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Original contribution

The utility of p63, p40, and GATA-binding protein 3 immunohistochemistry in diagnosing micropapillary urothelial carcinoma ☆,☆☆

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Summary Micropapillary urothelial carcinoma (MPUC) is an uncommon variant of urothelial carcinoma (UC) with an aggressive clinical course. There have been limited studies on the UC markers GATA-binding protein 3 (GATA3), p63, and p40 in MPUC. Our study investigated the immunoreactivity of these 3 markers in MPUC compared with conventional UC of different grades and stages. Immunohistochemistry was performed on 62 cases of high-grade urothelial carcinoma (HGUC), 16 low-grade urothelial carcinoma (LGUC), and 20 MPUC. p63 expression was strong and diffuse in all LGUC, significantly decreased in high stage and HGUC, and virtually absent in MPUC. p40 expression was decreased in HGUC and markedly decreased in MPUC relative to LGUC. These results suggest that loss of p63 expression in a UC appears to be associated with adverse features—including cases with micropapillary differentiation. Decreased GATA3 expression was seen frequently in high-grade and high–pathologic stage (≥pT2) tumors but was retained in MPUC cases. The findings of retained GATA3 expression in MPUC, which often shows a loss of expression of other urothelial markers such as p63, may be helpful for determining the origin of micropapillary carcinoma of unknown primary. Compared with the traditional markers p63 and p40, GATA3 is the most sensitive marker of conventional UC and MPUC.

1. Introduction

Urothelial carcinoma (UC) is the most common malignancy of the urothelial tract, and many morphologic subtypes of UC have been described to date. Micropapillary carcinoma (MPUC) of the urinary tract is a well-recognized subtype of UC with an incidence ranging from 0.7% to 8% [1,2]. MPUC is characterized clinically by an aggressive

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clinical course with a high rate of metastases to lymph nodes and other organs [3]. The diagnosis of MPUC is important for its implications regarding clinical management—with some centers offering early cystectomy due to the high likelihood of understaging MPUC with transurethral resection [4,5]. The morphologic features of primary MPUC of the urinary tract are well defined and include infiltrative nests of tumor cells arranged in ring-like configurations, which are often located in spaces referred to as lacunae [6]. Although these diagnostic features are well established for primary tumors, at metastatic sites the morphologic features of MPUC overlap significantly with micropapillary tumors from other organs, which include breast, ovary, salivary gland, lung, and colon. In the metastatic setting, an immunohistochemical (IHC) workup is often indicated to attempt to establish the primary site of a carcinoma with micropapillary features [1].

Currently, there are several biomarkers that are helpful in establishing a urothelial origin of a metastatic carcinoma. One of the most sensitive markers of urothelial origin is p63. The p63 gene, a homologue of the p53 tumor suppressor gene, encodes multiple splicing isoforms that may either transactivate p53 responsive genes (TAp63) or act as a dominant-negative factor toward p53 and p73 (ΔNp63) [7]. Unlike p53, which is induced in response to a myriad of stressors, p63 is constitutively expressed in the basal cell compartment of stratified epithelial tissues, including skin, prostate, breast, and urothelium. At all of these sites, ΔNp63 is the predominant isoform expressed [8]. 4A4, a widely used anti-p63 antibody, identifies both p63 isoforms (TAp63 and ΔNp63), whereas the p40 antibody identifies only the ΔNp63 isoform. Strong nuclear p63 expression has been reported in UC, and thus, IHC for p63 is frequently used in clinical practice to support a diagnosis of UC [9]. However, decreased or even absent p63 expression has been described in advanced stage UCs and many carcinomas that are not urothelial in origin can be strongly p63 positive [7,10,11]. A prior study by Lotan et al [12] found that p63 is less sensitive and specific for a diagnosis of MPUC when evaluating tumors from various organs with micropapillary growth patterns. The expression of ΔNp63 (p40) in UC has not been well studied. The few studies that have evaluated ΔNp63 (p40) expression in UC have suggested that p40 labeling is associated with more advanced disease and a poorer prognosis [7,13-15].

GATA-binding protein 3 (GATA3) is a zinc finger transcription factor with a diverse range of biological roles. The protein contains 2 GATA-type zinc fingers and is an important regulator of T-cell development and plays an important role in endothelial cell biology [16]. Recent studies have identified GATA3 as a sensitive IHC marker for UC [17-19]. Other studies have demonstrated that GATA3 is expressed in breast cancer, many different types of salivary gland neoplasms, a minority of endometrial carcinomas, occasional squamous cell carcinomas of the anus, cervix, lung, and also paraganglioma [5,20,21]. The expression of GATA3 has not, however, been specifically evaluated in cases of MPUC.

In this study, we evaluated the expression of p63, p40, and GATA3 in MPUC, high-grade urothelial carcinoma (HGUC), and low-grade urothelial carcinoma (LGUC). The study cohort included both low-stage (<pT2) and high-stage (≥pT2) tumors. Our aim was to characterize the expression of these markers in UCs of various grades and stages with a particular focus on MPUC.

2. Materials and methods

2.1. Case selection

This study was approved by the Institutional Review Board of Northwestern University. A total of 98 UC cases, which include biopsies, transurethral resection, or cystectomy at our institution between 2000 and 2012 were selected on the basis of histopathologic diagnosis, availability of hematoxylin and eosin–stained slides (4 μm), and corresponding paraffin blocks. The diagnostic criteria used to identify cases of MPUC are those outlined in the recent study by Sangoi et al [6]. For the purposes of this study, only tumors which had more than 10% micropapillary differentiation were considered to be MPUC. Grading of UC in this study followed the 1998 World Health Organization/International Society of Urologic Pathology grading system [3]. Pathologic stage for the cases included in this study was determined using the 2010 American Joint Committee on Cancer TNM classification [4].

2.2. Immunohistochemistry

IHC for p63 (1:600; Dako, Carpinteria, CA), p40 (1:500 dilution; Calbiochem/Millipore, Billerica, MA), and GATA3 (1:400; Santa Cruz Biotech, Santa Cruz, CA) was performed on 4-μm-thick sections of paraffin-embedded tissue with appropriate positive and negative controls. Paraffin-embedded blocks were sectioned, deparaffinized, rehydrated, and blocked with methanolic 3% hydrogen peroxide. Antigen retrieval was performed in citrate buffer. Only moderate to strong nuclear staining was considered positive for p63, p40, and GATA3. IHC staining in tumor samples was graded in a semiquantitative fashion as absent/weak (0 or 1+) or strong (2+ or 3+). A case was considered positive only when there was strong labeling in more than 25% of tumor cells in the section.

2.3. Statistic method

Student t test, χ^2 test, and Yates correction test were used for statistical analysis. The Yates correction test was applied, when group consists of less than 20 cases.