COMMENTARY

Distinct Mutational Landscape of Inverted Urothelial Papilloma†

Mahmut Akgul[1], Gregory T. MacLennan[2], Liang Cheng[3]*

[1]Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]Department of Pathology, University Hospitals Cleveland Medical Center, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA; and [3]Department of Pathology, Indiana University School of Medicine, Indianapolis, IN, USA

†Invited commentary for Isharwal *et al.* Genomic landscape of inverted urothelial papilloma and urothelial papilloma of the bladder. *J Pathol* 2019; https://doi.org/10.1002/path.5261

Running title: Genomic Landscape of Benign Papillary Lesions

Conflict of interest: None

This is the author's manuscript of the article published in final edited form as:

Akgul, M., MacLennan, G. T., & Cheng, L. (2019). Distinct Mutational Landscape of Inverted Urothelial Papilloma. The Journal of Pathology, 0(ja). https://doi.org/10.1002/path.5307

*Correspondence to: Liang Cheng, M.D., Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, 350 West 11th Street, IUHPL Room 4010, Indianapolis, IN 46202, USA. E-mail: liang_cheng@yahoo.com

Abstract

A recent study has identified gene mutations involving the MAPK/ERK pathway, particularly the *HRAS* gene, in all inverted urothelial papillomas, in the absence of pathway mutations in *TERT* promoter, *FGFR3*, and *TP53*/*RB1*genes. Neither recurrence nor progression was observed in inverted urothelial papillomas. These data support several longstanding hypotheses: 1) inverted urothelial papillomas are benign and do not recur or progress; 2) they harbor mutations that are different from those of urothelial carcinoma; and 3) they arise through different molecular mechanisms than low- or high-grade urothelial carcinoma. As the most critical differential diagnosis in this context is inverted-type urothelial carcinoma, more comprehensive studies are needed to compare and contrast these entities.

Keywords: urinary bladder; inverted urothelial papilloma; molecular genetics; MAPK/ERK pathway; *TERT* promoter mutation; FGFR3

Inverted urothelial papilloma (IUP) is a rare (<1% of bladder neoplasms) neoplasm characterized by inverted growth of anastomosing nests and cords of cytologically bland urothelial cells and peripherally palisading basal cells. Differential diagnosis includes a wide spectrum of nonneoplastic and neoplastic urothelial proliferations with an endophytic growth pattern, such as florid cystitis cystica et glandularis, inverted papillary urothelial neoplasm of low malignant potential, large nested variant urothelial carcinoma, and noninvasive low- and high-grade papillary urothelial carcinomas with inverted growth [1]. Although the diagnosis of IUP could be challenging, up to 25% of cases of inverted urothelial carcinoma may be misdiagnosed as IUP, highlighting the difficulty of morphologically distinguishing these entities in some instances.

Urothelial papilloma (UP) represents the least cytologically and architecturally atypical member of the papillary urothelial neoplasms of the urinary tract. In urothelial papilloma, delicate fibrovascular cores are lined by several layers of urothelial cells preserving polarity and lacking atypia [1]. When using strict criteria designated by the World Health Organization (WHO), both entities account for less than 1% of papillary urothelial neoplasms.

Urothelial carcinoma has two main oncogenic pathways. *FGFR3* mutations are frequently associated with low-grade noninvasive urothelial carcinoma (Figure 1), and the *TP53* pathway, in contrast, is associated with high-grade invasive cancer [2]. Moreover, about 70-80% of noninvasive urothelial carcinomas harbor promoter mutations in the *TERT* gene, especially concurrent with *FGFR3* mutations. *FGFR3* mutations might have therapeutic implications, considering the recent approval of

erdafitinib, a new *FGFR2/FGFR3* inhibitor, for clinical use in patients with locally advanced or metastatic bladder cancer that is unresponsive to platinum-based chemotherapy [3]. *STAG2* and *PIK3CA* mutations are also frequently described in noninvasive urothelial carcinoma and are seen in approximately 33% and 25% of the cases, respectively [2].

Several groups of investigators, using cohorts of various size and a variety of methodologies, have studied the genomic landscape of IUP (**Table 1**) [4-9]. In a recent issue of *The Journal of Pathology*, Isharwal and colleagues reported the findings from the most recent of such efforts. Their study, using next generation sequencing on IUPs and (urothelial papillomas) UPs, employing the MSK-IMPACT platform targeting 486 cancer associated genes, as well as whole exome sequencing in a subset of cases, is by far the most comprehensive study to date [9].

All IUPs and UPs had mutations in the MAPK/ERK pathway, *HRAS* and *KRAS* mutations being predominant in IUPs (10/11) and UPs (8/11), respectively. None of the IUPs and UPs had an APOBEC (apolipoprotein B mRNA editing catalytic polypeptide-like) mutational signature, which was identified in about 70% of muscle-invasive urothelial carcinomas [10], and all had low mutational burden. The vast majority of IUPs and UPs did not show mutations in *FGFR3*, *TERT*, *TP53*, *RB1*, or chromatin remodeling genes, which are known to be common mutations in urothelial carcinoma.

Urothelial carcinomas with *HRAS*/*KRAS* mutations also seem to have enriched mutations at the *TERT* promoter region as well as mutations at various *TP53*/cell cycle genes. It has been observed repeatedly that mutations at the *FGFR3*, *PI3KCA* and *TERT*

genes are present in the majority of the low-grade urothelial carcinomas, whereas mutations involving the MAPK/ERK pathway, mainly *HRAS*, *KRAS* and occasionally *BRAF*, have been identified in UPs and IUPs [8-9]. In the current study, all IUPs (10 *HRAS* and 1 *KRAS* mutations) and 91% of UPs (8 *KRAS* and 2 *HRAS*) had MAPK/ERK pathway mutations and no papilloma harbored *TP53* mutations [9]. The findings in the current study are comparable to those of a previous study [8]. One shortcoming of the current study is the absence of a clear comparison of the mutational status between IUP and inverted noninvasive urothelial carcinoma. Another limitation is the small sample size which may explain why certain mutations may not be detected in contrast to other studies.

In summary, the current study provides evidence for the hypothesis that IUPs and UPs harbor mutations that are different from those of urothelial carcinoma, and arise through molecular mechanisms that are different from those of low- or high-grade urothelial carcinoma (Figure 1)**.** Additional comprehensive studies to delineate the nature of IUP and to further clarify the molecular differences between IUP and inverted urothelial carcinoma are warranted.

References:

1. Cheng L, Lopez-Beltran A, Bostwick DG. Bladder Pathology. ed). Wiley-Blackwell: Hoboken, NJ, 2012.
2. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nat Rev Cancer* 2015; **15**: 25-41.

3. FDA approves first targeted therapy for metastatic bladder cancer 2019 [Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm635906.htm]. ClinicalTrials.gov. An Efficacy and Safety Study of JNJ-42756493 in Participants With Urothelial Cancer 2019 [cited 2019 April 14, 19:53]. Available from: https://clinicaltrials.gov/ct2/show/NCT02365597?term=NCT02365597&rank=1.

4. Cheng L, Davidson DD, Wang M*, et al.* Telomerase reverse transcriptase (TERT) promoter mutation analysis of benign, malignant and reactive urothelial lesions reveals a subpopulation of inverted papilloma with immortalizing genetic change. *Histopathology* 2016; **69**: 107-113.

5. Cheng L, Montironi R, Lopez-Beltran A. TERT promoter mutations occur frequently in urothelial papilloma and papillary urothelial neoplasm of low malignant potential. *Eur Urol* 2017; **71**: 497-498.

6. Lott S, Wang M, Zhang S*, et al.* FGFR3 and TP53 mutation analysis in inverted urothelial papilloma: incidence and etiological considerations. *Mod Pathol* 2009; **22**: 627-632.

7. Sung MT, Eble JN, Wang M*, et al.* Inverted papilloma of the urinary bladder: a molecular genetic appraisal. *Mod Pathol* 2006; **19**: 1289-1294.

8. McDaniel AS, Zhai Y, Cho KR*, et al.* HRAS mutations are frequent in inverted urothelial neoplasms. *Hum Pathol* 2014; **45**: 1957-1965.

9. Isharwal S, Hu W, Sarungbam J*, et al.* Genomic landscape of inverted urothelial papilloma and urothelial papilloma of the bladder. *J Pathol* 2019. **Editor's note: This reference will be published by *J Pathol*. Please update this reference during production. DO NOT ASK the authors to update this reference, here or in the citation list.**

10. Roberts SA, Lawrence MS, Klimczak LJ*, et al.* An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. *Nat Genet* 2013; **45**: 970-976.

11. Williamson SR, Zhang S, Lopez-Beltran A, *et al.*Telomere shortening distinguishes inverted urothelial neoplasms. *Histopathology* 2013; **62:** 595-601.

Author contributions statement: All authors were involved in writing and approving the final version of the manuscript.

Figure Legend

Figure 1. Activating mutations targeting various proteins forming MAPK/ERK and PI3KCA signaling pathways are commonly detected in low-grade urothelial neoplasms. These mutations cause stimulation at different genomic sites, including *FGFR3* and/or its receptor-linked tyrosine kinases, downstream proteins such as RAS (particularly *HRAS* and *KRAS*), and occasionally RAF (*BRAF*). The PI3CKA/AKT pathway is also coupled with the receptor tyrosine kinase complex and directly interacts with the MAPK/ERK pathway. Activation of both pathways results in nuclear transcription of

proteins that play a role in cell growth, cell cycle progression, and proliferation. Another frequently mutated gene, *TERT*, causes perpetual activation of the TERT enzyme and alters the delicate balance of telomere maintenance. Increased telomerase activity helps the neoplastic cells become immortal by avoiding the natural occurrence of telomere shortening, cell cycle arrest/senescence, and cell death.

Indicates proteins in which their associated genes are targeted by activating mutations in low grade urothelial carcinoma;

 Indicates proteins in which their associated genes are targeted by activating mutations in urothelial papilloma and inverted urothelial papilloma

Table 1. Molecular studies investigating inverted urothelial papilloma (IUP)

Citation	Number of cases	Age (range)	Gender M/F	Test used	Genes included	Findings
[7]	39	N/A	36/3	LOH	NA	3/37 D9S177, 4/38 *TP53*, 3/37 *IFNA*, 3/36 D3S1300
[6]	20	58 (37-75)	18/2	Sanger sequencing	*FGFR3* (exon 7, 10, 15), *TP53* (exons 5,7,8)	9/20 *FGFR3* mutations (most common exon 7), none had *TP53* mutations
[11]*	26	N/A	N/A	FISH	Telomere length	5/26 IUPs, 26/26 inverted TCC had telomere shortening
[8]†	5	59 (50-74)	5/0	NGS/Sanger sequencing	50 gene panel	3/5 *HRAS*, 1/5 *FGFR3*
[5]*†	26	59 (35-74)	22/4	Sanger sequencing	*TERT* promoter	4/26*TERT* mutations
[9]*	11	66 (53-90)	9/2	Whole exome sequencing/NGS	486 gene panel	10/11 *HRAS*, 1/11 *KRAS*

*Cases including urothelial carcinoma for comparison
†Cases including inverted urothelial carcinoma
FISH: fluorescence in situ hybridization; LOH, loss of heterozygosity; NGS, next generation sequencing; IUP, inverted urothelial papilloma; TCC, transitional cell (urothelial) carcinoma