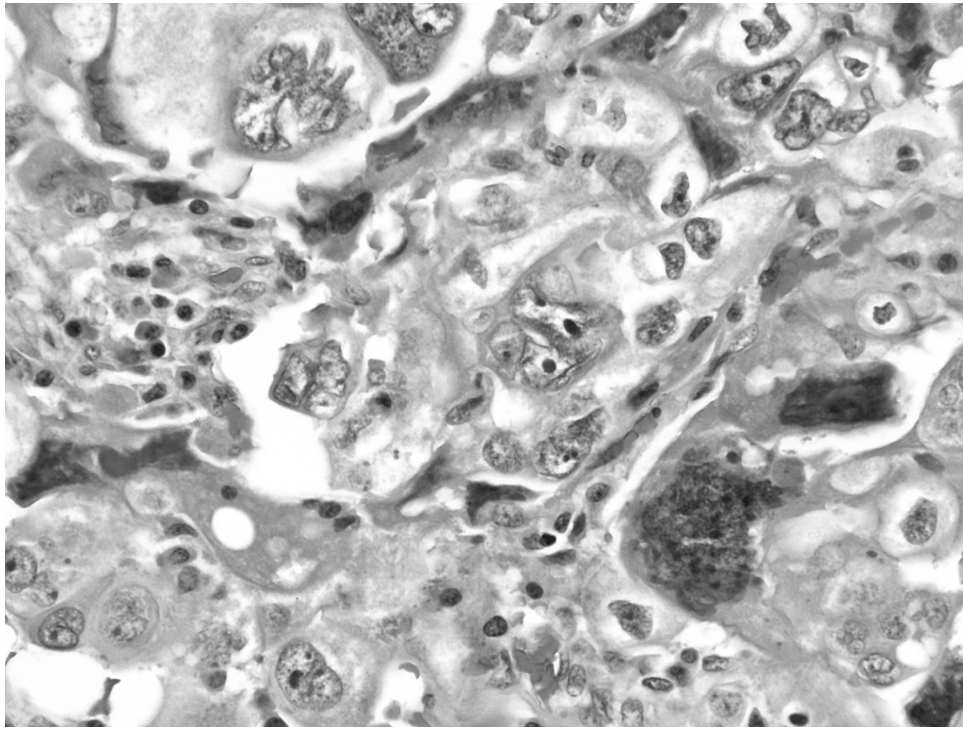
Figure 3.
High-grade serous carcinoma arising in a background of low-grade serous carcinoma. (A and B) Low-grade component. (C and D) High-grade component.
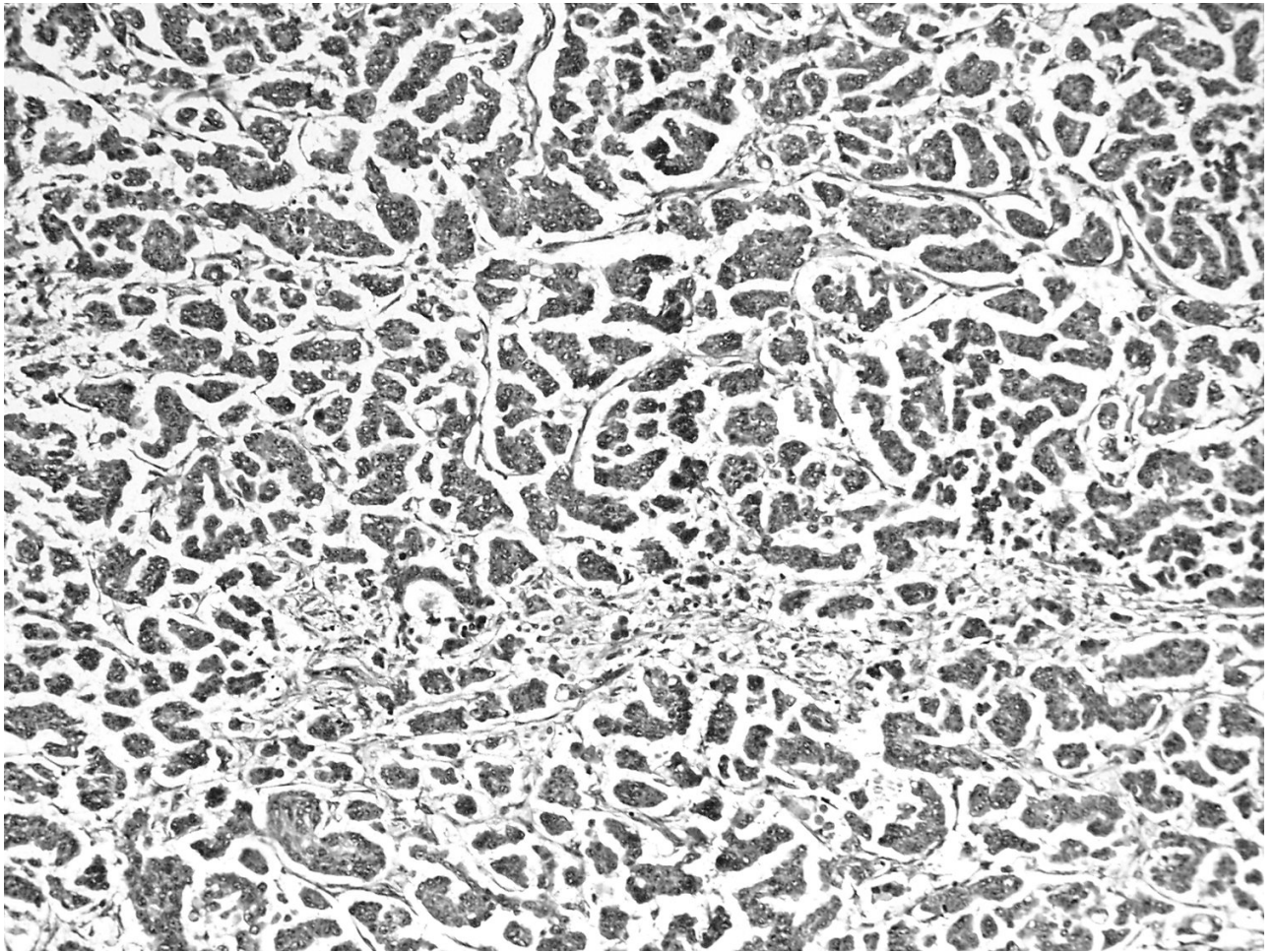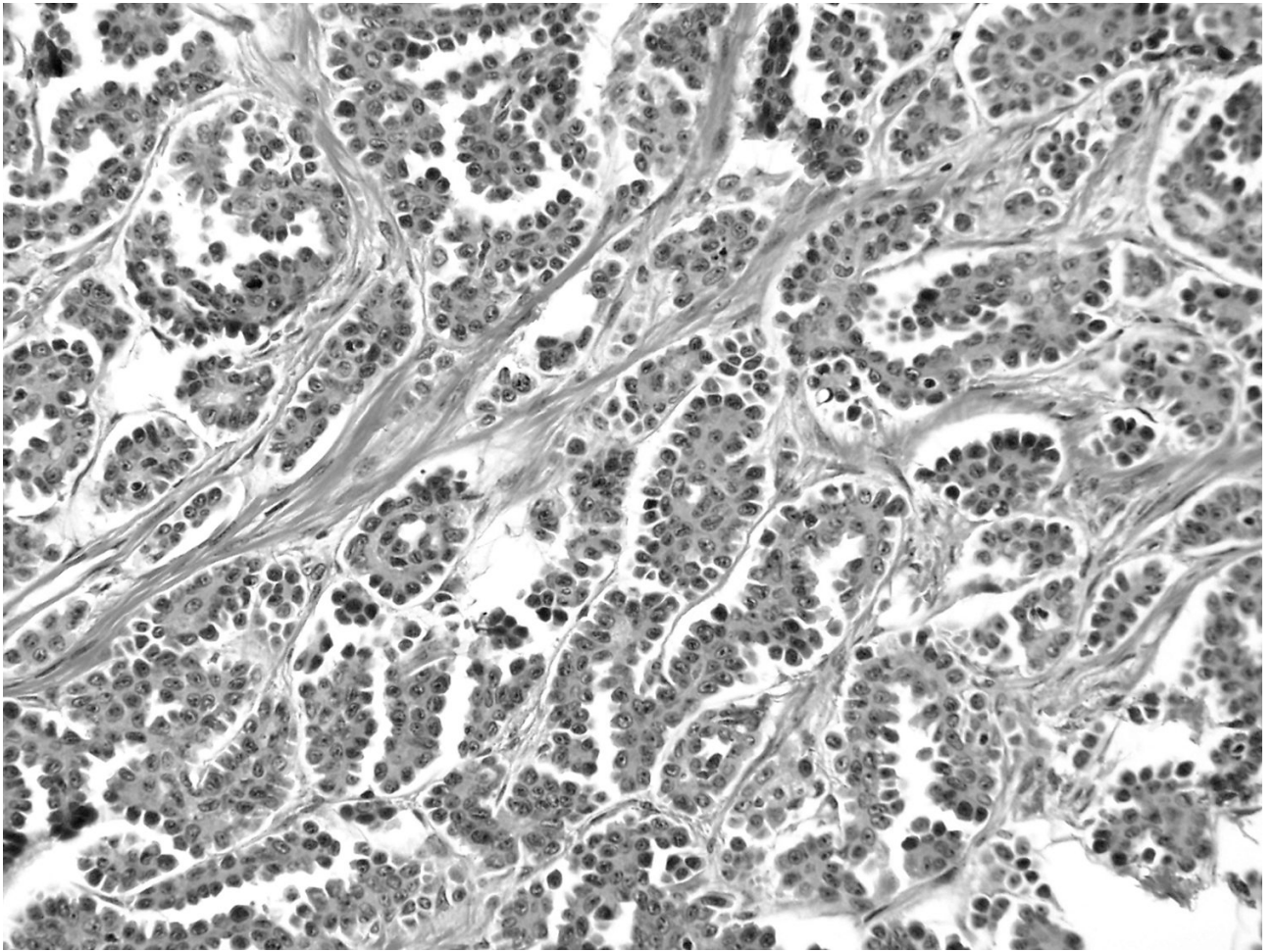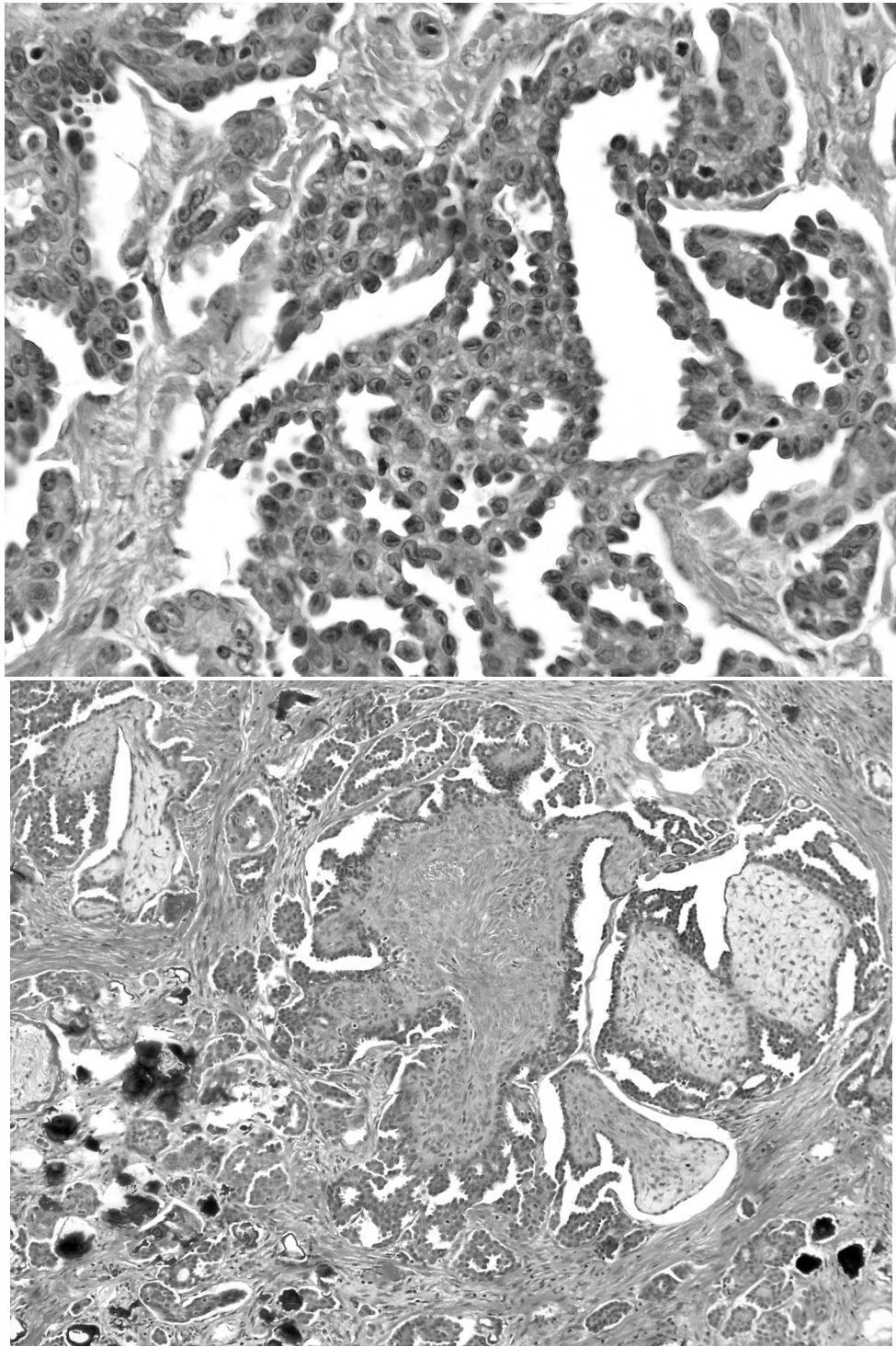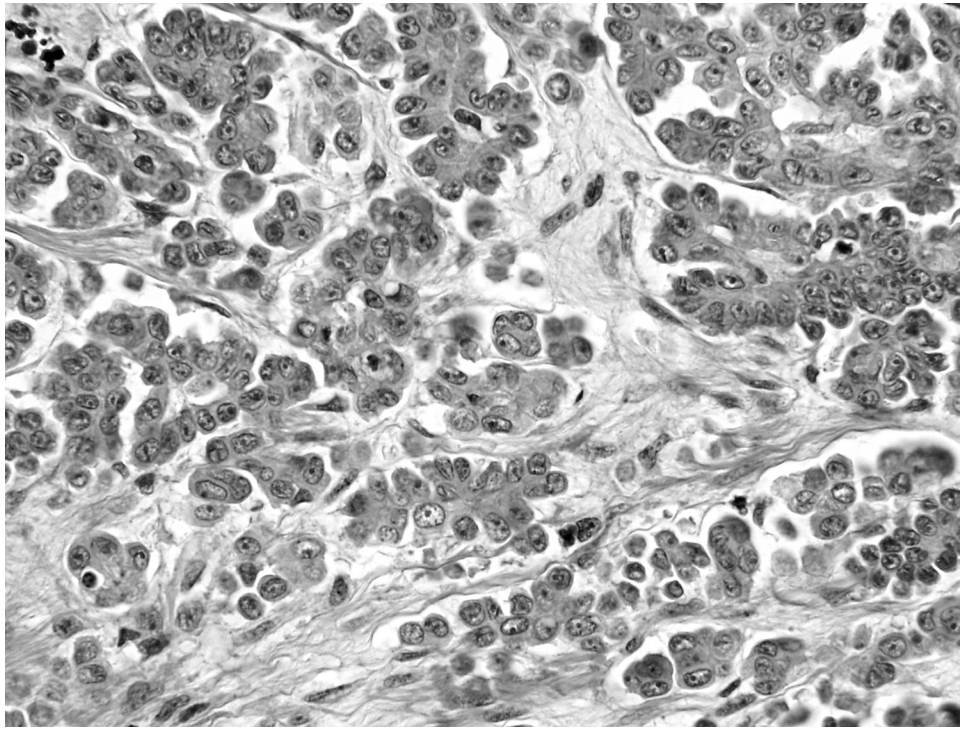
Figure 4.
Low-grade serous carcinoma. (A and B) Carcinoma invades stroma and is composed of small micropapillae. (B) The papillae either lack fibrous cores or contain thin attenuated fibrous cores and are surrounded by clear spaces. (C) In some instances, the papillae fuse to form anastamosing patterns. (D) Larger papillae may be seen. Psammoma bodies are also present. (E) The nuclei of the micropapillae are small, uniform, and round, with evenly dispersed chromatin and small nucleoli.
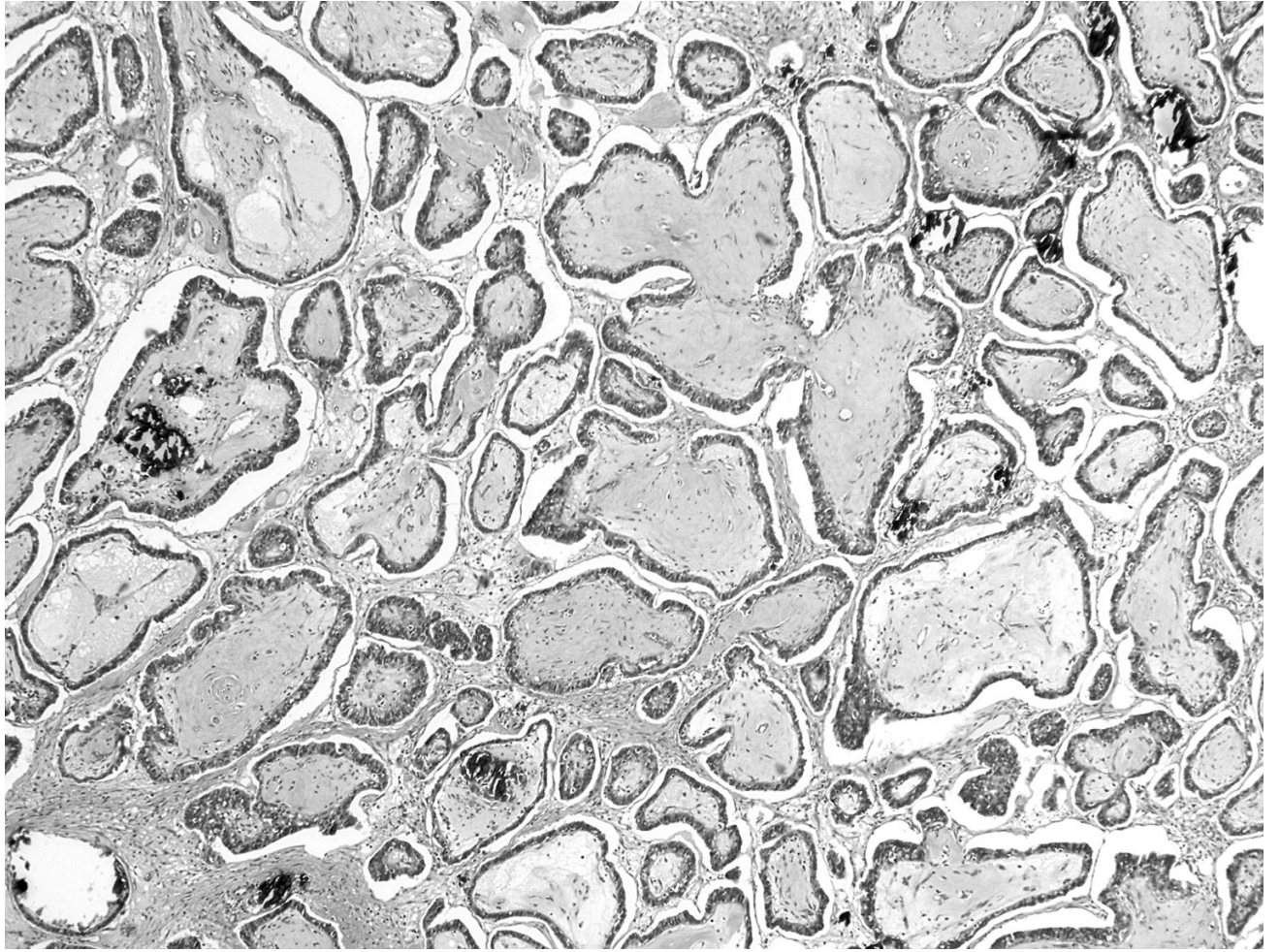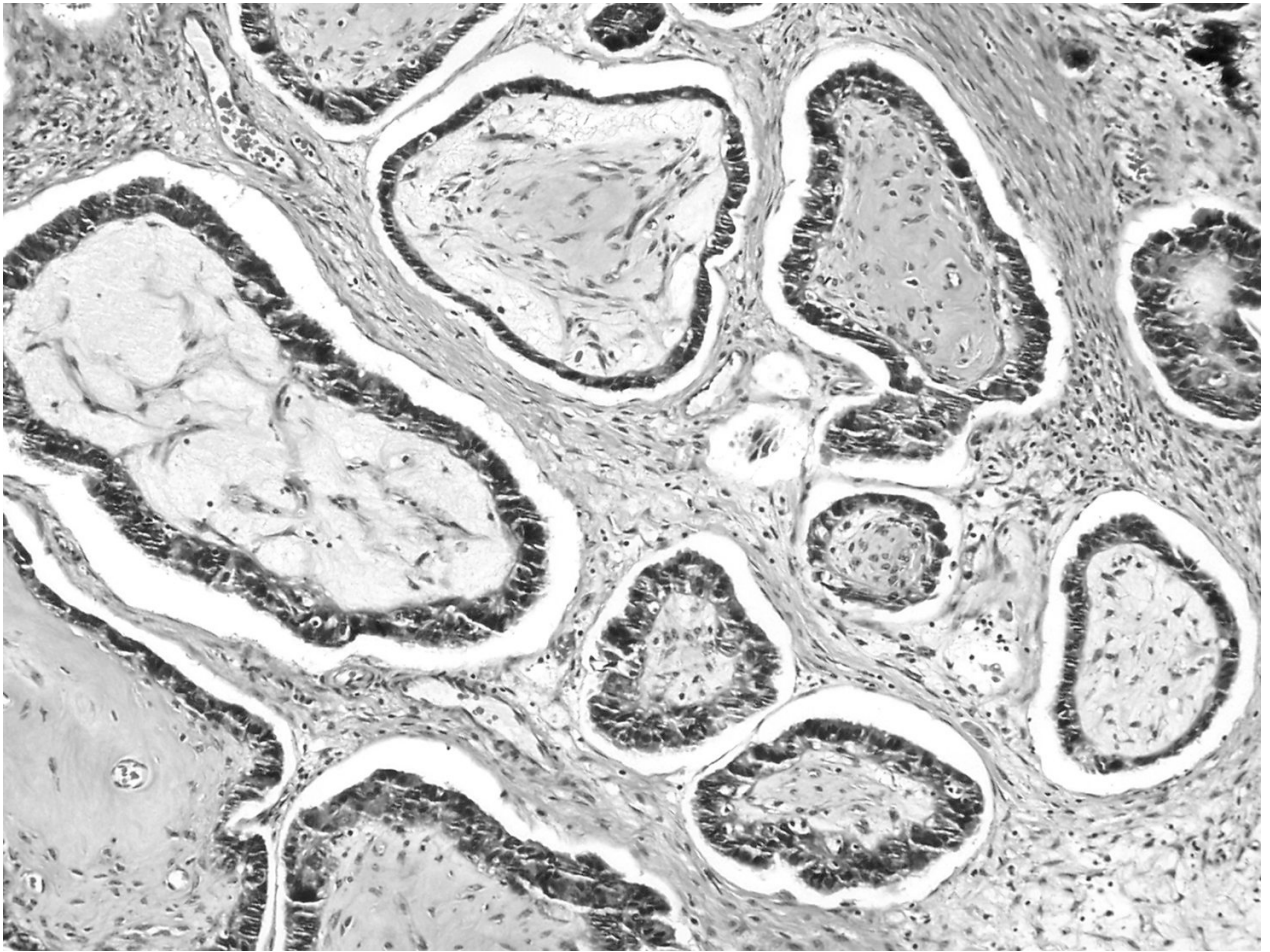
Figure 5.
Low-grade serous carcinoma with inverted macropapillary pattern. (A and B) The invasive papillae are medium to large in size, haphazardly arranged, and surrounded by clear spaces. The papillae are lined by cells with low-grade nuclei similar to those in the conventional micropapillary pattern.
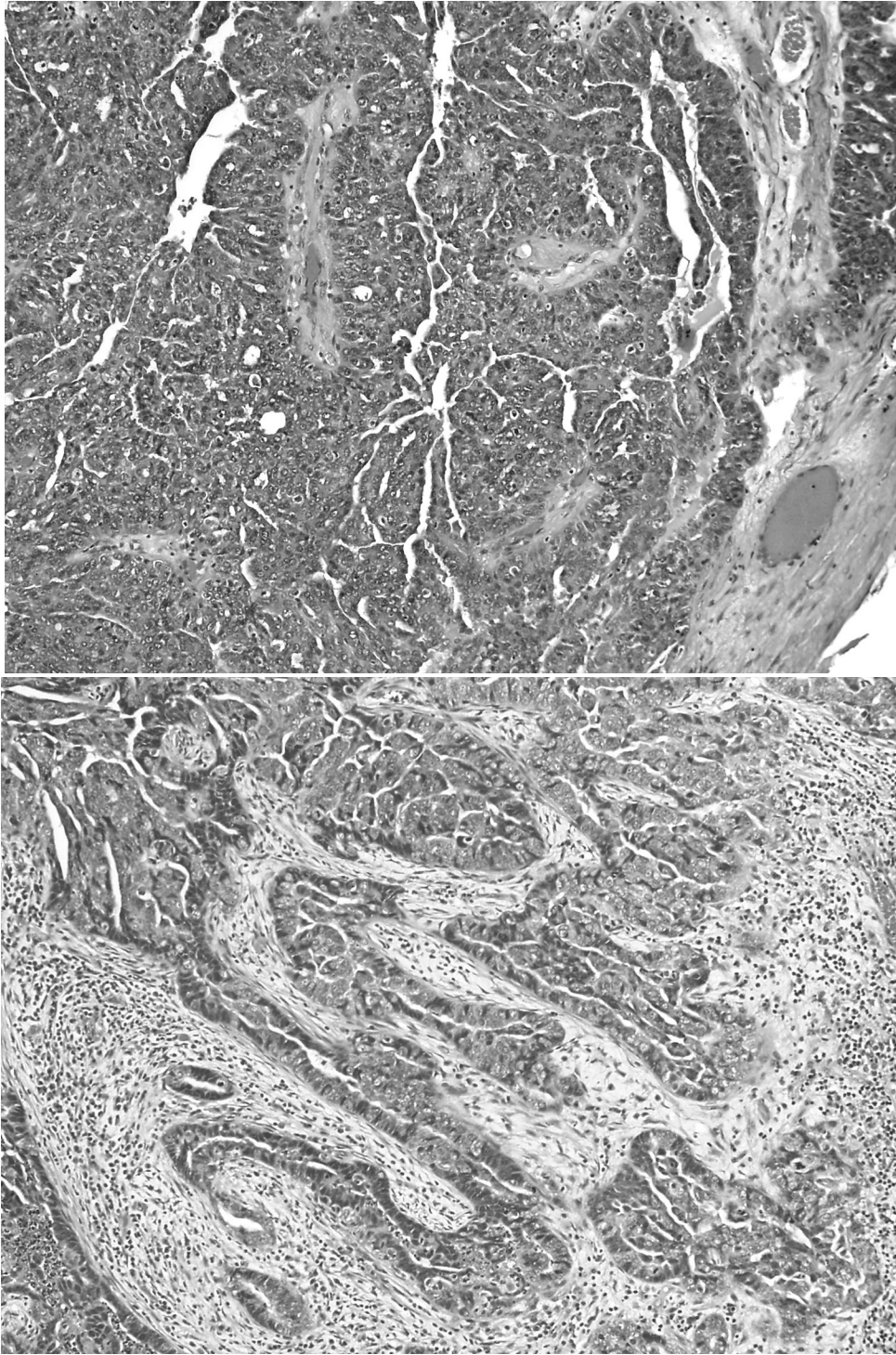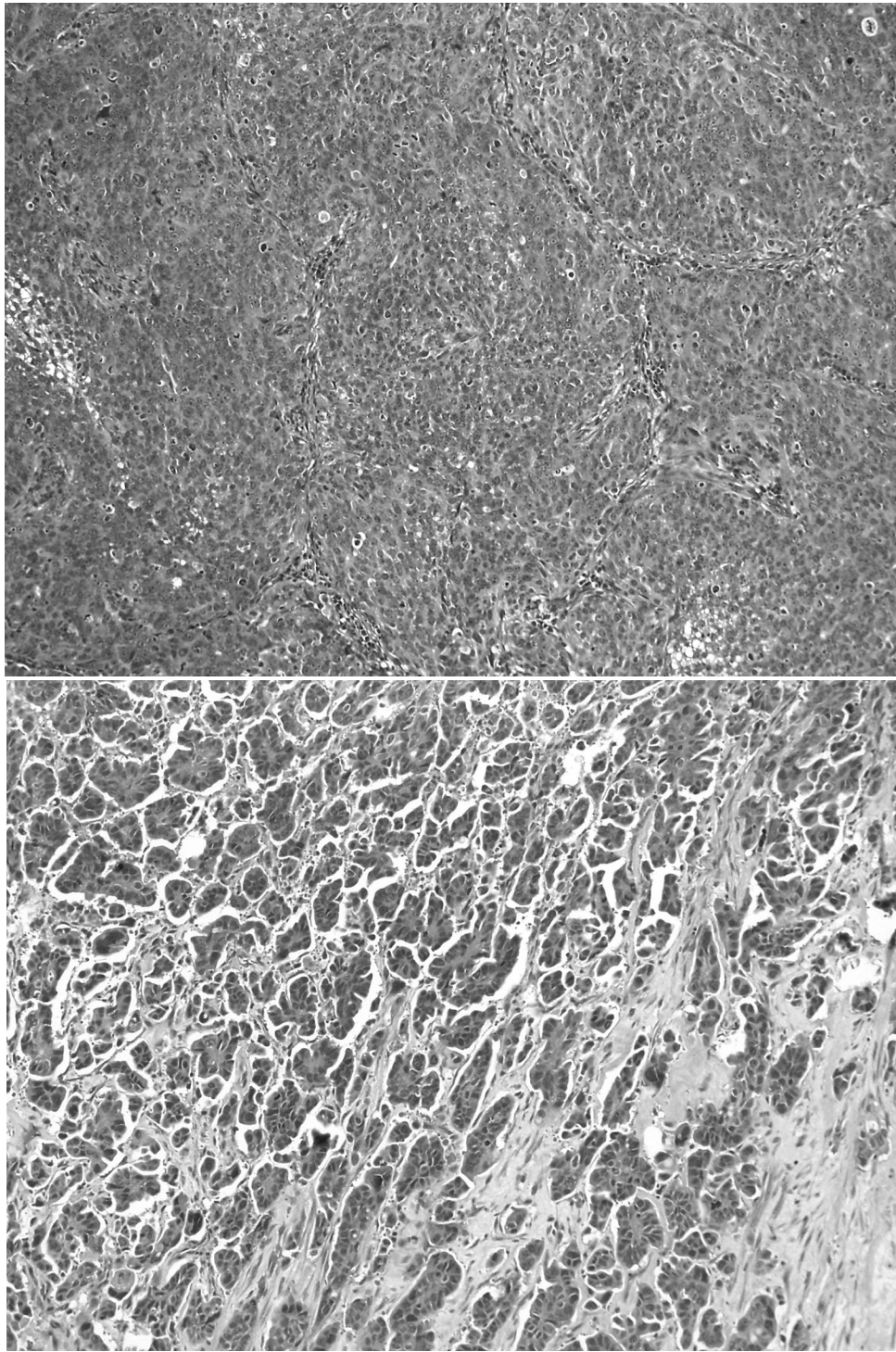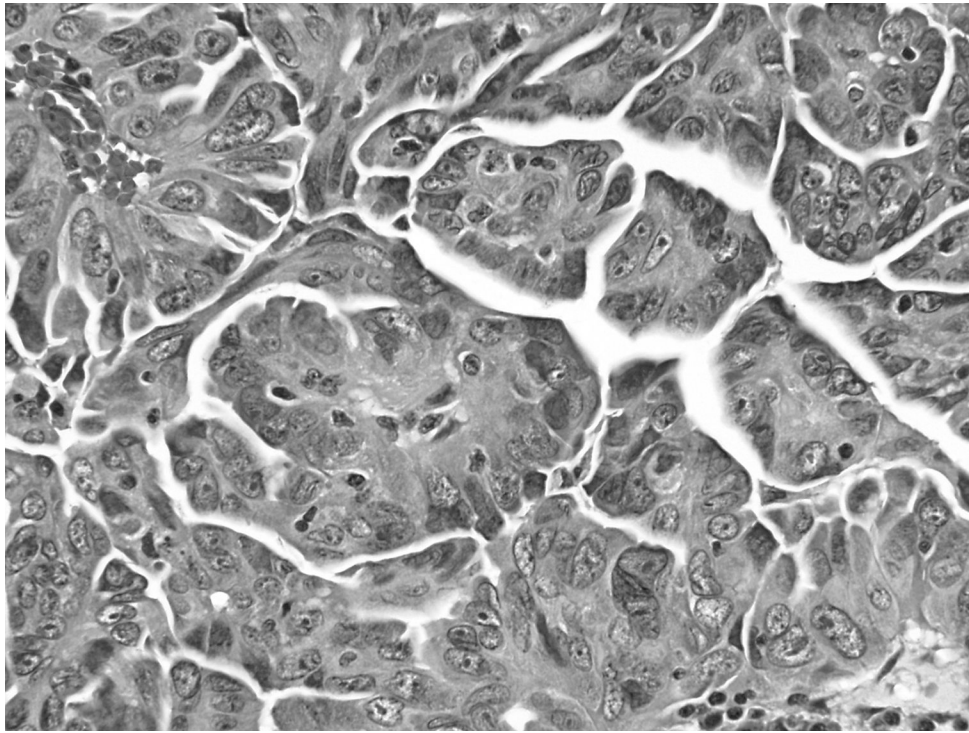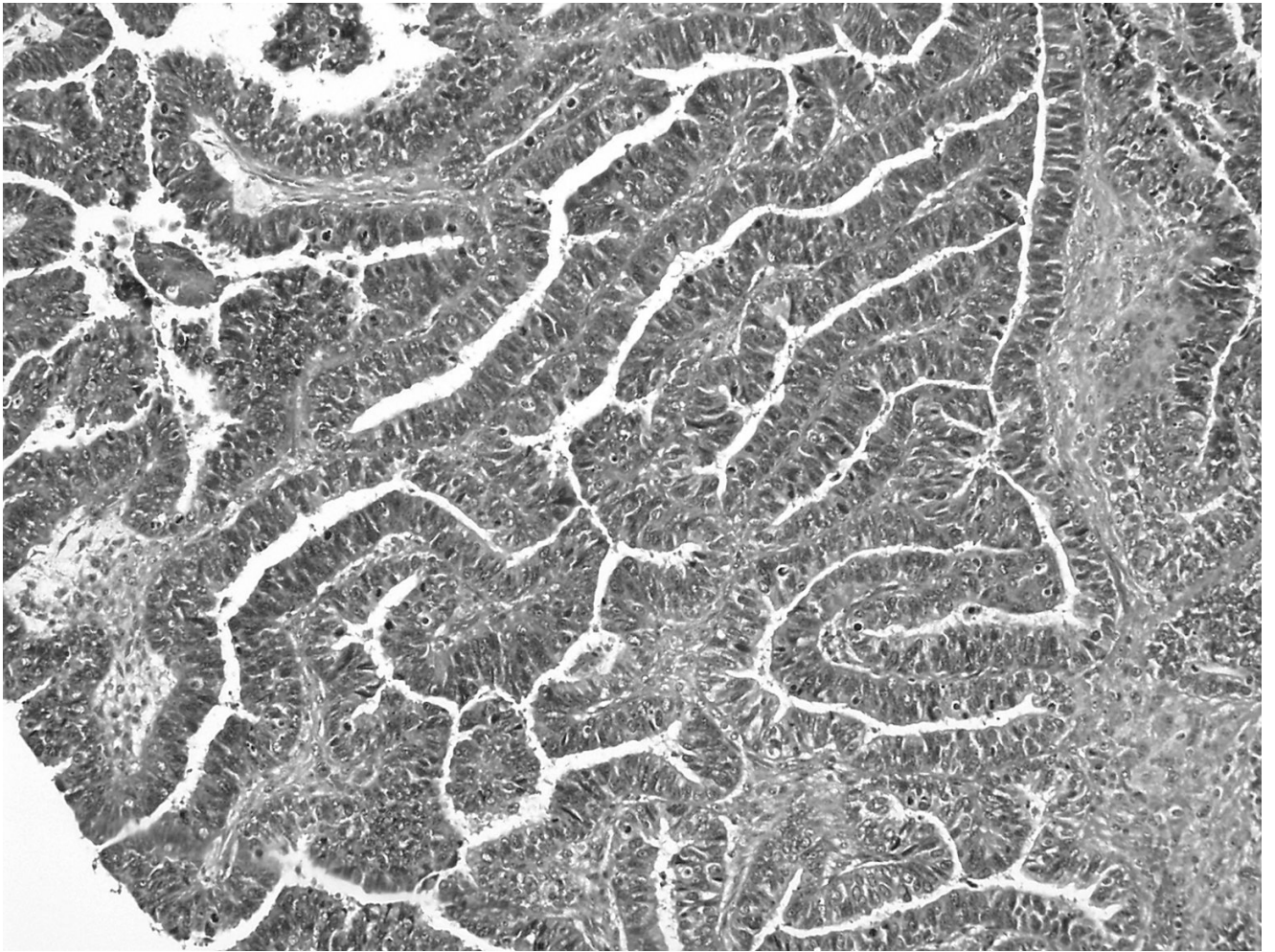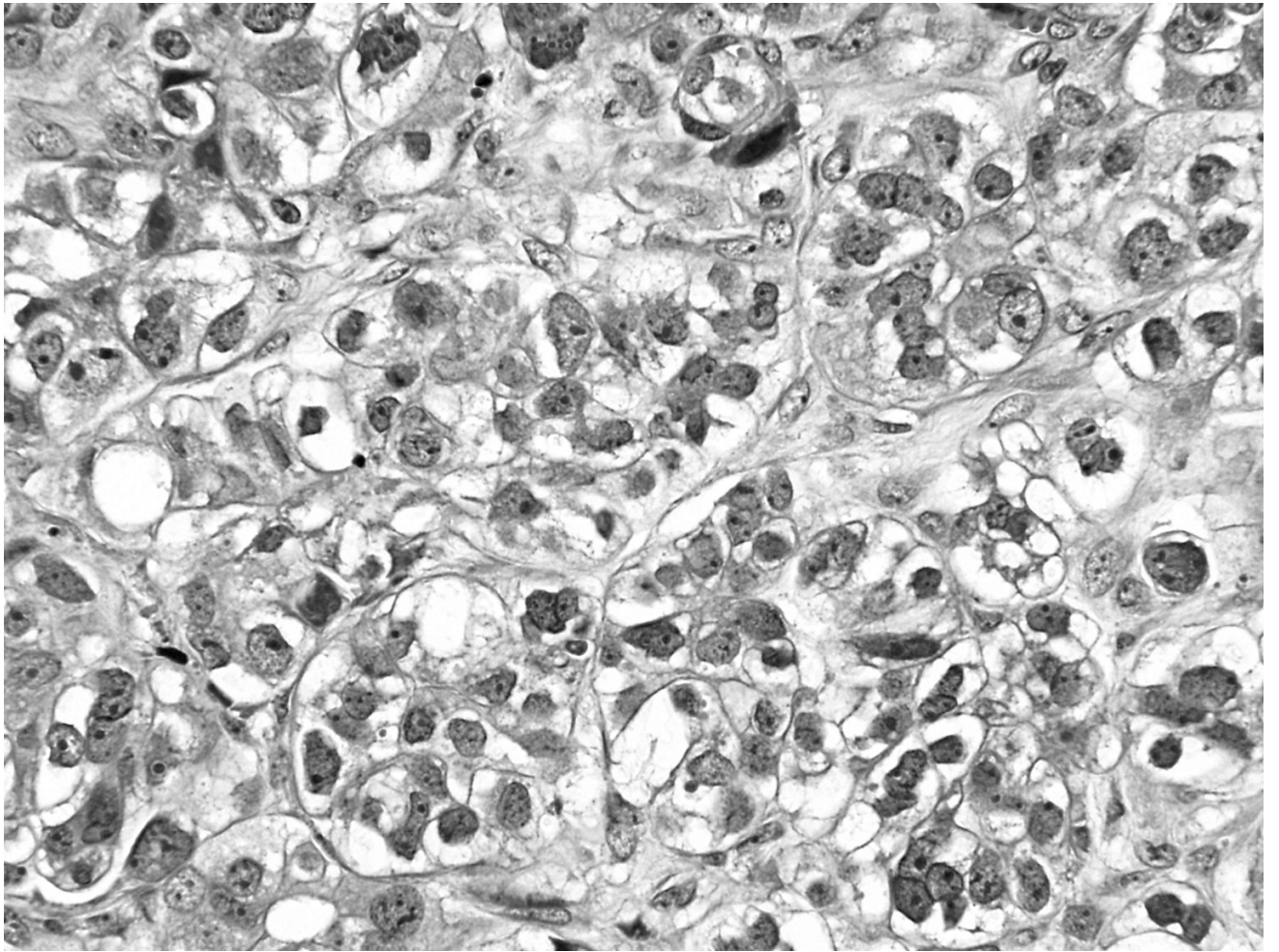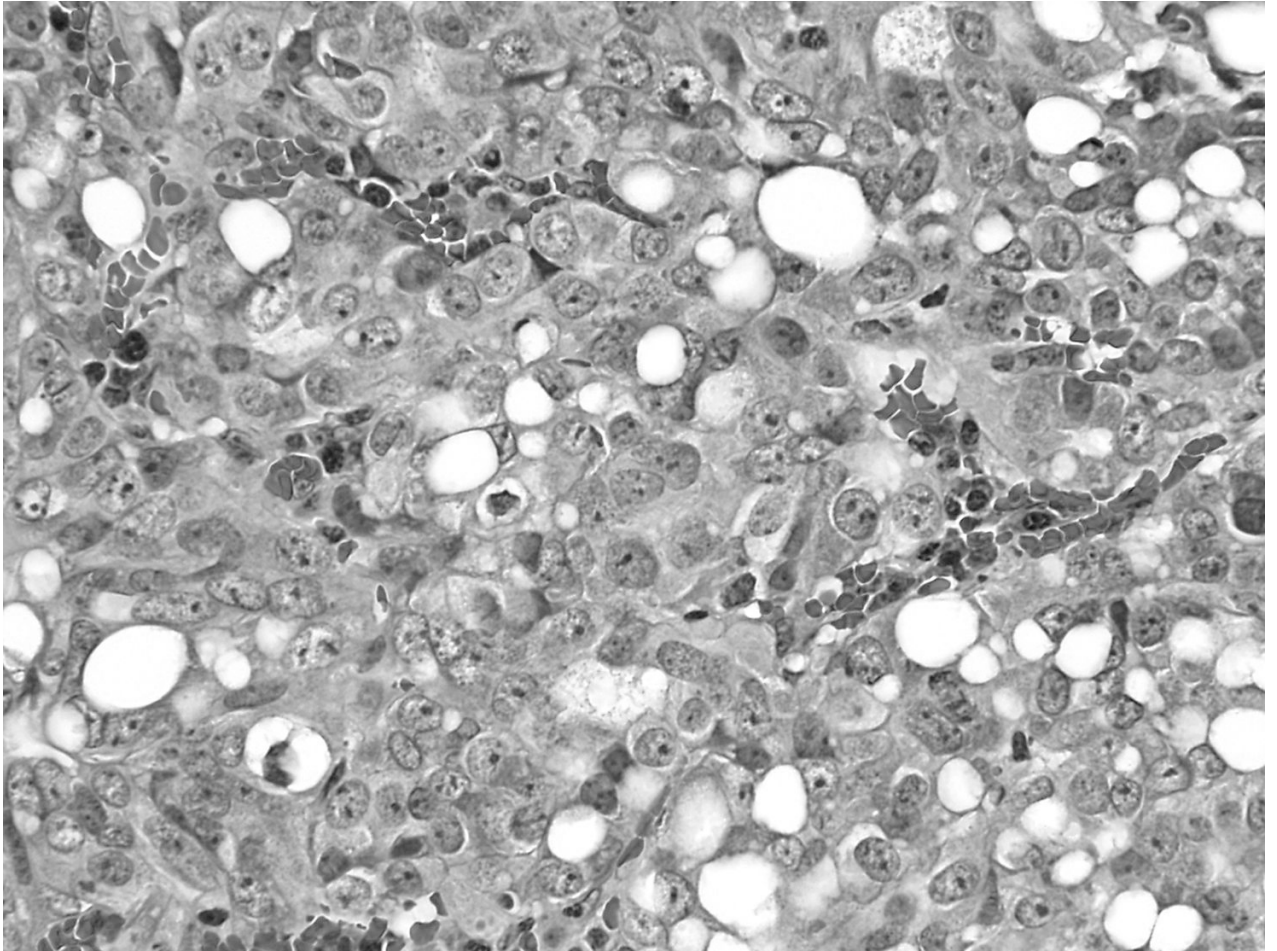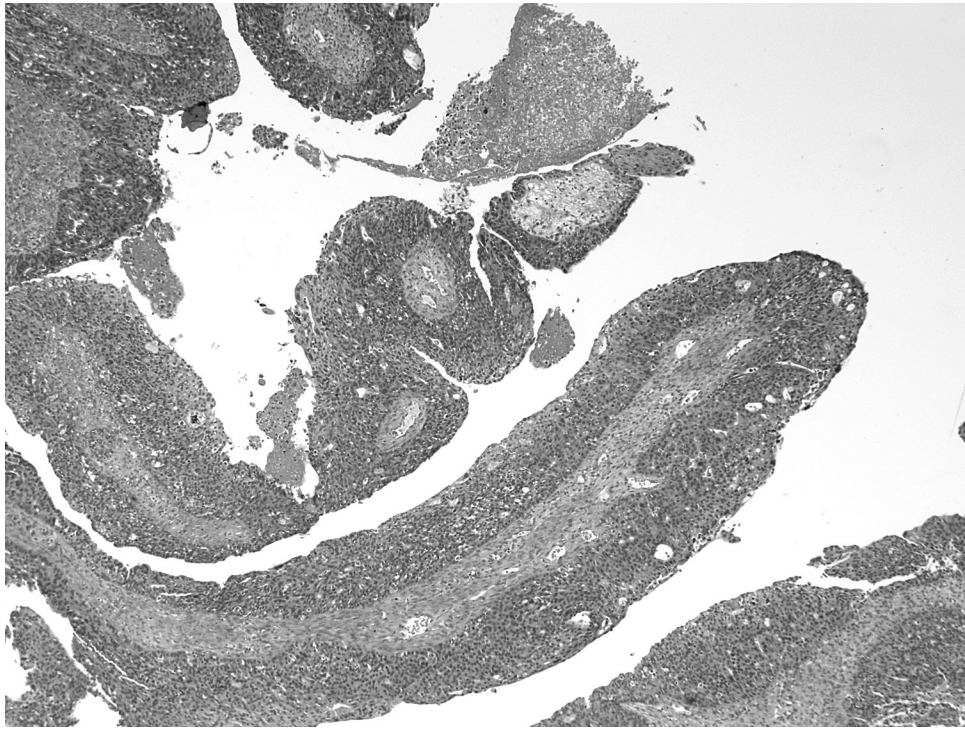
Figure 6.
High-grade serous carcinoma. (A) Typical papillary pattern showing irregular slit-like spaces. (B) Glandular pattern. (C) Diffuse, solid pattern. (D) Micropapillary pattern. (E) In contrast to low-grade serous carcinoma (Fig. 4E), the nuclei of high-grade serous carcinoma are larger with greater pleomorphism and larger nucleoli. (F) Endometrioid carcinoma-like pattern. (G) Serous carcinoma with clear cytoplasm. (H) Transitional cell carcinoma-like pattern. (I) Signet ring change simulating signet ring cells of metastatic adenocarcinoma involving the ovary.
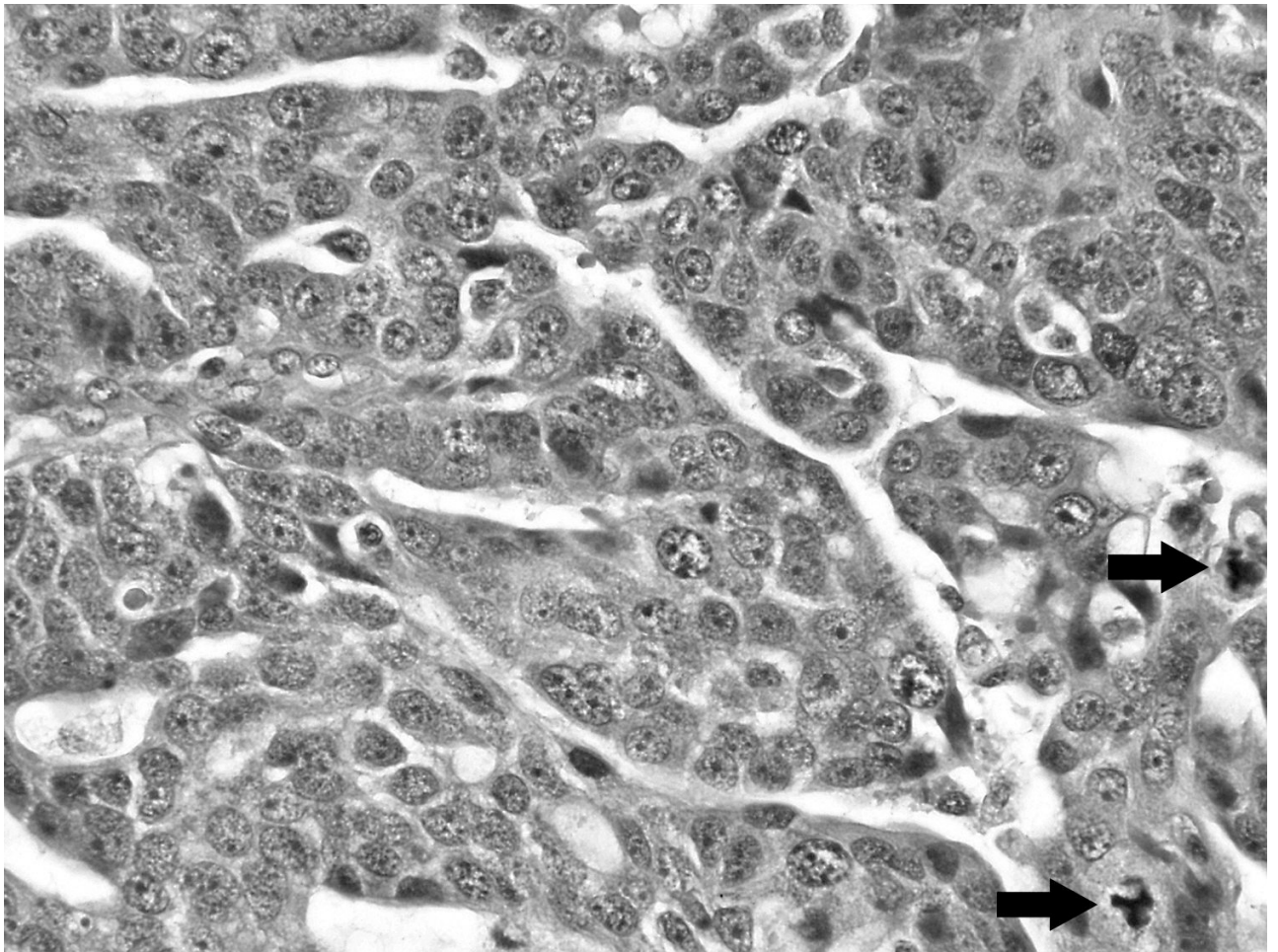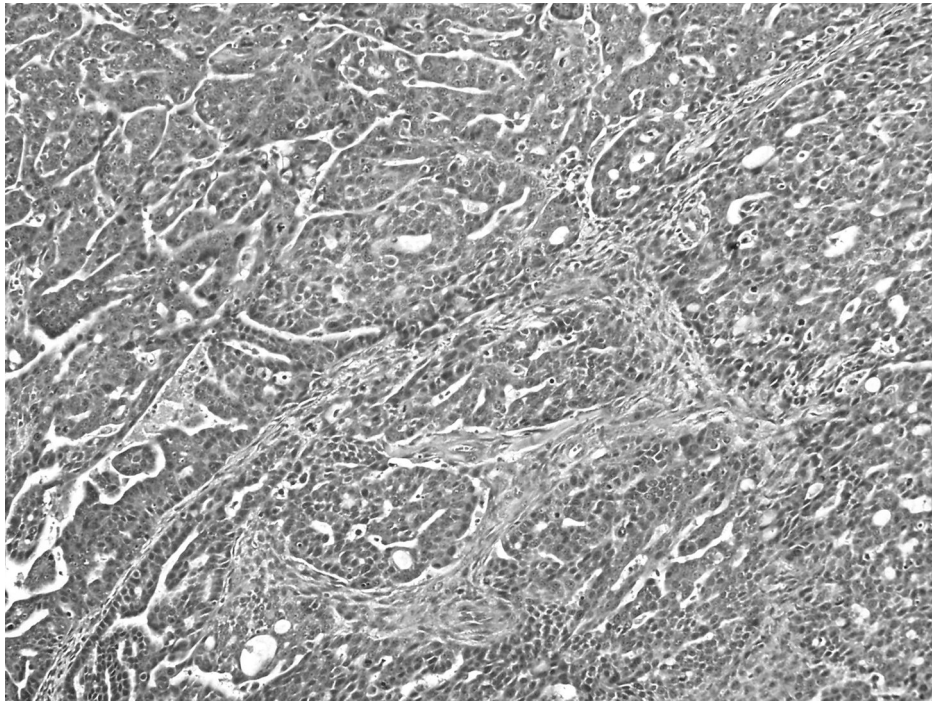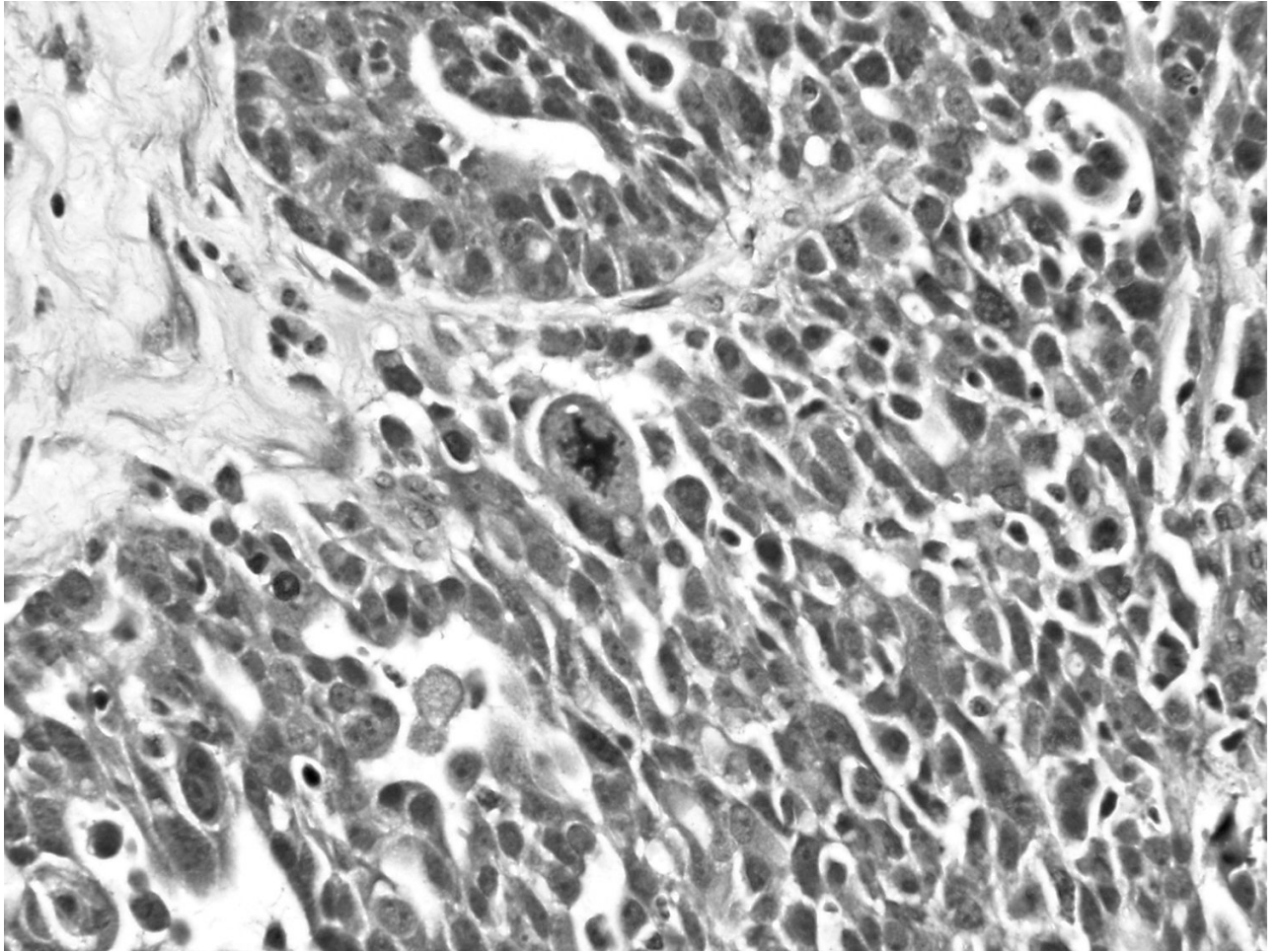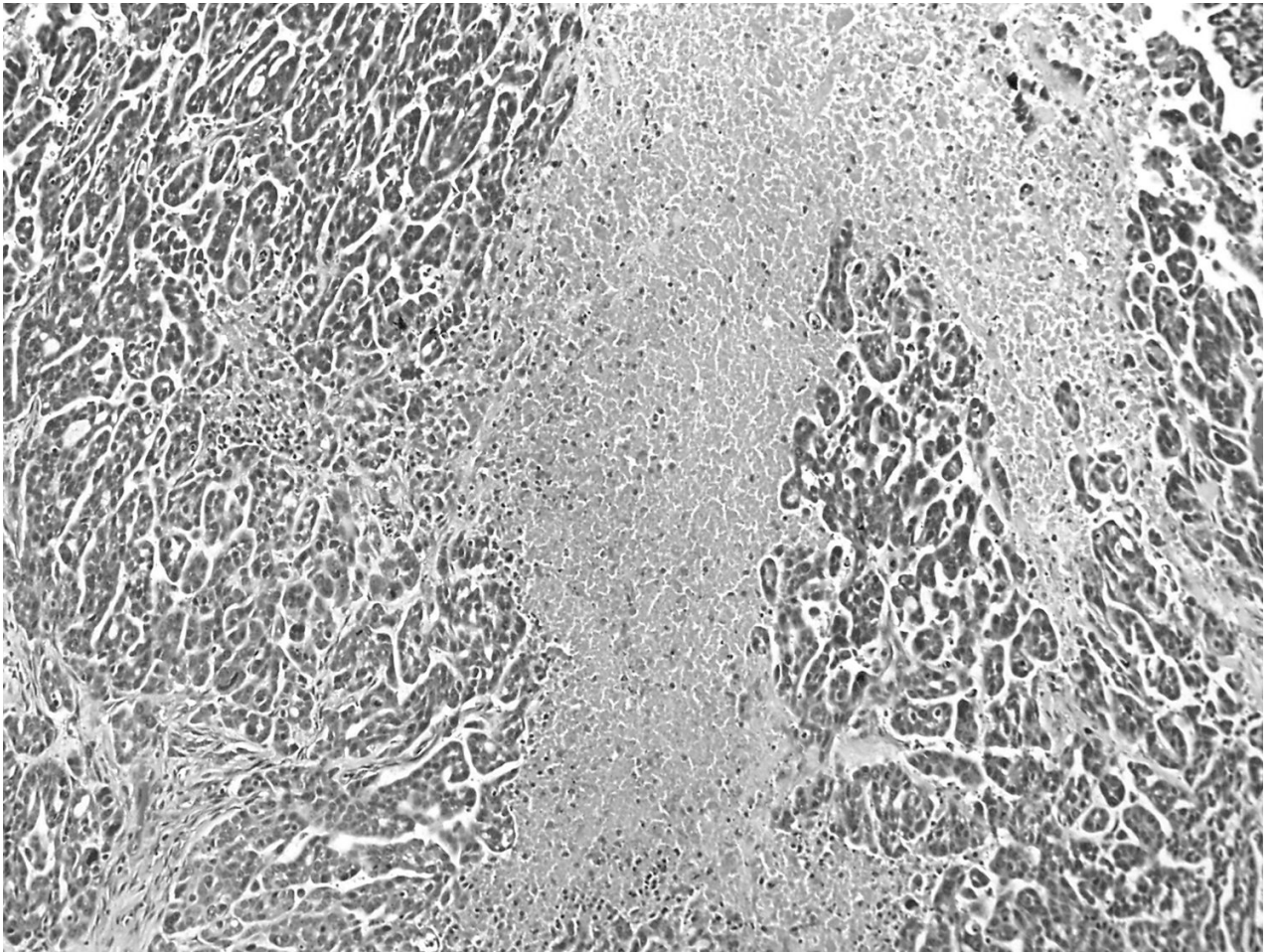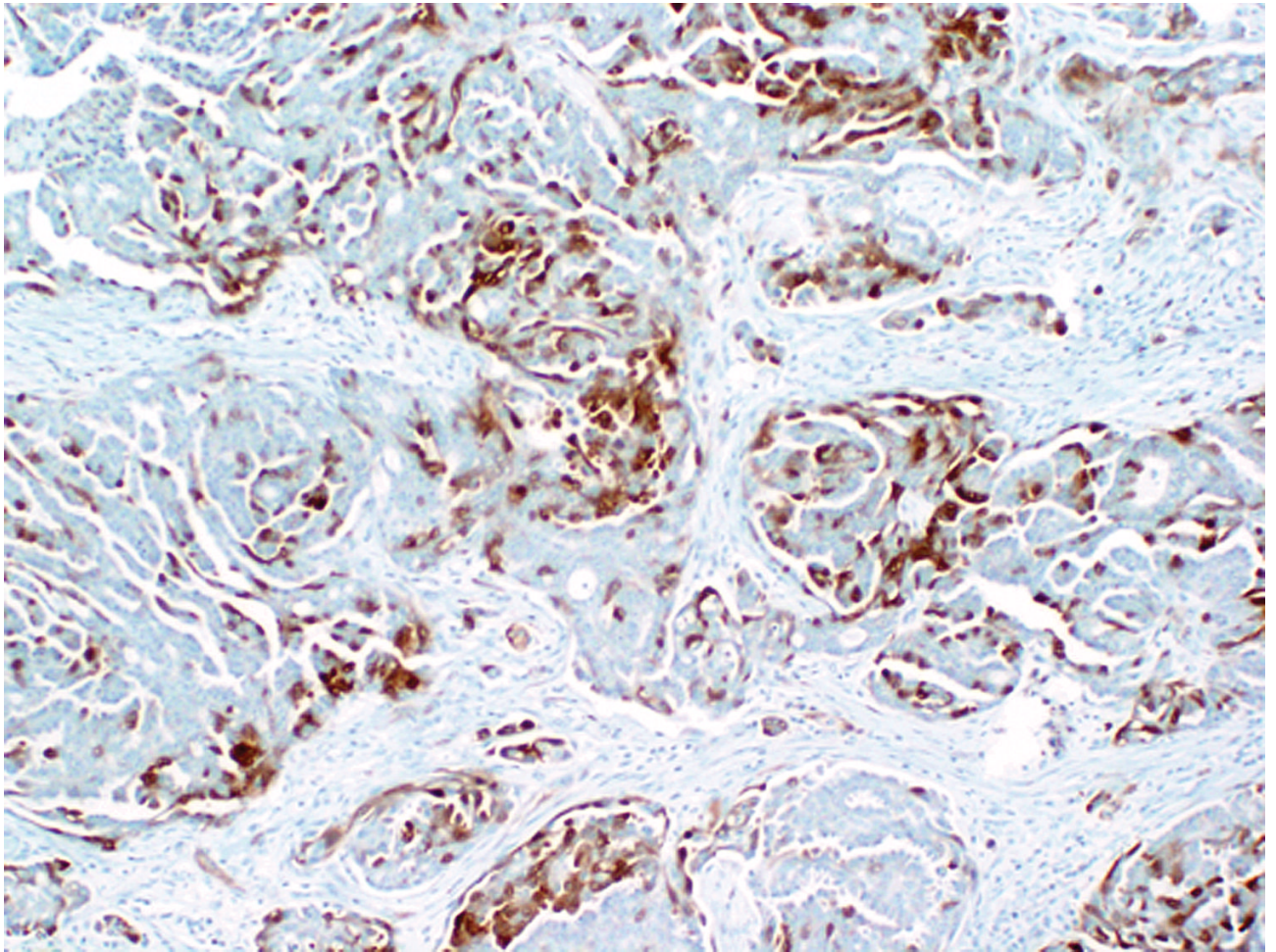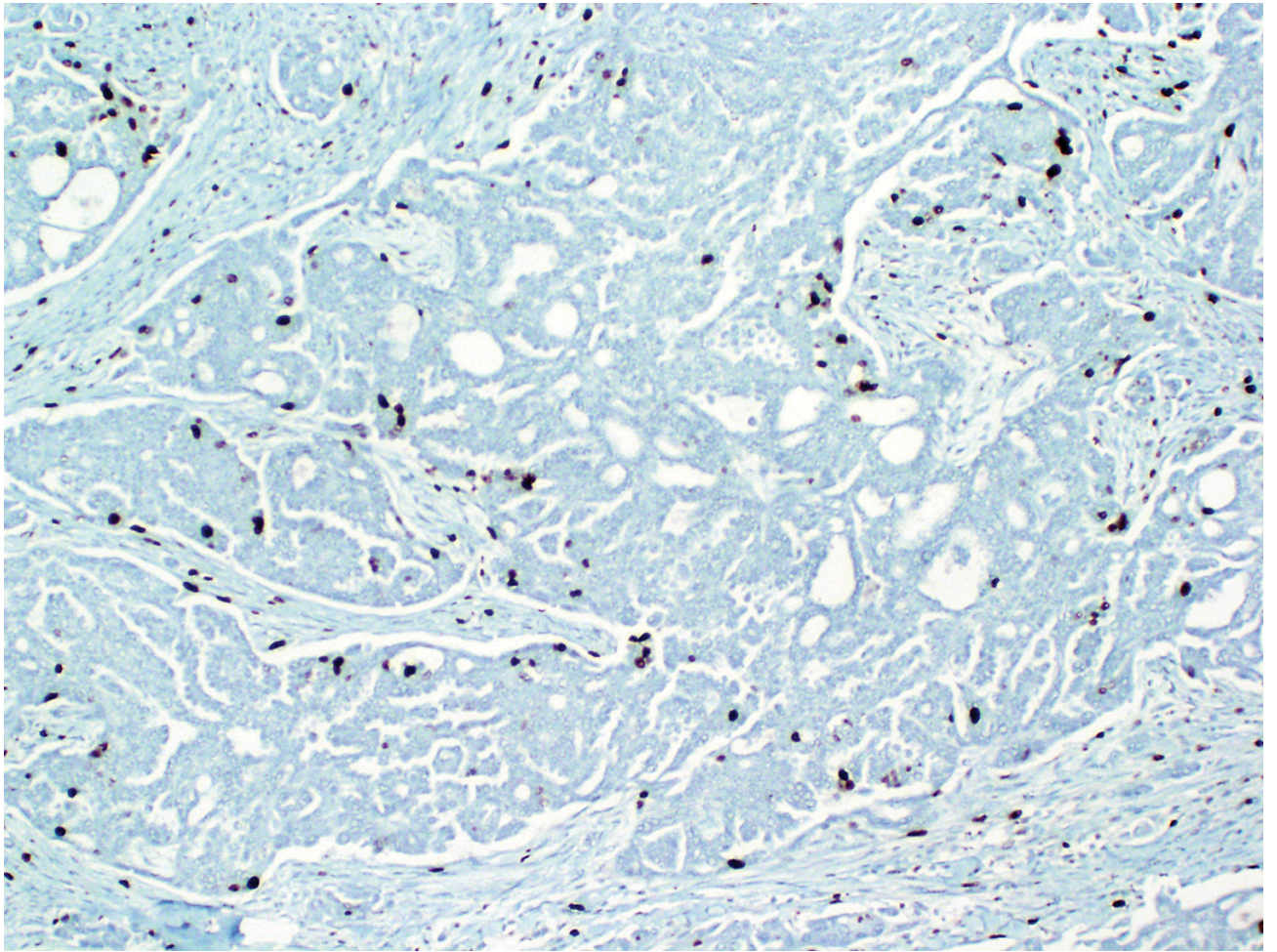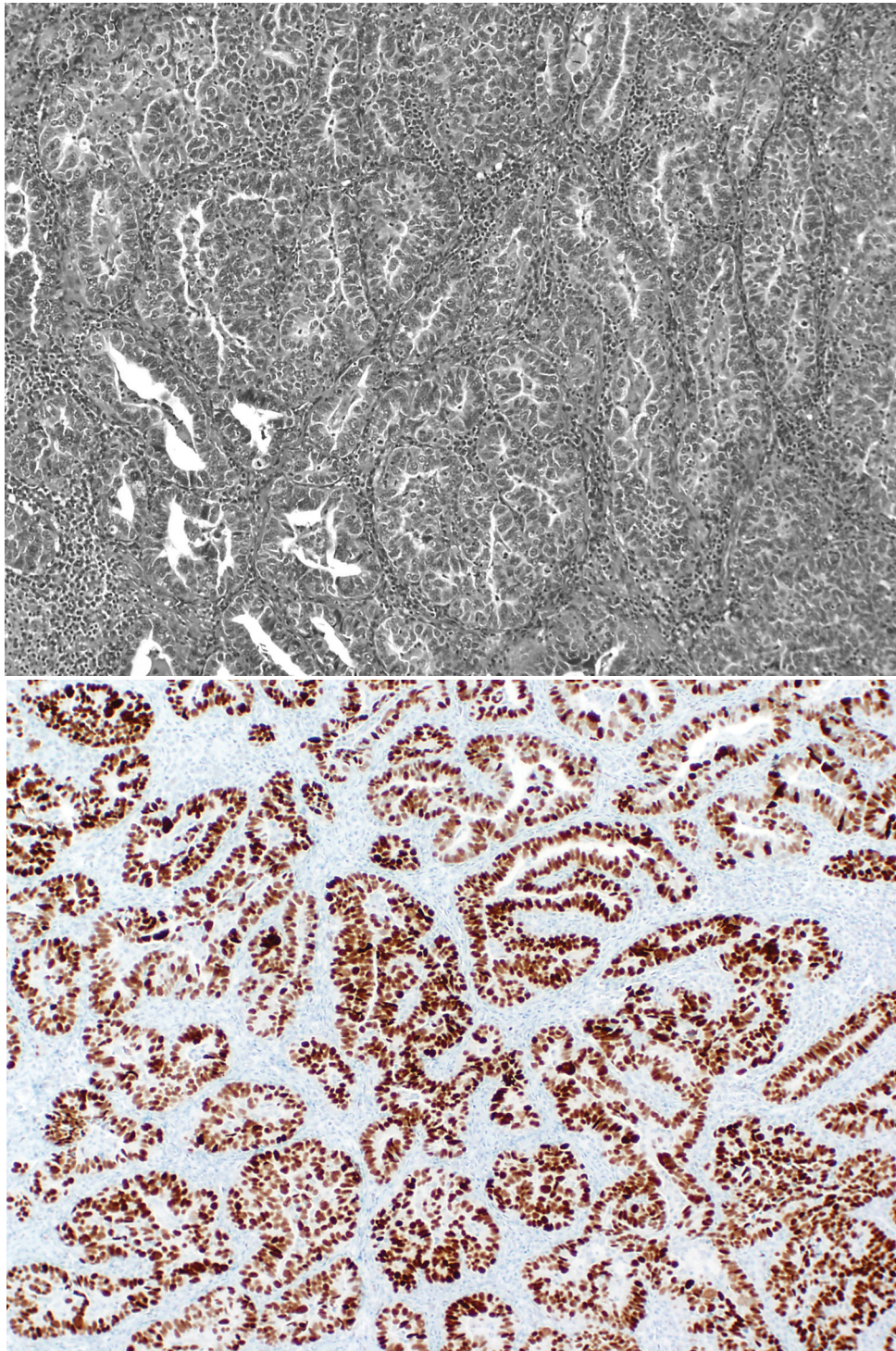
Figure 7.
High-grade serous carcinoma with "grade 2 nuclei." (A and B) The nuclei are more uniform and smaller than in typical high-grade serous carcinomas but larger than low-grade serous carcinoma. The combination of (B) slightly increased variation in nuclear size, coarser chromatin, nucleolar prominence, and increased mitotic activity (*arrows*), (C) abnormal mitotic figures, and (D) necrosis allows for distinction from low-grade serous carcinoma.
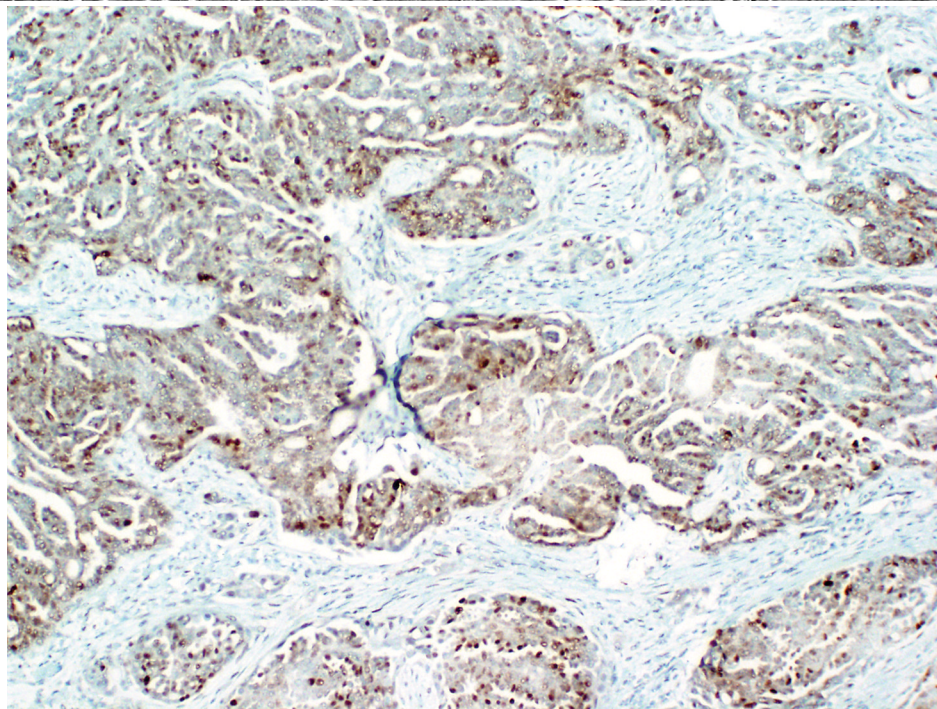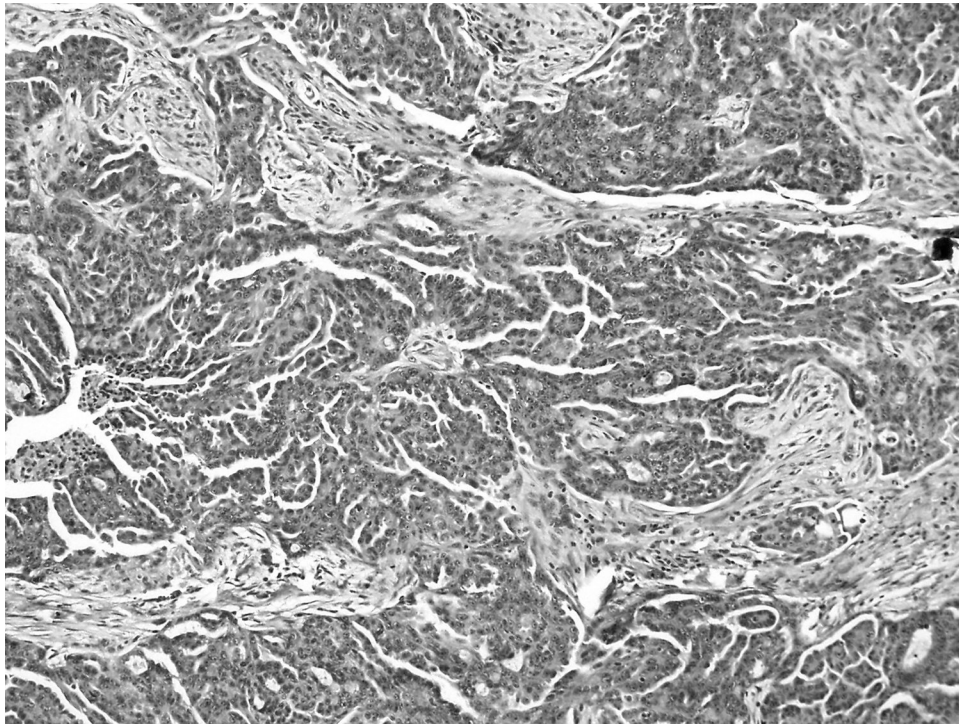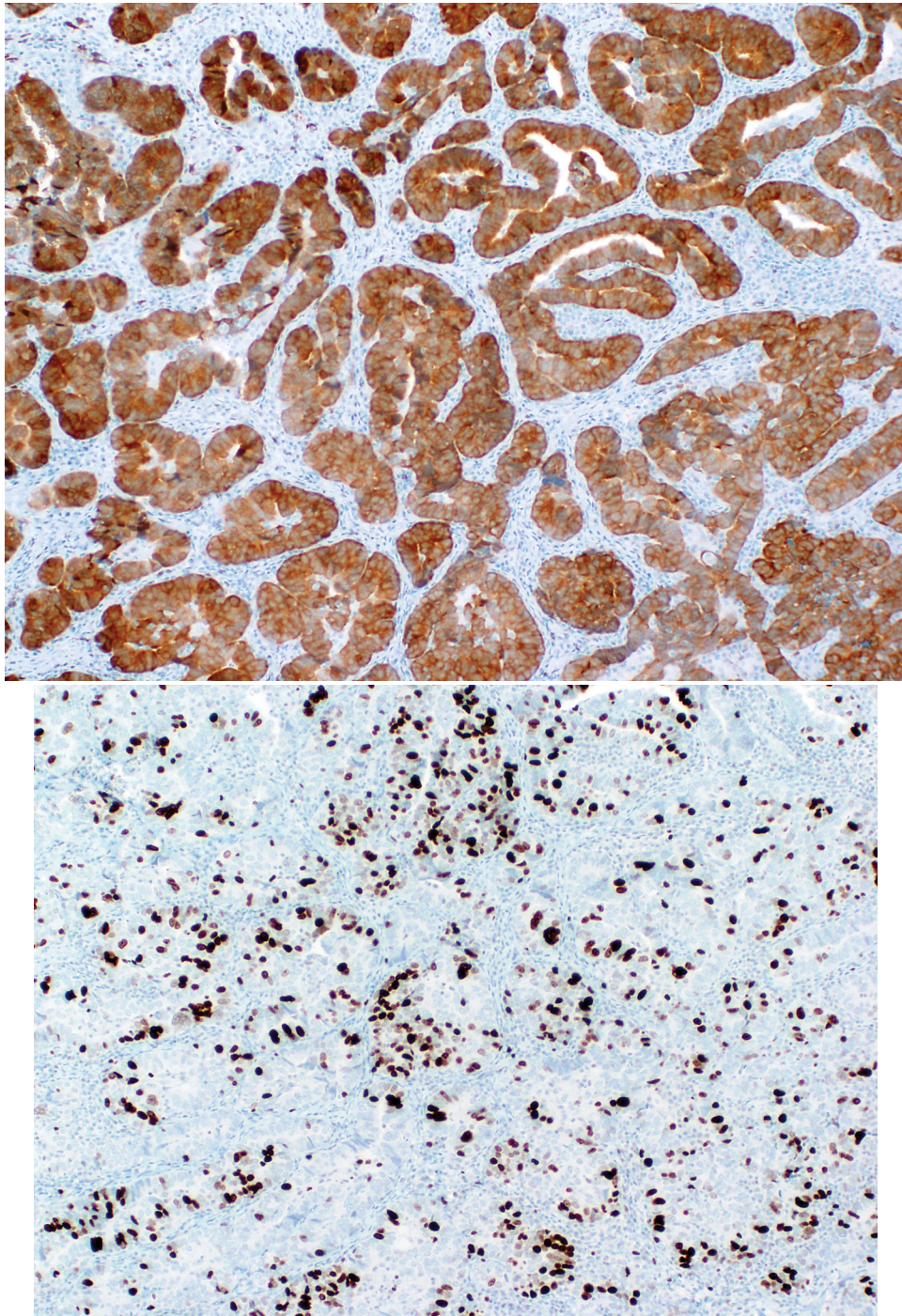
Figure 8.
Immunohistochemical staining for p53, p16, and Ki-67 in serous carcinoma. *Low-grade carcinoma*: (A) H&E, (B) p53 expression in scattered cells, (C) patchy p16 expression, and (D) low Ki-67 proliferation index. *High-grade carcinoma*: (E) H&E, (F) diffuse p53 expression, (G) diffuse p16 expression, and (H) high Ki-67 proliferation index. It should be noted that the H&E appearance in (E) is suggestive of a high-grade endometrioid carcinoma, but the immunophenotype is consistent with high-grade serous carcinoma.

Table 1

Pathogenesis of low-grade vs. high-grade ovarian serous carcinoma

	Low-grade serous carcinoma	High-grade serous carcinoma
Precursor lesion	Adenofibroma/cystadenoma →APST → non-inv MPSC → inv MPSC	Tubal intraepithelial carcinoma[*]
Level of chromosomal instability	Low	High
Genes typically mutated	• KRAS • BRAF • ERBB2	*TP53*
Tempo of tumor development	Slow, step-wise	Rapid

Key: APST, atypical proliferative serous tumor; inv MPSC, invasive micropapillary serous carcinoma; non-inv MPSC, non-invasive micropapillary serous carcinoma; and [*], Currently, precursor lesions in the ovaries or peritoneum have not been firmly established, and it appears that approximately half of high-grade serous carcinomas are associated with tubal intraepithelial carcinoma.

Table 2

Clinicopathologic features of low-grade and high-grade serous carcinomas

	Low-grade serous carcinoma	High-grade serous carcinoma
Age (mean)	45–57 years	55–65 years
Stage >I	>90%	>90%
Bilaterality	74–77%	84%
Growth pattern	• Micropapillary-rich • Macropapillary uncommonly	• Large papillae • Glandular • Solid • Micropapillary occasionally
Necrosis	Absent	Frequent
Nuclei	Low-grade	High-grade
Mitotic index	Low	High
In vitro drug resistance assays	• More frequent extreme drug resistance to paclitaxel and carboplatin • Less frequent extreme drug resistance to etoposide and doxorubicin	• Less frequent extreme drug resistance to paclitaxel and carboplatin • More frequent extreme drug resistance to etoposide and doxorubicin
5-year survival for stage >I	40–56%	9–34%

Table 3

Immunohistochemical features of low-grade and high-grade serous carcinomas[*]

	Low-grade serous carcinoma	High-grade serous carcinoma
p53 (pattern of expression)	Negative, focal, or patchy	Diffuse expression
p16 (pattern of expression)	Negative, focal or patchy	Diffuse expression
Ki-67 (proliferation index)	Low	Moderately elevated to high

[*] Typical staining patterns are listed here, but overlap between low- and high-grade serous carcinomas frequently occurs.