Plasmacytoid urothelial carcinoma (PC-UC) is an infrequent and clinically aggressive variant of urothelial carcinoma (UC) that was described in the early 1990s by Zukerberg et al and is still included in the World Health Organization/International Society of Urologic Pathology classification of bladder tumors. Initially, PC-UC was identified based on morphology as a potential mimic of malignant plasma cell and lymphoid neoplasms. Nevertheless, it is currently well known that PC-UC is not of plasma cell or lymphoid lineage. Instead, it is a poorly differentiated, discohesive carcinoma of urothelial lineage (ie, GATA3 and p63 positive), which may include a component with signet ring–like morphology, that should be distinguished from a poorly differentiated yet more cohesive UC. In fact, the National Comprehensive Cancer Network states that a more aggressive treatment plan should be considered for variant UC, including PC-UC, sarcomatoid UC (SUC), and micropapillary UC, as these variants have a higher risk for muscle-invasive disease compared with conventional UC. More recently, several studies have demonstrated that loss of E-cadherin (E-Cad) expression secondary to inactivating CDH1 mutations or methylation of the CDH1 promoter is a hallmark feature of PC-UC, a finding that distinguishes it from nonplasmacytoid, poorly differentiated UC.

Sarcomatoid UC, first described by Dent in 1955, is another rare and aggressive variant of UC that includes carcinomatous and sarcomatous components (ie, dedifferentiated carcinoma). The latter can be represented by a nondescript high-grade spindle cell proliferation and/or it can demonstrate more lineage-specific change, including, but not limited to, leiomyosarcomatous, rhabdomyosarcomatous, and chondrosarcomatous differentiation. Epithelial-to-mesenchymal transition, a process characterized by a change from epithelial to mesenchymal gene expression patterns, is thought to induce the transformation of the carcinoma into a sarcomatous component in SUC. Loss of E-Cad has been shown to be an important step in the process of epithelial-to-mesenchymal transition, and is strongly supportive of PC-UC and SUC from conventional UC. In particular, the combination of cytoplasmic p-120 and loss of E-Cad is strongly supportive of PC-UC and SUC from conventional UC. In contrast, most PC-UCs were negative for E-Cad (17 of 22; 77%) with an additional 2 of 22 cases (9%) showing cytoplasmic with partial membranous staining. p-120 catenin demonstrated cytoplasmic or negative staining in 21 of 22 cases (95%). Most SUCs showed an absence of E-Cad (5 of 6; 83%) and cytoplasmic or negative p-120 in 5 of 6 cases (83%). Staining for B-Cat was also abnormal in a subset of PC-UCs and SUCs. Five PC-UC cases that harbored CDH1 gene variants were p-120 cytoplasmic positive.

Results.—E-cadherin, B-Cat, and p-120 showed membranous staining in all conventional and micropapillary UCs. In contrast, most PC-UCs were negative for E-Cad (17 of 22; 77%) with an additional 2 of 22 cases (9%) showing cytoplasmic with partial membranous staining. p-120 catenin demonstrated cytoplasmic or negative staining in 21 of 22 cases (95%). Most SUCs showed an absence of E-Cad (5 of 6; 83%) and cytoplasmic or negative p-120 in 5 of 6 cases (83%). Staining for B-Cat was also abnormal in a subset of PC-UCs and SUCs. Five PC-UC cases that harbored CDH1 gene variants were p-120 cytoplasmic positive.
commonly observed in the sarcomatous components of SUC.8

The core structure of the CDH1 adhesion complex, which mediates homotypic cell-cell adhesion at adherens junctions of epithelia, consists of a transmembrane protein (ie, E-Cad) and several associated cytoplasmic proteins (ie, catenins). Catenins act as a link between E-Cad and actin microfilaments of the cytoskeleton, and regulate the stability and dynamic properties of the complex.9 β-Catenin (CTNNB1, also referred to as B-Cat) and γ-catenin are interchangeable and connect the carboxy-terminal intracytoplasmic domain of E-Cad to α-catenin, which contains actin-binding domains.7–120 catenin (p-120) also interacts with the cytosolic aspect of E-Cad, regulating the stability and adhesive strength of the complex.9,10 In vitro and animal models show that upon E-Cad loss or down-regulation, p-120 localizes to the cell cytoplasm, which ultimately increases resistance to anoikis, promotes cell migration, and induces a mesenchymal phenotype.11

Based on these biological interactions, prior immunohistochemistry (IHC) studies have shown that E-Cad is useful for diagnosing PC-UC and SUC; however, these findings are not entirely sensitive, as E-Cad expression can be retained in up to a third of PC-UCs and 15% of SUCs.3,8 Similar IHC results have been seen in lobular breast cancer, another tumor characterized by E-Cad loss, with a subset of lobular breast cancers retaining E-Cad expression or showing aberrant expression of this protein.12,13 In this context, IHC that targets different members of the E-Cad adhesion complex can be very useful.13,14 For example, in lobular breast cancer, p-120 has been shown to demonstrate cytoplasmic immunoreactivity without the characteristic membranous reinforcement seen in benign breast epithelia and invasive ductal breast carcinomas. Interestingly, cases of lobular breast carcinoma with retained or aberrant E-Cad expression often show an abnormal staining pattern for p-120 (ie, cytoplasmic expression).10

Based on the described molecular interactions, and the similar discohesive nature of PC-UC and lobular breast cancer, this study explored the utility of the cadherin-catenin adhesion complex proteins, E-Cad, B-Cat, and p-120, in the distinction of conventional versus variant UCs, including PC-UC, SUC, and micropapillary UC, with the hypothesis that abnormal (ie, absent or cytoplasmic) staining would be seen in PC-UCs and SUCs, but not in conventional or micropapillary UCs.

**MATERIALS AND METHODS**

**Selection of Cases**

This study was performed with approval of our Institutional Review Board. A search for reports including the terms urothelial carcinoma, plasmacytoid, sarcomatoid, and micropapillary was performed using the departmental laboratory information system, so that cohesive and discohesive variants of bladder cancer were included in the study. Approximately two-thirds of cases were biopsies/excisions performed at our institution and one-third of cases were reviewed in consultation; for the latter, original slides had been returned to the referring institution at the time of this study, and only material cut from one corresponding paraffin block was available for use. Hematoxylin-eosin–stained slides were reviewed to confirm the diagnoses and to choose the best block for IHC. Histologic features consistent with PC-UC included an infiltrative carcinoma with discohesive cells that resembled plasma cells or signet ring–like cells; SUC cases included those with a high-grade spindle cell component, not otherwise specified; micro-papillary UCs demonstrated small nests of epithelial cells with peripheral nuclei clustered in lacunar-like spaces; conventional UCs included tumors with small to large nests of invasive epithelial cells as well as poorly differentiated yet cohesive epithelial cells.2 Only classic (ie, morphologically recognizable) cases were included in this study; cases were excluded if the diagnosis was uncertain or if plasmacytoid, sarcomatoid, and micropapillary features could not be confirmed on the hematoxylin–eosin–stained slides available for review/use. One case was also excluded from the plasmacytoid group because the patient had a remote history of breast cancer and metastatic lobular breast carcinoma could not be entirely excluded.

The final cohort included a total of 58 samples from 55 patients, as 3 cases had areas of both PC-UC and SUC. The final breakdown included 25 conventional invasive UCs, 22 PC-UCs, 6 SUCs, and 5 micropapillary UCs. Specimen types included 45 transurethral resections/bladder biopsies, 3 cystoprostatectomies, 1 anterior pelvic exenteration, 1 abdominoperineal resection, 2 biopsies of tumor from metastatic sites (1 pelvic soft tissue nodule and 1 peritoneal nodule), 1 nephroureterectomy, 1 penectomy, and 1 autopsy case.

**Immunohistochemistry**

IHC was performed with the Envision Plus/horseradish peroxidase system (Dako, Carpinteria, California), a polyclonal antibody to p-120 (1:500 titer; clone 98/p-120, BD Biosciences, San Jose, California), and monoclonal antibodies against E-Cad (1:50 titer; clone M3612, Dako Agilent, Santa Clara, California) and B-Cat (1:1000 titer; clone 14, BD Biosciences). Briefly, paraffin-embedded sections were incubated in hydrogen peroxide and absolute alcohol for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was performed with pressure-cooker pretreatment in citrate buffer (pH 6.0). Tissue sections were subsequently incubated with the primary antibody for 40 minutes at 25°C. Following Tris-buffered saline rinses, the tissue was incubated with the Envision Plus or Novolink Polymer Detection system (Leica Biosystems, Buffalo Grove, Illinois) for 30 minutes and then with diaminobenzidine for 5 minutes. Appropriate positive and negative controls were stained in parallel for each round of IHC, and an internal positive control (ie, nonneoplastic epithelium) was noted when present.

Based on the availability of tissue sections, E-Cad and p-120 IHC was performed on all cases, and all cases except one micropapillary UC were stained for B-Cat. A membranous-only staining pattern for all biomarkers was considered normal, whereas cytoplasmic expression, cytoplasmic and membranous expression, or an absence of expression (ie, negative in tumor cells) was considered an abnormal staining pattern.

Location of the positive staining was reported as 1 of 4 different categorical variables: complete membranous (circumferential), incomplete membranous (from segmental to dotlike), incomplete membranous and cytoplasmic, or only cytoplasmic (without membranous staining). The extent of staining was reported as 1 of 3 categorical variables: scattered cells (individual positive cells within the tumor), focal (groups of cells representing <10% of the lesion), and nonfocal (diffuse or groups of cells representing >10% of the lesion). Intensity of E-Cad, B-Cat, and p-120 staining was semiquantitatively evaluated using 4 ordinal variables (ranks): 0 (absent), 1+ (weak), 2+ (moderate), and 3+ (strong).

**Next-Generation Sequencing**

Eight PC-UC cases had molecular characterization via the OncoPanel assay developed at our institution.15,16 OncoPanel molecular testing was performed using formalin-fixed, paraffin-embedded tissue or freshly frozen tissue. Tumor DNA was isolated per standard methods (Qiagen, Valencia, California) from macrodissected regions of tumor. Libraries were prepared from a minimum of 50 ng of DNA using a customized solution-phase hybrid capture approach (Agilent Technologies, Santa Clara, California). Depending on the version of OncoPanel used at the
time, bait sets covered either 298 genes or an extended panel of 447 genes; CDH1 was included in both panels. Next-generation sequencing was performed using an Illumina HiSeq 2500 (Illumina, San Diego, California). Sequencing was analyzed by a variety of internally developed and publicly available tools, as previously described.15

**Statistical Analysis**

Categorical and ordinal variables were compared between groups using the Fisher exact test. Statistical significance was set at $P < .05$. All analyses were performed using R version 3.0.0 (R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Immunohistochemical Results**

The IHC results are summarized in Tables 1 and 2.

**Conventional UC.**—All conventional UCs included in this study ($n = 25$), including both the noninvasive/in situ and invasive components, showed complete (circumferential) membranous staining for E-Cad, B-Cat, and p-120 (Figure 1, A through H; Table 1). Cases with squamous elements generally showed decreased E-Cad, B-Cat, and p-120 expression in the areas of squamous differentiation (not shown). A subset of poorly differentiated conventional UC cases showed variation in staining intensity for E-Cad, B-Cat, and p-120; none of these cases showed cytoplasmic positivity for p-120 (Table 1; Figure 1, G and H).

**Variant UCs.**—**Plasmacytoid UC.**—Nonneoplastic urothelial and overlying noninvasive/in situ carcinoma, when present, demonstrated a membranous staining pattern for all 3 biomarkers (Figure 2, A through D, I through L, and M through P). In contrast, more than three-quarters (77%; 17 of 22) of all PC-UCs (Figure 2, A, E, I, M, and Q) showed a complete absence of E-Cad staining (Tables 1 and 2; Figure 2, B, F, J, and N), and an additional 2 cases (9%) showed incomplete membranous staining with cytoplasmic staining, for an overall abnormal staining pattern rate of 86% (19 of 22) (Table 3). The remaining 3 cases (14%) showed incomplete membranous staining without cytoplasmic positivity (Figure 2, R).

Fewer PC-UCs were completely negative for B-Cat (8 of 22; 36%) (Tables 1 and 2; Figure 2, C), with an additional 8 cases showing cytoplasmic expression without membranous (5 of 22; 23%) or with incomplete membranous (3 of 22; 14%) staining patterns (overall abnormal rate was 72% of cases; 16 of 22) (Table 3). The remaining 6 cases demonstrated a normal complete or incomplete membranous staining pattern (1 of 22 [5%] and 5 of 22 [15%, respectively), sometimes in only a subset of cells (Figure 2, G and S).

Nearly all PC-UC cases (21 of 22; 96%) demonstrated abnormal expression of p-120, lacking the characteristic complete membranous staining pattern seen in conventional UCs (Tables 1 and 2; compare Figures 1 and 2). Eighteen cases (82%) showed only cytoplasmic p-120 reactivity (Tables 1 through 3; Figure 2, D, L, P, and T), whereas 2 cases demonstrated incomplete membranous staining with concurrent cytoplasmic p-120 expression, and 1 case was completely negative. Only 1 case (5%) that was morphologically believed to be a PC-UC showed complete (ie, circumferential), albeit weak, staining (Figure 2, H). It should be noted that 3 cases that retained membranous E-Cad expression and lacked cytoplasmic E-Cad (Table 2, cases 18–20) were all positive for p-120 with a cytoplasmic staining pattern (Table 2; Figure 2, Q through T).

Three PC-UC cases were treated with neoadjuvant chemotherapy prior to surgical resection and material from these postneoadjuvant surgical specimens was available for IHC in this study (Table 2, cases 1, 19, and 20). The staining patterns in these 3 cases did not appear to differ from those that did not receive neoadjuvant chemotherapy, with all 3 cases demonstrating abnormal cytoplasmic p-120 expression; E-Cad and B-Cat staining was variable (negative, cytoplasmic or incomplete membranous) in these cases (Table 2).

**Sarcomatoid UC.**—Most SUCs were negative for E-Cad (5 of 6; 83%), and showed cytoplasmic-only or an absence of staining with p-120 in 4 of 6 (67%) and 1 of 6 cases (17%), respectively (Tables 1 and 2; Figure 3, A through D). In contrast, B-Cat was positive in all SUC cases with complete membranous (1 of 6; 17%), incomplete membranous (3 of 6; 50%), incomplete membranous and cytoplasmic (1 of 6; 17%), and cytoplasmic only (1 of 6; 17%) staining patterns. Only 1 case showed complete membranous positivity for all 3 biomarkers, and this case was treated with neoadjuvant chemotherapy prior to resection (Table 2, case 28; Figure 3, E through H). Overall, complete loss of both E-Cad and cytoplasmic p-120 had identical sensitivity for SUC (83%), and this was greater than that seen for B-Cat (abnormal staining pattern rate 33%) (Table 3).

**Micropapillary UC.**—All micropapillary UCs showed diffuse, strong complete membranous staining for E-Cad, B-Cat, and p-120 in the majority of tumor cells. However, it should be noted that an absence of staining for these biomarkers was seen at the periphery of clustered tumor cells (“inside-out” morphology) in a cuplike staining pattern (Figure 1, I through L) similar to that seen with other epithelial biomarkers in micropapillary UC.17

**Molecular Results and Correlation With Immunohistochemical Staining**

Molecular data were obtained via next-generation sequencing (OncoPanel) in 8 PC-UC cases (Table 2). Five cases showed genetic aberrations involving the CDH1 gene, which encodes for E-Cad (cases 6, 13, 17, 19, and 22). Four of these cases (cases 6, 13, 19, and 22) demonstrated shallow deletions of the chromosomal region encompassing the CDH1 locus; case 6 also showed molecular evidence of biallelic inactivation of CDH1, and case 17 showed a nonsense mutation.

Two of the 3 cases with single-copy loss of CDH1 only (Table 2, cases 19 and 22) showed incomplete membranous staining for E-Cad and B-Cat (case 13 was negative for E-Cad), whereas the cases that harbored a frameshift single-nucleotide deletion combined with CDH1 single-copy loss (Table 2, case 6) or a nonsense CDH1 variant (Table 2, case 17) lacked membranous staining for E-Cad. In contrast, p-120 showed a cytoplasmic-only pattern in all 5 cases that harbored CDH1 gene aberrations.

Three cases lacked demonstrable CDH1 mutations (Table 2, cases 4, 12, and 20). Corresponding biomarker results were variable in these cases, with 1 case showing loss of E-Cad and B-Cat expression plus cytoplasmic p-120 (case 4, abnormal results), 1 case showing loss of E-Cad expression with retained membranous B-Cat and p-120 expression (case 12, mixed results, normal p-120), and 1 case showing incomplete membranous expression of E-Cad plus cytoplasmic B-Cat and p-120 expression (case 20, mixed results, abnormal p-120).
Table 1. Staining Patterns for E-Cadherin (E-Cad), β-Catenin (B-Cat), and p-120 Catenin (p-120) in Conventional and Variant Urothelial Carcinoma

| Locationb | Complete membranous | Incomplete membranous | Cytoplasmic and incomplete membranous | Cytoplasmic | Negative | Not performed | Extent of staining | Nonfocal | Focal | Scattered cells | Negative | Not performed | Intensity |
|-----------|---------------------|-----------------------|---------------------------------------|-------------|----------|-------------|-------------------|----------|-------|----------------|----------|-------------|----------|
| E-Cad, No. (%) | 0 (4.5) | 1 (4.5) | 1 (17) | 0 | 1 (16.7) | 4 (66.6) | 0 | 17 (77.5) | 8 (36) | 1 (4.5) | 5 (83) | 0 | 1 (20) |
| B-Cat, No. (%) | 1 (4.5) | 1 (4.5) | 1 (17) | 0 | 1 (16.7) | 4 (66.6) | 0 | 17 (77.5) | 8 (36) | 1 (4.5) | 5 (83) | 0 | 1 (20) |
| p-120, No. (%) | .34 | .27 | .38 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| E-Cad, No. (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B-Cat, No. (%) | 1 (4.5) | 1 (4.5) | 1 (17) | 0 | 1 (16.7) | 4 (66.6) | 0 | 17 (77.5) | 8 (36) | 1 (4.5) | 5 (83) | 0 | 1 (20) |
| p-120, No. (%) | 1 (4.5) | 1 (4.5) | 1 (17) | 0 | 1 (16.7) | 4 (66.6) | 0 | 17 (77.5) | 8 (36) | 1 (4.5) | 5 (83) | 0 | 1 (20) |
| Extent of staining | .99 | .22 | .19 | .001 | <.001 | >.99 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| E-Cad, No. (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B-Cat, No. (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| p-120, No. (%) | .37 | .27 | .44 | <.001 | .36 | .30 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Extent of staining | .99 | .22 | .19 | .001 | <.001 | >.99 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |

a P values indicate the significance of the difference between the different nonplasmacytoid variants (sarcomatoid, micropapillary, conventional) and plasmacytoid urothelial carcinoma (Fisher exact test).
b Membranous staining only (complete or incomplete) is considered a normal staining pattern for diagnostic purposes; the presence of any or all cytoplasmic staining is considered an abnormal staining pattern for diagnostic purposes; negative staining is considered an abnormal staining pattern for diagnostic purposes.
Additional mutations identified in the 8 cases evaluated by next-generation sequencing included those frequently seen in urothelial cancer, including TP53 (6 of 8), TERT promoter (4 of 8), RB1 (3 of 8), and FGFR3 (2 of 8) (see Table 2 for all mutations/indels found). An APOBEC mutational signature was identified in 3 of 8 cases.

**DISCUSSION**

Plasmacytoid UC, SUC, and micropapillary UC are less-common to rare variants of UC that have been associated with an aggressive clinical course and adverse outcomes. Plasmacytoid UCs are characterized by an invasive dis cohesive proliferation of malignant epithelioid cells of urothelial origin that mimic plasma cells or lymphoma, with large hyperchromatic nuclei that are typically eccentricaly located within the cells (Figure 2, A, E, I, M and Q). According to the more recent World Health Organization classification of bladder neoplasms, tumors with signet ring–like morphology are now also included in the category of PC-UC. Sarcomatoid UC is a biphasic neoplasm of urothelial origin that contains both epithelial and mesenchymal elements (Figure 3, A and E). The latter can be composed of a high-grade spindle cell component, not otherwise specified, or it may demonstrate homologous (ie, malignant smooth muscle or vascular) or heterologous (ie, malignant osteoid or chondroid) differentiation. Micropapillary UC is characterized by small nests of malignant epithelioid cells within a lacunar-like space that demon-

### Table 2. Immunohistochemistry and Molecular Results in Plasmacytoid and Sarcomatoid Urothelial Carcinomaa

| Case No. | E-Cadherinb | β-Cateninb | p-120 Cateninb | CDH1 Gene Status | Additional Mutations/Indels |
|----------|-------------|------------|----------------|-----------------|-----------------------------|
| PC-UCs   |             |            |                |                 |                             |
| 1        | NEGATIVE    | NEGATIVE   | CYTOPLASMIC    | NP              | NP                          |
| 2        | NEGATIVE    | NEGATIVE   | CYTOPLASMIC    | NP              | NP                          |
| 3        | NEGATIVE    | NEGATIVE   | CYTOPLASMIC    | NP              | NP                          |
| 4        | NEGATIVE    | NEGATIVE   | CYTOPLASMIC    | None detected   | FGFR3, ARID1A, KDM5A, MTOR |
| 5        | NEGATIVE    | NEGATIVE   | CYTOPLASMIC    | NP              | NP                          |
| 6        | NEGATIVE    | NEGATIVE   | CYTOPLASMIC    | CDH1 single-copy deletion | FGFR3, TP53, TERT promoter, APOBEC signature |
| 7        | NEGATIVE    | NEGATIVE   | CYTOPLASMIC    | NP              | NP                          |
| 8        | NEGATIVE    | Membranous (i) | CYTOPLASMIC   | NP              | NP                          |
| 9        | NEGATIVE    | Membranous (i) | CYTOPLASMIC   | NP              | NP                          |
| 10       | NEGATIVE    | Membranous (i) | CYTOPLASMIC   | NP              | NP                          |
| 11       | NEGATIVE    | Membranous (i) | CYTOPLASMIC   | NP              | NP                          |
| 12       | NEGATIVE    | Membranous (c) | Membranous (c) | None detected   | TP53                         |
| 13       | NEGATIVE    | CYTOPLASMIC | CYTOPLASMIC    | CDH1 single-copy deletion | TP53, TERT promoter, PIK3CA, RB1, ARID1B, EP300, RBM10 |
| 14       | NEGATIVE    | CYTOPLASMIC | NEGATIVE       | NP              | NP                          |
| 15       | NEGATIVE    | CYTOPLASMIC | CYTOPLASMIC    | NP              | NP                          |
| 16       | NEGATIVE    | CYTOPLASMIC | CYTOPLASMIC    | NP              | NP                          |
| 17       | NEGATIVE    | Membranous (i) | CYTOPLASMIC   | CDH1 p.E547*4  | ARID1A                     |
| 18       | Membranous (i) | NEGATIVE   | CYTOPLASMIC    | NP              | NP                          |
| 19       | Membranous (i) | Membranous (i) | CYTOPLASMIC   | CDH1 single-copy deletion | TP53, RB1 NF1, APOBEC signature |
| 20       | Membranous (i) | CYTOPLASMIC | Membranous (i) | Membranous (i) | None detected | TP53, TERT promoter, KMT2D, EP300 |
| 21       | Membranous (i) | Membranous (i) | Membranous (i) | Membranous (i) | NP | NP                          |
| 22       | Membranous (i) | Membranous (i) | CYTOPLASMIC    | CDH1 single-copy deletion | TP53, TERT promoter, ARID2 |
| SUCs     |             |            |                |                 |                             |
| 23       | NEGATIVE    | Membranous (i) | CYTOPLASMIC   | NP              | NP                          |
| 24       | NEGATIVE    | Membranous (i) | CYTOPLASMIC   | NP              | NP                          |
| 25       | NEGATIVE    | Membranous (i) | CYTOPLASMIC   | NP              | NP                          |
| 26       | NEGATIVE    | CYTOPLASMIC | NEGATIVE       | NP              | NP                          |
| 27       | NEGATIVE    | Membranous (i) | Membranous (i) | Membranous (i) | NP | NP                          |
| 28       | Membranous (c) | Membranous (c) | Membranous (c) | Membranous (c) | NP | NP                          |

Abbreviations: c, complete/circumferential; i, incomplete; NP, not performed; PC-UC, plasmacytoid variant of urothelial carcinoma; SUC, sarcomatoid variant of urothelial carcinoma.

*Abnormal results are presented in all capital letters and bold font.*

**Disc**

Plasmacytoid UC, SUC, and micropapillary UC are less-common to rare variants of UC that have been associated with an aggressive clinical course and adverse outcomes. Plasmacytoid UCs are characterized by an invasive dis cohesive proliferation of malignant epithelioid cells of urothelial origin that mimic plasma cells or lymphoma, with large hyperchromatic nuclei that are typically eccentricaly located within the cells (Figure 2, A, E, I, M and Q). According to the more recent World Health Organization classification of bladder neoplasms, tumors with signet ring–like morphology are now also included in the category of PC-UC. Sarcomatoid UC is a biphasic neoplasm of urothelial origin that contains both epithelial and mesenchymal elements (Figure 3, A and E). The latter can be composed of a high-grade spindle cell component, not otherwise specified, or it may demonstrate homologous (ie, malignant smooth muscle or vascular) or heterologous (ie, malignant osteoid or chondroid) differentiation. Micropapillary UC is characterized by small nests of malignant epithelioid cells within a lacunar-like space that demon-
strate cell molding and inside-out (ie, peripherally located nuclei) cytology (Figure 1, I). The diagnosis of these variant UCs is typically possible with morphology alone; however, in a subset of cases, distinction from conventional UC is more difficult, especially when the tumor is poorly differentiated. Nevertheless, the distinction of conventional and variant morphology UC is clinically important. Patients with PC-UC, SUC, and/or micropapillary UC may proceed directly to surgery, as neoadjuvant chemotherapy is thought to be less effective on these variants of UC. In contrast, poorly differentiated UC, even with small-cluster or single-cell infiltration (Figure 1, A, E, and G) may benefit from neoadjuvant chemotherapy. In recent years, several groups have focused their efforts on providing better phenotypic and genetic characterization of PC-UC, SUC, and micropapillary UC. However, except for CDH1 mutation and loss of E-Cad expression in PC-UC, IHC criteria for the diagnosis of these UC variants are still lacking. Loss of E-Cad expression, which is considered the hallmark IHC finding of PC-UC, is seen in many, but not all cases; in this study, complete loss of E-Cad was present in 77.5% of PC-UC cases. Loss of E-Cad expression in PC-UC is secondary to deletions or frequent promoter hypermethylation in CDH1. However, some cases have no detectable genetic or epigenetic abnormalities. Moreover, a significant number of PC-UCs (between one-fourth and one-third of cases) have residual E-Cad expression by IHC. In this study, 3 cases lacked a CDH1 mutation; 2 were negative for E-Cad by IHC (Table 2, cases 4 and 12) and 1 demonstrated partial loss of membranous staining (Table 2, case 20). Similarly, up to 15% of SUCs have been reported to retain membranous E-Cad; this was also seen in 1 of 6 cases (17%) in this study. Importantly, the abnormal expression of cytoplasmic p-120 in PC-UC was not affected by IHC. In this study, 3 cases lacked a CDH1 mutation; 2 were negative for E-Cad by IHC (Table 2, cases 4 and 12) and 1 demonstrated partial loss of membranous staining (Table 2, case 20). Similarly, up to 15% of SUCs have been reported to retain membranous E-Cad; this was also seen in 1 of 6 cases (17%) in this study. Importantly, the abnormal expression of cytoplasmic p-120 in PC-UC was not affected.
by neoadjuvant chemotherapy, and the presence of membranous p-120 was retained in poorly differentiated areas of conventional UC that might be difficult to distinguish from a PC-UC (Figure 1, G and H). In contrast, B-Cat was slightly less reliable, as it demonstrated a cytoplasmic staining pattern, with or without membranous staining, in only 36% (8 of 22) of PC-UCs and there was an absence of staining in an additional 8 cases (36%). Two-thirds (4 of 6) of SUCs and all conventional and micropapillary UCs retained membranous B-Cat staining (Table 1).

p-120 catenin alone may be considered a superior biomarker to E-Cad IHC in the distinction of PC-UC for 2 reasons: first, it is generally accepted that expression of a biomarker (ie, the presence of cytoplasmic p-120) is always more reliable than loss of expression (ie, loss of E-Cad); and second, cytoplasmic expression of p-120, with or without focal/incomplete membranous staining (20 of 22 cases; 91%), is shown in this

**Figure 2.** Immunostaining patterns for E-cadherin (E-Cad), β-catenin (B-Cat), and p-120 catenin (p-120) in plasmacytoid urothelial carcinoma (PC-UC). Case 6 is a bladder PC-UC (A) with an absence of E-Cad (B) and B-Cat expression (C) and strong cytoplasmic positivity for p-120 (D) in the invasive plasmacytoid component. Case 12 shows frequent signet ring–like cells (E), an absence of E-Cad (F), and membranous staining for both B-Cat (G) and p-120 (H). Cases 13 (I through L) and 17 (M through P) also show a mixture of plasmacytoid and signet ring cells, but with the characteristic immunoprofile seen in many PC-UCs, that is, negative E-Cad (J and N), focal membranous and/or cytoplasmic B-Cat (K and O), and cytoplasmic p-120 (L and P). Case 19 shows densely packed discohesive PC-UC cells with a high nuclear to cytoplasmic ratio (Q) and focal expression of membranous E-Cad (R) and B-Cat (S). However, this tumor shows diffuse cytoplasmic expression of p-120 positivity (T), consistent with PC-UC. Note that the high nuclear to cytoplasmic ratio in this case might mimic membranous p-120 staining at lower magnification and is a potential pitfall (hematoxylin-eosin, original magnification ×400 [A, E, I, M, and Q]; original magnification ×400 [B through D, F through H, J through L, N through P, and R through T]).
study to have slightly higher sensitivity than the complete loss of E-Cad (17 of 22 cases; 78%). Nevertheless, the combination of E-Cad loss and/or p-120 cytoplasmic staining is most reliable and detects 100% of cases of PC-UC.

The combination of E-Cad and p-120 IHC may reflect the functional status of the cadherin-catenin adhesion complex and the biology of the tumor better than E-Cad alone. In this regard, the most useful staining pattern is complete loss of E-Cad and cytoplasmic expression of p-120. However, a cytoplasmic staining pattern for p-120 might be particularly helpful when there is intact or residual membranous E-Cad expression. In this study, cases with residual membranous E-Cad (Table 2, cases 18–22) invariably showed abnormal cytoplasmic staining patterns for p-120, with cytoplasmic-only or predominantly cytoplasmic plus residual (incomplete) membranous positivity for this biomarker. Retained membranous E-Cad expression in some cases with single-copy deletion or an absence of CDH1 variants (Table 2, cases 19, 20, and 22), but not in others (Table 2, cases 4, 6, 12, and 13) could potentially be explained by epigenetic modifications or posttranslational change. Regardless, cytoplasmic p-120 was present more frequently than E-Cad loss in cases with known CDH1 molecular alterations (5 of 5 [100%] versus 3 of 5 [60%], respectively) (Table 2).

A recent study by Genitsch et al28 showed that there is common ancestry between conventional UC and adjacent sarcomatoid areas, and that a loss of E-Cad expression in SUC is consistent with epithelial-to-mesenchymal transition. In our study, E-Cad was lost in 83% of SUC cases. However, in contrast to PC-UC, p-120 did not appear to be superior to E-Cad in the distinction of SUC; instead, results were more similar/complementary, with the presence of cytoplasmic p-120 in 4 of 6 cases (67%) and the loss of p-120 in 1 additional case (17%) (83% abnormal results in total) (Tables 1 and 3). Four of the total 6 cases had both of these IHC findings (E-Cad loss, p-120 cytoplasmic); 1 additional E-Cad–negative case was also negative for p-120 (abnormal result), and the sixth case retained membranous staining for both E-Cad and p-120. β-Catenin staining was variable in SUCs, retaining membranous staining in 4 of 6 cases (67%). Interestingly, the 3 cases that showed areas of plasmacytoid and sarcomatoid differentiation in the same specimen (Table 2, cases 7 and 24, 11 and 25, and 16 and 26), had similar staining patterns in both areas, suggesting that these 2 variants share some biologic properties in at least a subset of cases.

In the context of complete or partial loss of E-Cad expression, translocation of p-120 to the cytoplasm has also shown to play a central role in epithelial-to-mesenchymal transition.11,29,30 The mechanisms by which cytoplasmic p-120 elicits these effects appear to be highly dependent on the cell type and surrounding microenvironment, and they usually involve modulation of RHO signaling via differential regulation of members of the RHO family of small GTPases and their downstream effectors.29,31 The functional role of p-120 as a regulator of RHO GTPases is highly dependent on its subcellular niche, and cytoplasmic localization appears to be necessary for p-120 to promote migration, invasion, and anchorage-independent growth in E-Cad–deficient tumor cells.29–32 From a biological perspective, combined absence of E-Cad and cytoplasmic p-120 expression by IHC might indicate a more mesenchymal phenotype of UC with

| Table 3. Summary of Normal Versus Abnormal Immunostaining Results in Plasmacytoid Urothelial Carcinoma (PC-UC) and Sarcomatoid Urothelial Carcinoma (SUC)* |
|--------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                    | Normal, No. (%) | Abnormal, No. (%) | Normal, No. (%) | Abnormal, No. (%) |
| E-Cad              | 3 (13.5)        | 19 (86.5)       | 1 (17)          | 5 (83)          |
| B-Cat              | 6 (27.5)        | 16 (72.5)       | 4 (67)          | 2 (33)          |
| p-120              | 1 (4.5)         | 21 (95.5)       | 1 (17)          | 5 (83)          |

Abbreviations: B-Cat, β-catenin; E-Cad, E-cadherin; p-120, p-120 catenin.

*Membranous staining only (complete or incomplete) is considered a normal staining pattern for diagnostic purposes; the presence of any or all cytoplasmic staining is considered an abnormal staining pattern for diagnostic purposes.

Figure 3. Staining patterns for E-cadherin (E-Cad), β-catenin (B-Cat) and p-120 catenin (p-120) in sarcomatoid urothelial carcinoma. Case 23 shows a pleomorphic spindled sarcomatoid carcinoma (A) that is negative for E-Cad (B), shows incomplete membranous staining for B-Cat (C), and expresses cytoplasmic p-120 (D). Case 28 (E), in contrast, shows membranous expression of all 3 biomarkers with various intensity (E-Cad, F; B-Cat, G; p-120, H) (hematoxylin-eosin, original magnification ×400 [A and E]; original magnification ×400 [B through D and F through H]).
increased invasive or metastatic potential, regardless of the presence of any specific morphologic findings; however, further studies are needed to test this hypothesis. This study has a few limitations. First, the number of SUC and micropapillary UC cases is small, limiting the interpretation of sensitivity and specificity. Second, because several cases were received and reviewed as consult cases, homogeneous conditions for tissue fixation and processing are not guaranteed, and not all slides remained available for review at the time of this study. Finally, only a subset of our variant cases had characterisation by next-generation sequencing, and none of them had CDH1 promoter methylation analysis. Nevertheless, the main strength of this study is that all cases were centrally reviewed by experts in genitourinary pathology who were able to agree on the morphologic diagnoses prior to study inclusion. Also, E-Cad, B-Cat, and p-120 IHC were performed in the same laboratory and under the same conditions in all cases.

In conclusion, this study illustrates the diagnostic utility of p-120 as a superior and/or adjunct biomarker to E-Cad as part of the diagnostic workup of PC-UC and possibly SUC. More specifically, the presence of cytoplasmic p-120 expression can be used to distinguish PC-UC from conventional and micropapillary UCs, and it may be most useful when E-Cad expression is partially retained or when a conventional UC includes poorly differentiated invasive component. This distinction of PC-UC and SUC from conventional UC is clinically relevant and can affect patient management, especially when the decision includes drug-naive radical cystectomy versus neoadjuvant chemotherapy prior to cystectomy.

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