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BRAF^{V600E} mutations and immunohistochemical expression of VE1 protein in low-grade serous neoplasms of the ovary

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Abstract

Aims—The most common *BRAF* mutation in ovarian low-grade serous neoplasms (LGSNs) involves substitution of valine by glutamic acid at position 600 (V600E). Small studies have demonstrated high specificity of immunohistochemistry with mutation-specific monoclonal antibody VE1. We sought to investigate expression of VE1 protein in LGSNs and its correlation with *BRAF* mutation-associated histological features and *BRAF* mutation status.

Methods and results—We reviewed pathology reports and available slides from ovarian serous borderline tumours (SBTs) and low-grade serous carcinomas (LGSCs) diagnosed between 2000-2012. VE1 immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections. Tumours with ≥50% positive cells were considered positive. Statistical analyses were performed using SPSS 24.0. Of 121 LGSNs, there were 73 SBTs, 8 SBTs with micropapillary features (mpSBT), and 40 LGSCs (22 primary, 18 metastatic). VE1 was positive in 52% (38/73) of SBTs and 9% (2/22) of primary LGSCs, and in none of the mpSBTs and metastatic LGSCs ($p<0.0001$). Of 76 tumours with known mutation status, 42 (55%) harbored mutations, including *BRAF*^{V600E} (26, 34%), *KRAS*^{G12D} (8, 11%), and *KRAS*^{G12V} (8, 11%). *BRAF*^{V600E} mutations were present in 48% (25/52) of SBTs and 5% (1/22) of LGSCs ($p<0.0001$). VE1 was positive in 96% (25/26) of *BRAF*^{V600E}-mutated tumours and correlated with *BRAF* mutation-associated histological features ($p<0.0001$).

Conclusions—*BRAF*^{V600E} mutations are significantly more common in SBTs than in LGSCs. Immunohistochemical expression of VE1 protein is strongly associated with *BRAF*^{V600E} mutation and *BRAF* mutation-associated histological features. VE1 immunohistochemistry is a reliable method for the detection of *BRAF*^{V600E} mutations.

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Keywords

BRAF^{V600E} mutation; low-grade serous carcinoma; serous borderline tumour; ovary; VE1 immunohistochemistry

Introduction

Low-grade serous carcinomas (LGSCs) of the ovary represent slow-growing, genetically stable neoplasms, typically confined to the ovary, and lacking *TP53* mutations (1–3). LGSCs may occur de novo or evolve in a stepwise fashion from epithelial inclusions to benign serous cystadenomas or adenofibromas and serous borderline tumours (SBTs) (2, 4, 5). SBTs and LGSCs are commonly characterised by activating somatic mutations in *KRAS* oncogene and its downstream mediator, *BRAF*. *KRAS* and *BRAF* mutations are usually mutually exclusive in these tumours, with reported mutation frequencies of up to 54% for *KRAS* and up to 48% for *BRAF*. *BRAF* mutations have been reported in 23-48% of SBTs but in only 0-6% of LGSCs (6–11). *BRAF* encodes a protein from the RAF family of serine/threonine protein kinases, involved in regulating the mitogen-activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) cell signaling pathway. The most common *BRAF* mutation involves substitution of valine by glutamic acid at position 600 (V600E) and has been described in various tumours such as melanoma, hairy cell leukaemia, colorectal carcinoma, papillary thyroid carcinoma and non-small cell carcinoma of lung (12, 13).

Immunohistochemistry with anti-*BRAF*^{V600E} monoclonal antibody VE1 has been shown to be a sensitive and specific method for detection of *BRAF*^{V600E} mutation in ovarian serous tumours in small studies (14–16). Given that *BRAF*^{V600E}-mutated SBTs exhibit morphological features that may be misinterpreted as micropapillary SBT (mpSBT; a variant of SBT more frequently associated with advanced stage and LGSC) (17), accurate identification of *BRAF*^{V600E}-mutated SBTs is clinically important. We sought to investigate expression of VE1 protein in low-grade serous neoplasms of the ovary, and its association with *BRAF*^{V600E} mutation-associated histological features and *BRAF*^{V600E} mutation status.

Materials and Methods

This study was performed in accordance with institutional ethics committee guidelines (protocol no. 16-1684; Memorial Sloan Kettering Cancer Center Institutional Review Board; approved 23 December 2016).

Through a search of our institutional database, we identified patients diagnosed with SBTs and LGSCs of the ovary between 2000 and 2012. We reviewed pathology reports and available haematoxylin-eosin stained slides. Briefly, diagnostic criteria used were as follows – SBT: hierarchical branching of irregular papillae with detached cell clusters and single cells (budding), fibromatous to edematous fibrovascular cores, with or without luminal mucin; mpSBT: fibromatous to edematous papillae lined by delicate, filiform papillae lacking fibrovascular cores (5× longer than wide; the minimum size criterion of 5 mm is not used at our institution) or fused papillae imparting a cribriform appearance; and LGSC:

destructive stromal invasion with micropapillae, macropapillae or single cells, or solid growth measuring >5 mm. We recorded histological features associated with *BRAF*^{V600E} mutation, including the presence of epithelial cells with abundant eosinophilic cytoplasm, discrete cell borders, cell budding and detachment, as previously described (18, 19). Tumours exhibiting at least two of these features (eosinophilic cytoplasm and cell budding) were considered positive for *BRAF*^{V600E} mutation-associated histological features.

Immunohistochemistry was performed on 4-micron thick archival formalin-fixed paraffin-embedded (FFPE) tissue sections using the mouse monoclonal anti-*BRAF*^{V600E} antibody, clone VE1 (dilution 1:800, catalogue number E19294, Spring Bioscience, Pleasanton, CA, USA) on a Bond III automated staining system (Leica Biosystems, Buffalo Grove, IL, USA) according to the manufacturer's instructions. Heat-induced antigen retrieval was performed using epitope retrieval solution 2 (ER2, pH 8.0) (Leica Biosystems) for 40 minutes. Primary antibody was incubated for 30 minutes followed by the 3,3′-diaminobenzidine-based Bond Polymer Refine detection system (Leica Biosystems). Cytoplasmic expression of VE1 was scored based on the percentage of positive cells as follows: 0-10%, 25%, 50%, 75% and 90%. Tumours with ≥50% positive tumour cells were considered positive for VE1 expression, as previously described (20).

A subset of tumours was tested for *BRAF*^{V600E} and *KRAS* mutations. FFPE tissue samples were macrodissected to enrich for tumour cellularity of at least 80%. DNA extraction was performed using the DNeasy tissue kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). *BRAF* and *KRAS* hotspot mutations were then detected using a custom iPLEX assay (Sequenom, Inc, San Diego, CA, USA). These variants were manually reviewed and in tumours with sufficient DNA and hotspot mutations mutation status, confirmed with an orthogonal method, as previously described (9).

Statistical analyses were performed using the software package SPSS 24.0. Associations between clinico-pathological covariates and gene mutation or VE1 protein expression status were assessed using contingency tables, and significance of associations was determined using Pearson's χ^2 test or Fisher's exact test, as appropriate. A significance level of $p<0.05$ was used for all comparisons.

Results

The study cohort comprised 121 low-grade serous neoplasms, including 73 SBTs, 8 mpSBTs, and 40 LGSCs (22 primary, 18 metastatic). Immunohistochemical studies showed that VE1 was positive in 52% (38/73) of SBTs (Fig. 1A-D, 2A, 2C), 9% (2/22) of primary LGSCs (Fig. 2B, 2D), and in none of the mpSBTs and metastatic LGSCs ($p<0.0001$). The distribution of the percentage of positive cells in VE1-positive tumours was not significantly different amongst the groups ($p>0.05$).

Mutation status was available in 76 (63%) tumours, including 52 SBTs, 2 mpSBTs, and 22 LGSCs (12 primary, 10 metastatic). Of these 76 tumours, 42 (55%) harbored *BRAF*^{V600E} or *KRAS* mutations. Mutations were more common in SBTs compared to other groups ($p<0.0001$): mutations were identified in 75% (39/52) of SBTs and 14% (3/22) of all

LGSCs. Identified mutations were: *BRAF*^{V600E} in 26 (34%) tumours; *KRAS*^{G12D} in 8 (11%) tumours; and *KRAS*^{G12V} in 8 (11%) tumours.

BRAF^{V600E} mutations were present in 48% (25/52) of SBTs and 5% (1/22) of all LGSCs (p=0.006) (Table 1). Immunohistochemical expression of VE1 was identified in 96% (25/26) of tumours with *BRAF*^{V600E} mutation, while only 2 of 27 (7%) VE1-positive tumours were negative for *BRAF*^{V600E} mutation (p<0.0001). The sensitivity, specificity, positive predictive value and negative predictive value for VE1 immunohistochemistry were 96%, 96%, 93% and 98%, respectively.

Of the 52 SBTs with known mutation status, *BRAF* mutation-associated histological features were identified in 38 (73%) tumours. VE1 was positive in 26 (50%) tumours, and 25 (48%) harbored a *BRAF*^{V600E} mutation (p<0.0001). All of the 25 *BRAF*^{V600E}-mutated tumours were positive for VE1 by immunohistochemistry. In addition, one of two mpSBTs showed *BRAF* mutation-associated histological features. However, none of the mpSBTs was positive for VE1 by immunohistochemistry or *BRAF*^{V600E} mutation. The sensitivity, specificity, positive predictive value and negative predictive value for *BRAF* mutation-associated histological features were 92%, 70%, 62% and 95%, respectively.

Discussion

SBTs and LGSCs are low-grade serous neoplasms of the ovary that co-exist in approximately 75% of cases. These tumours are typically diagnosed in younger women with a mean age of 42 years for SBTs and 56 years for LGSCs. Although most patients with SBTs have a benign clinical course, recurrences and progression to LGSC have been described in approximately 15% and 5% of patients, respectively (21, 22). Transformation to high-grade serous carcinoma is extremely rare. Predictors for progression include advanced stage, type of extraovarian disease, micropapillary architecture and the presence of microinvasive carcinoma (22). Primary treatment of LGSC includes surgery combined with chemotherapy or hormonal therapy, while patients with recurrent disease generally receive multiple lines of systemic therapy and ultimately die from their disease (23). Median overall survival rates for LGSC range from 82 to 126 months (24, 25). Secondary cytoreduction without gross residual disease has been shown to improve overall survival to 167.5 months versus 88.9 months in patients with gross residual disease (26). Furthermore, patients with microscopic residual disease had a longer median progression-free (33.2 months versus ≤14.7 months) and overall survival (96.9 months versus ≤44.5 months) compared with those with >0.1 cm residual disease (p<0.001). Patients with measurable residual LGSC had a similar adjusted hazard ratio for death (2.12 versus 2.31; p=0.002 and p<0.001, respectively) as patients with high-grade serous carcinoma (27). This is most likely due to the relative resistance of LGSCs to cytotoxic chemotherapy (27, 28).

In genomic profiling studies, LGSCs cluster with SBTs but differ from high-grade serous carcinomas in having higher expression of hormone receptors, frequent *KRAS* and *BRAF* mutations, lower rates of *TP53* mutations and activation of the MAPK pathway (23, 29). Recurrent disease is generally treated with salvage chemotherapy, hormonal therapy or targeted therapies, such as inhibitors of vascular endothelial growth factor A (e.g.

bevacizumab); inhibitors of MAPK enzymes MEK1/2 (e.g. selumetinib, binimetinib, trametinib) and BRAF inhibitors (e.g. vemurafenib), have been explored in clinical trials (23, 29–34).

In our study, V600E *BRAF*^{V600E} mutations were present in 48% (25/52) of SBTs and 5% (1/22) of all LGSCs. *BRAF* mutations have been described in up to 48% of SBTs (6–11). Some studies have reported *BRAF* mutations in 19-33% of LGSCs (6, 35). However, lower mutation frequencies (0-6%) demonstrated by later studies, especially in advanced stage LGSCs (7, 9, 33, 36), may suggest that biologically aggressive LGSCs are more likely to arise from SBTs lacking *BRAF* mutations (7, 9). *KRAS* and *BRAF* mutation status has been shown to be a prognostic factor in LGSC. Patients with *KRAS* or *BRAF* mutations show significantly better overall survival than those lacking mutations (21 patients versus 58 patients, 106.7 months (95% CI, 50.6-162.9) versus 66.8 months (95% CI, 43.6.0-90.0)), respectively (p=0.018) (36). Patients with LGSC harboring *BRAF*^{V600E} mutation also have a better prognosis compared with those without mutation (9). Patients with *BRAF*-mutated SBTs have been shown to present at earlier stages (37). However, no significant differences were found in overall survival or disease-free survival among *BRAF*-mutated and non-mutated SBTs or LGSCs in some studies (11, 37).

Several small studies have reported sensitivity and specificity of VE1 immunohistochemistry. One study described VE1 positivity in 13% (4/31) of SBTs and 5% (3/62) of LGSCs, with 1 of 6 SBTs being falsely positive (15). Another study reported positive VE1 in 27% (3/11) of SBTs, with 100% concordance with competitive allele-specific hydrolysis probe polymerase chain reaction (CAST-PCR) and 68% concordance with Sanger sequencing (20). The largest study of VE1 immunohistochemistry reported positive results in 71% (22/31) of SBTs and 14% (1/7) of LGSCs, with 100% concordance with PCR (16). The latest report found VE1 expression in 14% (1/7) of SBTs and 0% (0/35) of LGSCs. In addition, one benign lesion described as “serous superficial papilloma” and one serous cystadenoma with focal proliferation were positive. All three VE1-positive lesions showed *BRAF*^{V600E} mutation by PCR (38). Our study includes the largest number of SBTs reported to date. In our study, VE1 was positive in 52% (38/73) of all cases of SBTs and 9% (2/22) of primary LGSCs, and in none of the mpSBTs or metastatic LGSCs. Of the 76 tumours with known mutation status, VE1 was positive in 50% (26/52) of SBTs compared with only 5% (1/22) of all LGSCs, and all 25 SBTs with *BRAF*^{V600E} mutation were positive for VE1.

BRAF-mutated SBTs have been reported to show specific morphological features, including abundant eosinophilic cytoplasm, well-defined cell borders, bland nuclei and cell budding (18). At least two of these features were identified in all 25 SBTs with *BRAF*^{V600E} mutation, in contrast to only 11% (5/47) of SBTs lacking *BRAF*^{V600E} mutation. The eosinophilic cells have been reported to show immunohistochemical expression of p16, a senescence-associated marker, associated with a lower Ki-67 proliferation index compared to adjacent non-eosinophilic cells (18). In our study, the presence of *BRAF*^{V600E} mutation-associated histological features was significantly associated with positive VE1 immunohistochemistry (p<0.0001), and most tumours with *BRAF*^{V600E} mutation (96%; 25/26) were VE1-positive (p<0.0001). Of note, *BRAF*^{V600E} mutation associated SBTs can

form micropapillae that differ from those of mpSBT but may lead to diagnostic confusion. Given the association of mpSBTs with invasive implants (LGSC) (17), it is very important to distinguish the two entities. Identification of at least two specific histological features and/or VE1 immunohistochemistry can be discriminatory in diagnostically challenging cases. *BRAF* mutations are thought to mediate progression of serous cystadenomas to SBTs (39). In contrast, in vitro experiments have demonstrated that induction of $BRAF^{V600E}$ expression leads to the development of the aforementioned *BRAF* mutation-associated histological features in cultured epithelial cells (18). The association of *BRAF* mutations with this cellular senescence phenotype and upregulation of tumour suppressor genes in SBTs suggest that *BRAF* mutations may have a protective role against the progression of SBT to LGSC (18, 39). *BRAF* mutation analysis of SBTs also suggests that peritoneal implants are derived from the primary ovarian tumour with overall concordance of *BRAF* mutations between ovarian SBTs and peritoneal implants reported to be 95% (59/62) ($p<0.00001$) (40).

In conclusion, $BRAF^{V600E}$ mutations which are significantly more common in SBTs than in LGSCs. Immunohistochemical expression of VE1 protein is strongly associated with $BRAF^{V600E}$ mutation status and specific histological features. VE1 immunohistochemistry is a reliable method for the detection of $BRAF^{V600E}$ mutations and is faster, less expensive and readily accessible to more laboratories than mutation testing.

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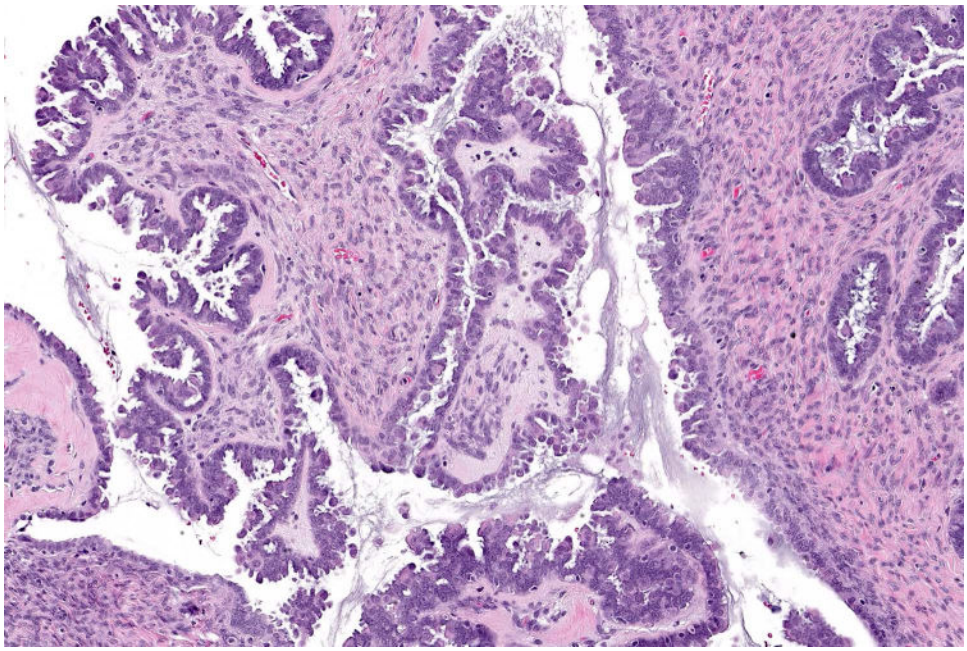
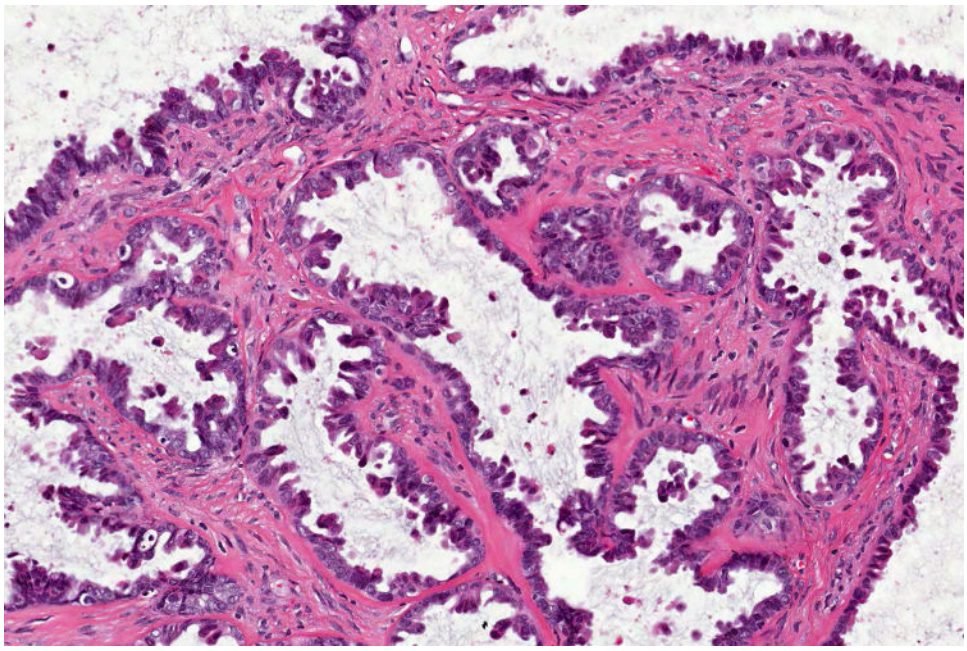




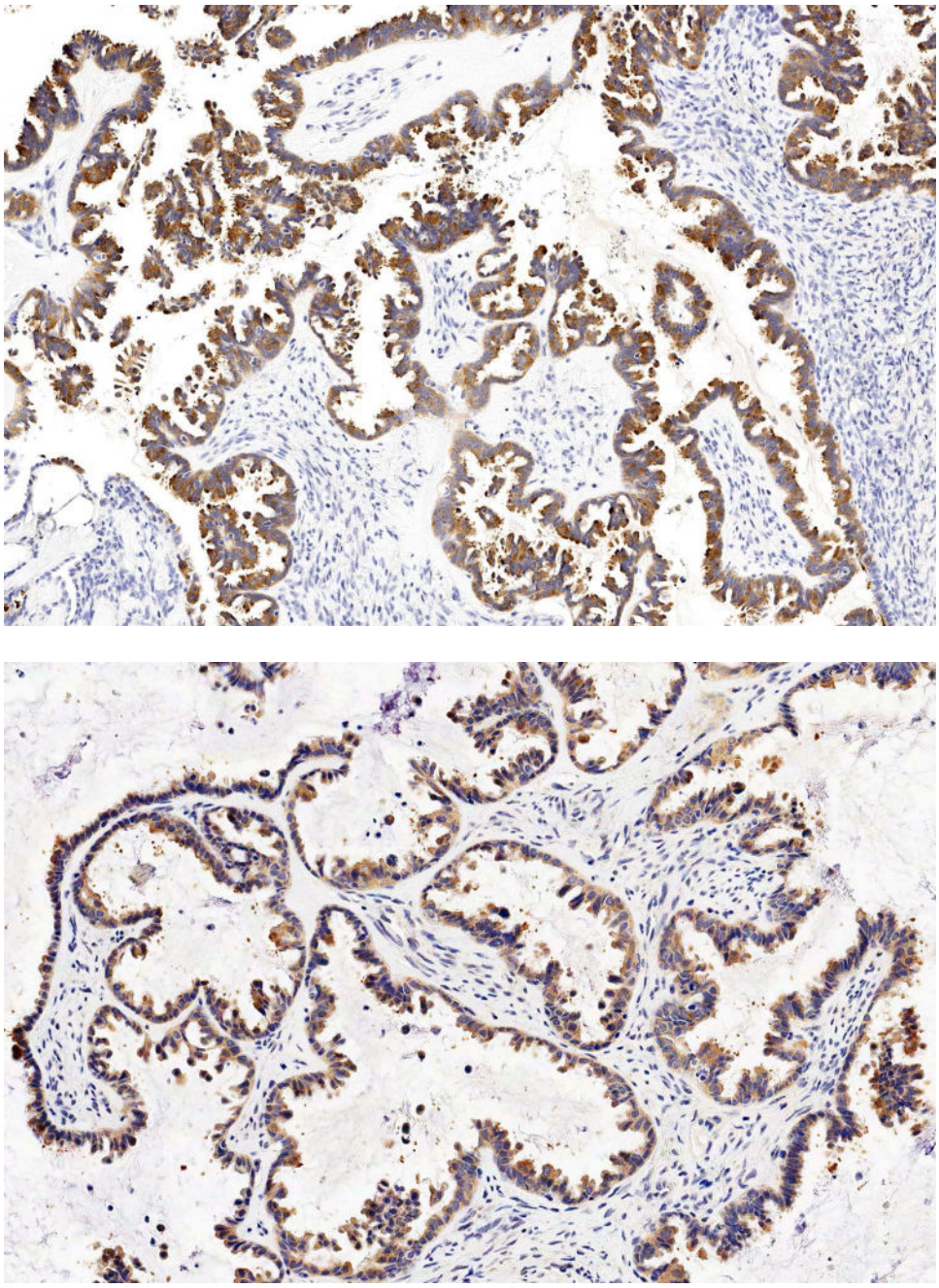
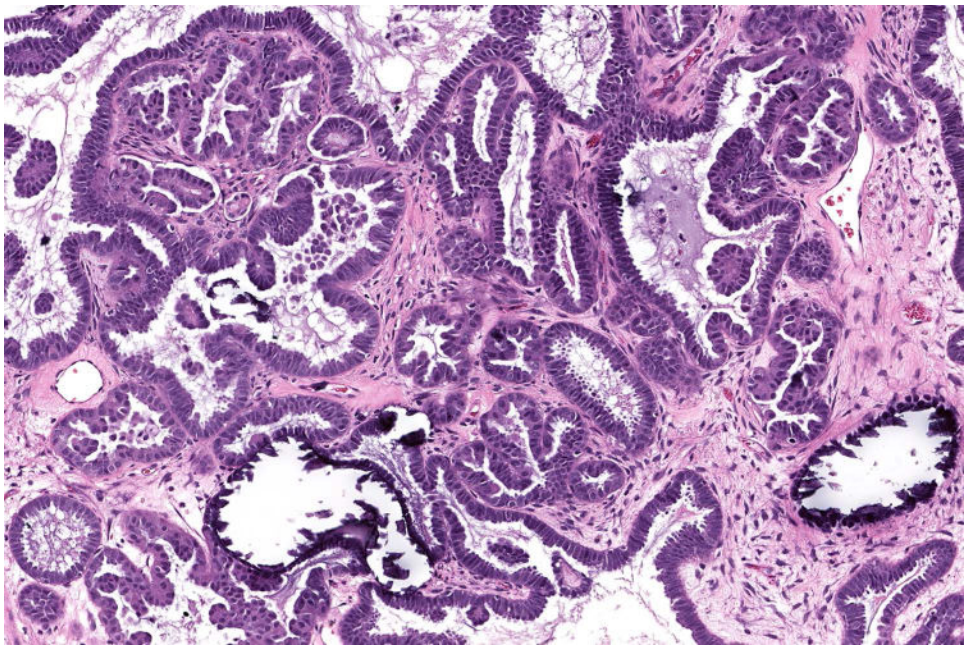
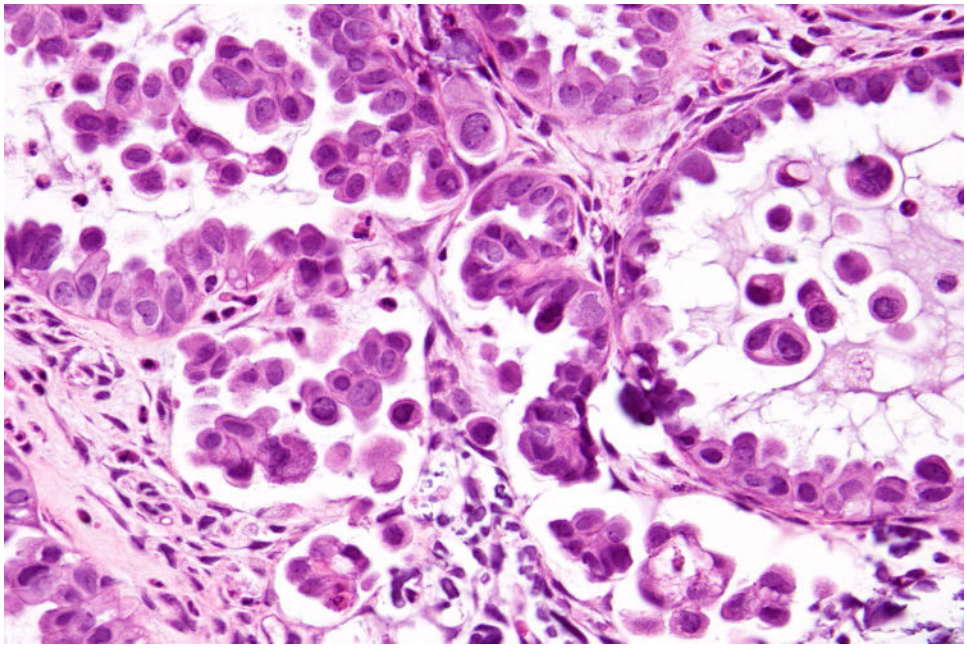


Figure 1.

Examples of serous borderline tumour with eosinophilic cells and budding (A, B), with cytoplasmic staining for VE1 (C, D). Haematoxylin-eosin stain (A, B); VE1 immunohistochemical stain (C, D). Original magnification ×100 (A, C); ×200 (B, D).





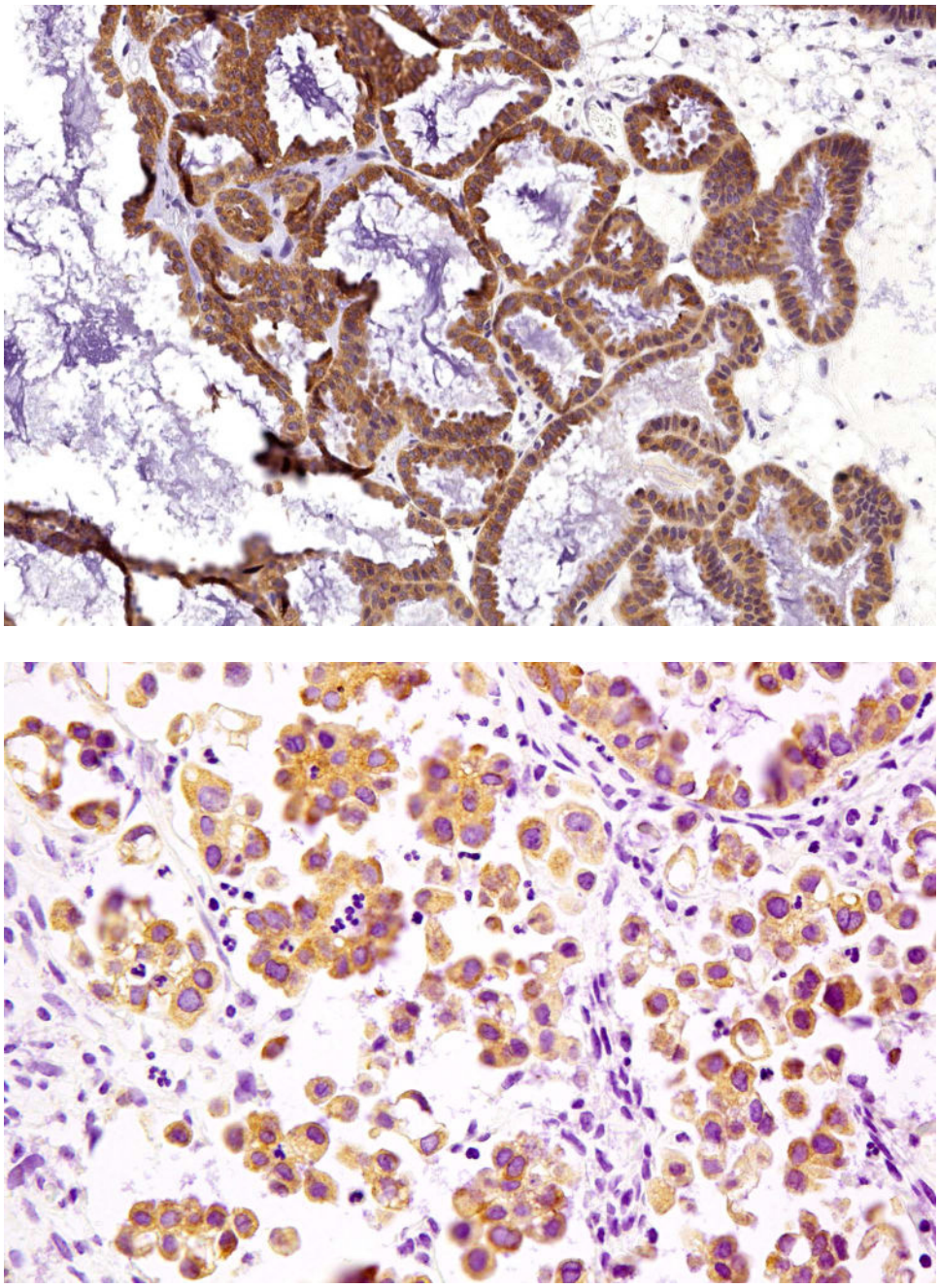


Figure 2.

Serous borderline tumour (A) associated with low-grade serous carcinoma (B), both tumours exhibiting cytoplasmic expression of VE1 (C and D, respectively). Haematoxylin-eosin stain (A, B); VE1 immunohistochemical stain (C, D). Original magnification ×100 (A, C); ×400 (B, D).

Table 1

BRAF mutation-associated histological features, VE1 immunohistochemistry and mutation frequencies in 76 tumours with known mutation status (all p<0.0001)

	N	SBT (n=52)	mpSBT (n=2)	LGSC (n=22)
BRAF mutation-associated histological features	39	38 (73%)	1 (50%)	0
Positive VE1 immunohistochemistry	27	26 (50%)	0	1 (5%)
$KRAS^{G12D}$ or $KRAS^{G12V}$ mutation	16	14 (27%)	0	2 (9%)
$BRAF^{V600E}$ mutation	26	25 (48%)	0	1 (5%)
Overall mutation frequency	42	39 (75%)	0	3 (14%)