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Molecular characteristics of urothelial neoplasms in children and young adults: a subset of tumors from young patients harbors chromosomal abnormalities but not *FGFR3* or *TP53* gene mutations

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Urothelial neoplasms in children and young adult patients are rare and hypothesized to have a lower rate of recurrence and progression than those of older adults. Because of their rarity, data regarding molecular abnormalities in these tumors are limited. We studied molecular characteristics of urothelial neoplasms from patients under age 30 years using UroVysion fluorescence *in situ hybridization* (chromosomes 3, 7, 17, and 9p21) and DNA mutational analysis for the *FGFR3* and *TP53* genes. Seventeen tumors were identified in patients 6–26 years of age, including low-grade papillary urothelial carcinoma ($n=10$), high-grade papillary urothelial carcinoma ($n=5$), urothelial papilloma ($n=1$), and papillary urothelial neoplasm of low malignant potential ($n=1$). No tumor demonstrated mutation of *FGFR3* or *TP53*. Chromosomal abnormalities were detected only in patients aged $\geq$19 years: two low-grade urothelial carcinomas had loss of 9p21 as a sole chromosomal abnormality and three high-grade urothelial carcinomas had other or multiple chromosomal abnormalities. Under age 19 years, no tumor showed molecular abnormalities with either method (five low-grade papillary urothelial carcinomas and one each of high-grade papillary urothelial carcinoma, papillary urothelial neoplasm of low malignant potential, and urothelial papilloma). Our results support the idea that mutations of the *FGFR3* and *TP53* genes are rare or absent in urothelial neoplasms of young patients. In contrast, chromosomal abnormalities detected by UroVysion fluorescence *in situ* hybridization are sometimes present in patients above 19–20 years of age. This finding supports the recently proposed hypothesis that an age of 19–20 years separates distinct molecular pathways of urothelial carcinogenesis.
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In children and young adults, urothelial neoplasms such as urothelial carcinoma are rare, in contrast with older adults, particularly men, in whom the urinary bladder is among the most common sites for development of a new cancer.[1–3] When urothelial carcinoma does occur in the pediatric and young adult population, it is generally thought that the

biologic behavior differs, including a lower rate of recurrence and progression.[2,4–9] Because of the rarity of urothelial neoplasms in these patients, data regarding the molecular alterations of urothelial carcinoma in this age group are limited. Nonetheless, there is emerging evidence to indicate that the characteristic molecular alterations of urothelial carcinomas of older adults are either absent or uncommon in young patients.[10–12] In order to better understand the pathogenesis of urothelial neoplasms in young patients and compare and contrast them with those of older patients, we assessed molecular features in a series of 17 tumors from children and young adults (under age 30 years) using fluorescence *in situ* hybridization with the UroVysion probe set and DNA sequencing mutational analysis for the *FGFR3* and *TP53* genes.

Materials and methods

Specimens

Formalin-fixed and paraffin-embedded tissue blocks from urothelial neoplasms of patients under 30 years of age were retrieved via search of the in-house and consultation files of the contributing authors to include lesions originally diagnosed as urothelial papilloma, papillary urothelial neoplasm of low malignant potential (PUNLMP), papillary urothelial carcinoma (any grade), urothelial carcinoma *in situ*, or invasive (papillary and nonpapillary) urothelial carcinoma. Hematoxylin and eosin-stained microscopic slides were reviewed and neoplasms were reclassified according to the World Health Organization and International Society of Urologic Pathology classification system.[13] Nonneoplastic urothelial proliferations, such as polypoid or papillary cystitis, and putative precursor lesions, such as urothelial hyperplasia, were not studied. Tumors developing in the setting of urinary bladder augmentation, in which segments of the gastrointestinal tract are utilized in reconstruction of the urinary bladder, were also excluded from the study.[14–16]

Fluorescence *In Situ* Hybridization

Unstained sections were prepared from the available tissue blocks and were deparaffinized with xylene for fluorescence *in situ* hybridization using the UroVysion probe set, using the methods previously described.[17] For this method, the slides were treated with absolute ethanol, and then air dried and boiled in a glass staining jar with 1 × citrate buffer (pH 6.0) (Zymed, CA, USA) within a beaker filled with distilled water on a hot block for 10 min. Slides were washed with distilled water and transferred to 2 × sodium citrate buffer (SSC) for 5 min. The slides were air dried and digested with 0.75 ml pepsin (5 mg/ml in 0.01 N HCl with 0.9% NaCl; Sigma, St Louis, MO, USA) at 37 °C for 40 min. The slides were then washed with distilled water and 2 × SSC, followed by air drying. Chromosome enumeration probes (CEPs) for chromosomes 3, 7, and 17, and the locus-specific indicator (LSI) probe for 9p21 were labeled with fluorophores. CEP3, CEP7, and CEP17 probes were labeled with Spectrum Red, Spectrum Green, and Spectrum Aqua, respectively. LSI p16 (9p21) was labeled with Spectrum Gold (Vysis, Downers Grove, IL, USA). The probes were diluted 1:10 with tDenHyb2 (Insitus, Albuquerque, NM, USA). To each slide, 5 μl of diluted probes was added in reduced light conditions. The slides were then covered with a 22 × 22 coverslip, sealed with rubber cement, and put into an opaque plastic box wrapped with aluminum foil. The slides were denatured at 83 °C for 12 min and hybridized at 37 °C overnight. After hybridization, the slides were washed and counterstained with 10 μl DAPI (Insitus) and sealed with a 50 × 22 coverslip.

The stained slides were observed and documented using MetaSystem software (Belmont, MA, USA) under 100 × oil objective using filters: SP-100 for DAPI, FITC MF-101 for Spectrum Green, Gold 31003 for Spectrum Gold, Aqua 31036V2 for Spectrum Aqua, and Texas Red Sp103 for Spectrum Red signals. Five sequential focus stacks with 0.4 μm intervals were acquired and integrated into a single image to reduce thickness-related artifacts.

For each case, 200 nuclei were counted. Each cell was simultaneously analyzed for the signals of chromosomes 3, 7, 17, and 9p21. Chromosomal gain or loss was defined based on the Gaussian model and relative to normal controls. As previously reported, the percentage of cells with chromosomal abnormalities in formalin-fixed, paraffin-embedded tissue sections from urothelial malignancies are typically high, with abnormalities detected in the majority of cells.[18] In contrast, normal urothelium from patients without a known bladder malignancy typically exhibits only rare cells with numerical chromosomal abnormalities, potentially because of section truncation artifact, unrecognized overlapping nuclei, and rare normal cells in G2 or M phase of the cell cycle.[19,20] Therefore, cutoff values for an abnormal result were set at the mean plus 3 standard deviation of the number of disomic cells in control individuals. The mean plus 3 standard deviation represents a specificity of 99.9%. Any tumor cases with a score beyond the cutoff value were considered to have either a gain or a loss of the designated chromosome(s).

Mutational Analysis

Mutational analysis of the *FGFR3* and *TP53* genes was performed using methods similar to those previously described, and was compared with previously published rates of mutations of these genes in urothelial carcinomas of older adults.[21–30] For this technique, laser-assisted microdissection of

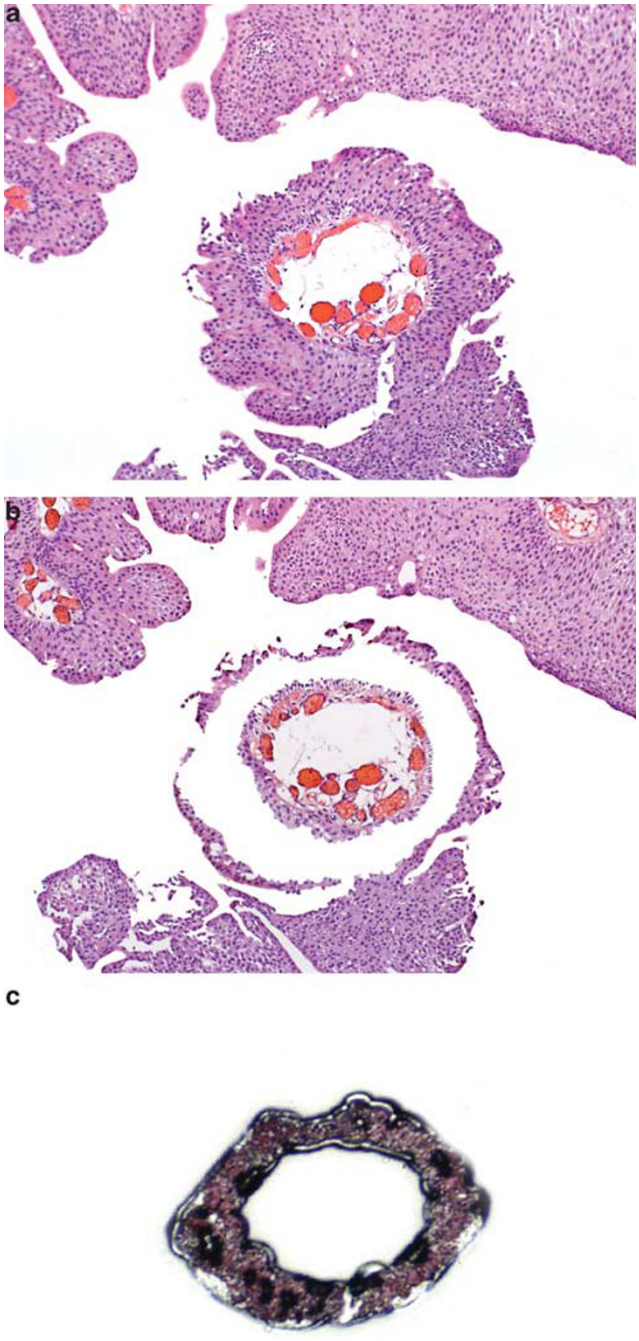

Figure 1 Tumor tissue for DNA mutational analysis of the *FGFR3* and *TP53* genes was collected using laser capture microdissection from formalin-fixed, paraffin-embedded tissue blocks containing urothelial neoplasms from young patients. (**a**) Hematoxylin and eosin-stained sections were examined to identify the neoplastic cells for laser capture microdissection. After microdissection, the neoplastic epithelial cells (**b**) have been selectively removed and the enriched sample (**c**) can be utilized for DNA mutational analysis.

the tumor tissues was performed (Figure 1) on lightly hematoxylin and eosin-stained sections using a PixCell II Laser Capture Microdissection System (Arcturus Engineering, Mountain View, CA, USA).[31,32] Approximately 600–1000 cells of each tumor were microdissected from the 4 μm histological sections. Microdissected normal tissue from the same patient served as a control. The dissected tissue was incubated in 50 ml of digesting buffer containing 10 mM Tris-HCl, 1 mM EDTA, 1% Tween-20, and 5 mg/ml of proteinase K (pH 8.3) at 37 °C overnight. The samples were boiled for 10 min to inactivate proteinase K. The genomic DNA from each sample was dissolved in 30 ml of dd H2O after phenol–chloroform extraction (phenol/chloroform/isoamyl alcohol 1/4 25:24:1).

For the *FGFR3* gene, exons 7, 10, and 15 were amplified by polymerase chain reaction (PCR) using previously established primers.[21,25,33,34] PCR was performed with 3 ml of isolated genomic DNA in a final volume of 50 ml containing 2.3 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM deoxynucleotide triphosphates, 2 mM each primer, and 2 U Taq DNA polymerase (Bio-Rad, Hercules, CA, USA). Each PCR protocol had an initial denaturing step of 95 °C for 5 min, followed by 40 cycles of: 95 °C for 30 s, 55 °C (for exons 7 and 15) or 58 °C (for exon 10) for 30 s, and 72 °C for 30 s. There was a single final extension step at 72 °C for 7 min. The PCR products were purified using the QIAquick PCR Purification kit (Qiagen Sciences, Germantown, MD, USA). DNA concentration of PCR products was measured and adjusted to 20 ng per μl. Sequencing of the purified PCR product was then performed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

For the *TP53* gene, DNA from exons 5, 7, and 8 was similarly amplified by PCR using previously established primers.[29,35,36] PCR was performed with 3 ml of isolated genomic DNA in a final volume of 50 ml containing 2.3 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM deoxynucleotide triphosphates, 2 mM each primer, and 2 U Taq DNA polymerase (Bio-Rad). Each PCR protocol had an initial denaturing step of 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a single final extension step at 72 °C for 7 min. The PCR products were purified by QIAquick PCR Purification kit (Qiagen Sciences). DNA concentration of the PCR products was measured and adjusted to 20 ng per μl. The purified PCR product was sequenced using an ABI Prism 3100 Genetic Analyzer.

Results

Patients and Tumors

Seventeen urothelial neoplasms were retrieved, all of which were initially removed by transurethral resection. The patients' ages ranged from 6 to 26 years (median 19). Twelve patients were male and 5 were female (M/F 2.4:1) After reassessment of histologic classification and grade, the tumors included low-grade papillary noninvasive urothelial

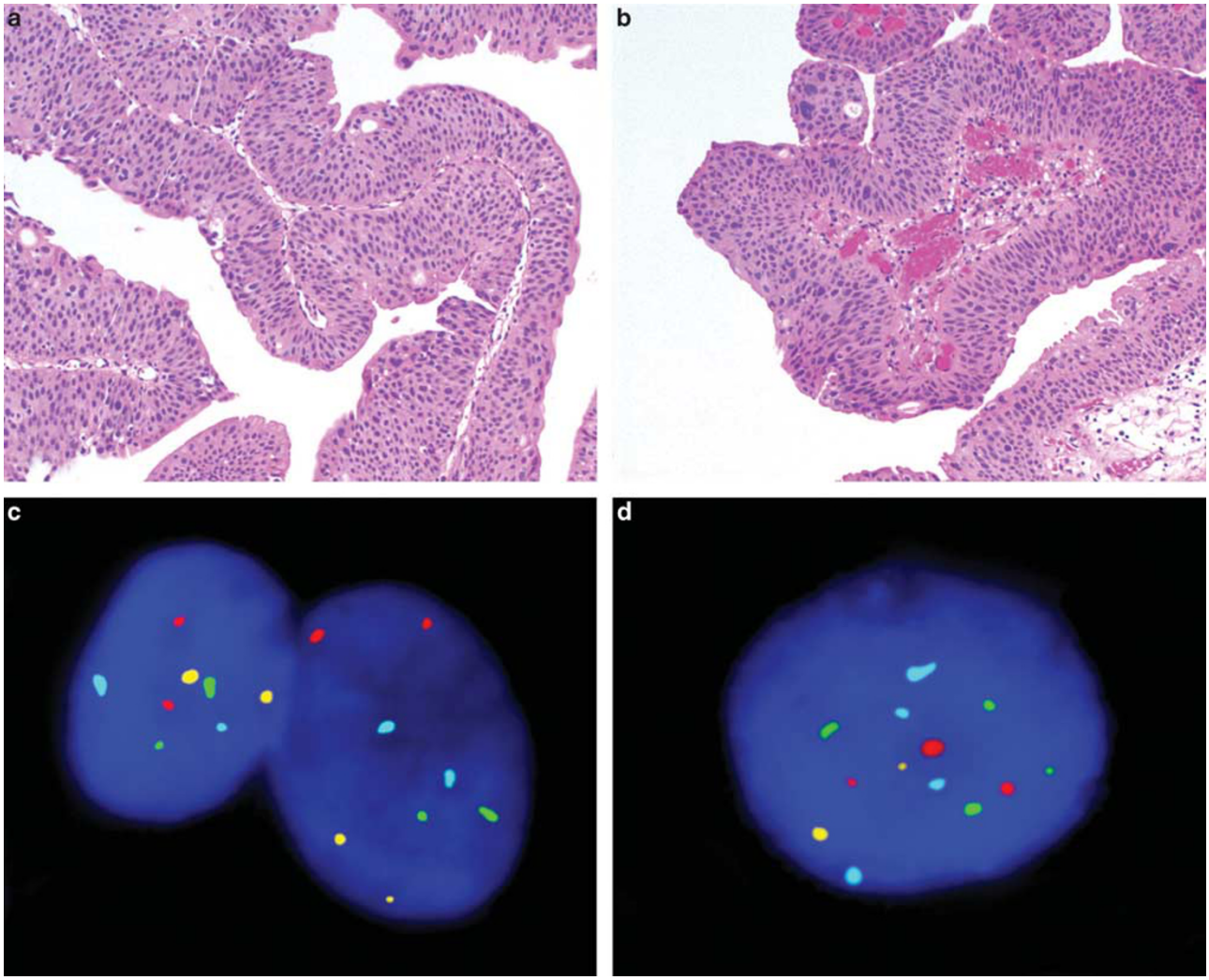

Figure 2 Results of UroVysion fluorescence *in situ* hybridization in urothelial neoplasms from young patients: microscopically, low-grade papillary urothelial carcinomas (**a**) were composed of papillary structures lined by urothelial cells with a limited degree of cytologic atypia and disorganized architecture. In contrast, high-grade urothelial carcinomas (**b**) exhibited more pronounced cytologic atypia, including scattered markedly enlarged, hyperchromatic, and irregularly shaped nuclei. Using fluorescence *in situ* hybridization, most tumors showed a wild-type or disomic pattern for each of the studied chromosomes (**c**), as evidenced by two red, green, aqua, and gold signals in each tumor cell nucleus. A subset of tumors from patients ≥19 years of age showed chromosomal abnormalities with this technique, such as gains of chromosomes 3, 7, and 17 (**d**), evidenced by more than two red, green, and aqua signals in the tumor cell nuclei (same high-grade tumor as depicted in **b**).

carcinoma (10 tumors, 59%; Figure 2a), high-grade papillary urothelial carcinoma (5 tumors, 29%; Figure 2b), PUNLMP (1 tumor, 6%), and urothelial papilloma (1 tumor, 6%). One patient (19 years of age) had a high-grade papillary urothelial carcinoma with an invasive component and was found to have deep muscularis propria invasion (pathologic stage pT2b) with lymph node metastases in a subsequent resection specimen.

Fluorescence *In Situ* Hybridization

Using fluorescence *in situ* hybridization with the UroVysion probe set for chromosomes 3, 7, 17, and 9p21, the majority of tumors showed a wild-type or disomic pattern for each of the studied chromosomes and the 9p21 locus (12 of 17 tumors, 71%; Figure 2c). Two low-grade papillary urothelial carcinomas had loss of chromosomal locus 9p21 as the only detected abnormality (Table 1), accounting for 12% of all tumors studied and 20% of the group of low-grade papillary urothelial carcinomas. Three high-grade papillary urothelial carcinomas (of 5 total high-grade tumors) had other chromosomal abnormalities or multiple abnormalities, including: loss of chromosome 7 (1 patient); gain of chromosomes 3, 7, and 17 (1 patient; Figure 2d); and loss of chromosome 7 and the 9p21 locus (1 patient; Table 1). Loss of 9p21 as a sole abnormality occurred only in low-grade tumors ($n=2$), whereas other abnormalities occurred only in high-grade tumors

Table 1 Molecular characteristics of urothelial neoplasms of young patients

	Gender	*Age*	*Classification*	*CEP3*	*CEP7*	*9p21*	*CEP17*	*Overall*	*Exon 7*	*Exon10*	*Exon 15*	*Exon 5*	*Exon7*	*Exon 8*
1	M	26	HG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
2	M	26	HG	Gain	Gain	Negative	Gain	Positive	No	No	No	No	No	No
3	F	25	LG	Negative	Negative	Loss	Negative	Positive	No	No	No	No	No	No
4	M	24	HG	Negative	Loss	Loss	Negative	Positive	No	No	No	No	No	No
5	M	24	LG	Negative	Negative	Loss	Negative	Positive	No	No	No	No	No	No
6	M	23	LG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
7	M	22	LG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
8	M	20	LG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
9	M	19	HG	Negative	Gain	Negative	Negative	Positive	No	No	No	No	No	No
10	F	18	LG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
11	M	18	LG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
12	F	18	HG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
13	F	18	LG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
14	M	12	Papilloma	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
15	M	12	PUNLMP	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
16	F	9	LG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No
17	M	6	LG	Negative	Negative	Negative	Negative	Negative	No	No	No	No	No	No

Abbreviations: M, male; F, female; HG, high-grade urothelial carcinoma; LG, low-grade urothelial carcinoma; PUNLMP, papillary urothelial neoplasm of low malignant potential; CEP, chromosome enumeration probe; No, no mutation identified.

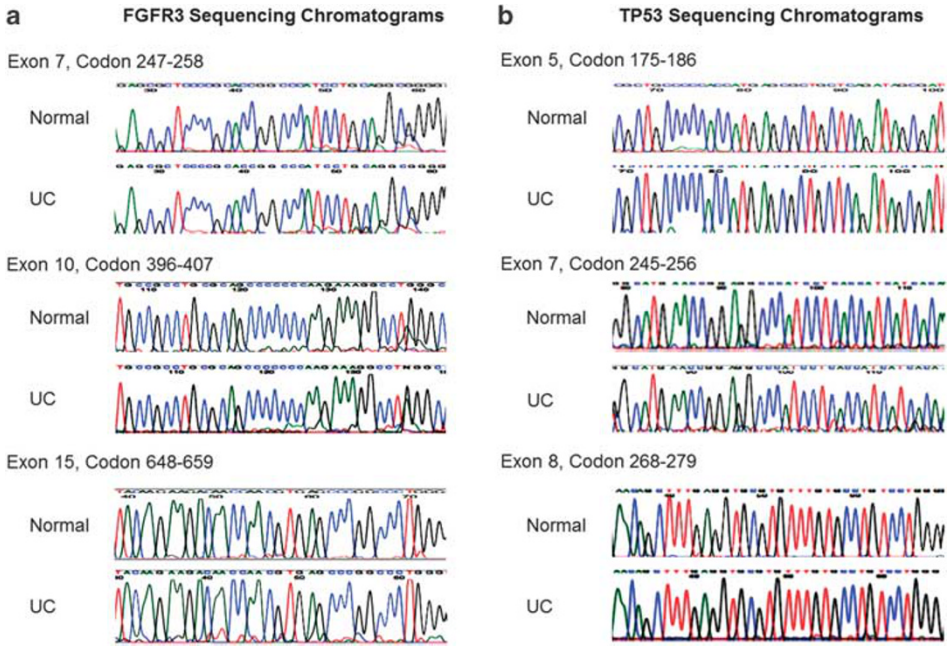

Figure 3 DNA sequencing chromatograms from the *FGFR3* (**a**) and *TP53* (**b**) genes did not reveal mutations at the studied exons in the neoplastic tissue from any tumor (bottom rows), when compared with normal control tissue (top rows). UC, urothelial carcinoma; normal, normal control tissue.

($n = 3$). All tumors from patients under 19 years of age exhibited no abnormalities (five low-grade papillary urothelial carcinomas, one high-grade papillary urothelial carcinoma, one urothelial papilloma, and one PUNLMP).

DNA Mutational Analysis

Using DNA sequencing mutational analysis for the *FGFR3* and *TP53* genes, no tumor showed mutation of either gene. Studied exons included 7, 10, and 15 for *FGFR3* and 5, 7, and 8 for *TP53* (Figure 3 and Table 1).

Discussion

In children and young adults, the development of urothelial neoplasms such as urothelial carcinoma is rare, particularly at the youngest end of this age group.[4–7,37] As such, the etiology and pathogenesis

of these tumors are not well understood.[2] In general, it appears that most urothelial carcinomas in these patients are of low grade, with infrequent recurrence and more indolent behavior when compared with those of adults;[4,5,9,38] however, other data suggest that recurrence and invasion do sometimes occur.[4,7–9,37,39] Through the first four decades of life, the incidence of urothelial carcinoma appears to increase slightly overall, although it remains rare.[2] As such, understanding of the molecular pathogenesis of tumors in this setting is quite limited, in contrast to tumors of older adults, in whom molecular pathways of urothelial tumorigenesis have been more thoroughly characterized.[10–12,23,40] To date, it appears that the typical molecular abnormalities of urothelial neoplasms of older patients are often lacking in tumors of children and young adults.[10,11] To better understand the pathogenesis of tumors that occur in this patient population, we assessed molecular features of urothelial neoplasms from patients under age 30 years using UroVysion fluorescence *in situ* hybridization and mutational analysis for the *FGFR3* and *TP53* genes.

Urothelial carcinoma is characteristically a disease of older patients, preferentially affecting men.[23] In older adult patients, a variety of risk factors such as cigarette smoking and exposure to other carcinogens, such as those used in the dye, rubber, textile, and chemical industries, have been well established.[41,42] In children and young adults, however, understanding of the etiology and pathogenesis of urothelial tumors remains more elusive.[2] An association with cigarette smoking has been noted in some patients;[4] however, in others no history of exposures is known.[6] Combined with the substantially shorter duration of exposure for a young patient, these risk factors seem unlikely to account entirely for the occurrence of urothelial carcinoma in this setting. Similarly, hereditary tumor predisposition syndromes appear to have a limited contribution to the occurrence of lower urinary tract urothelial carcinomas.[2]

In adults, loss of heterozygosity of chromosome 9 and abnormalities of *FGFR3* are characteristically associated with noninvasive papillary tumors, whereas mutation of the *TP53* gene (17p13.1) is typical of urothelial carcinoma *in situ*, and high-grade and invasive carcinomas.[23,43–45] In particular, mutations of *FGFR3* have been reported in 72–84% of low-grade papillary urothelial carcinomas and >70% of noninvasive papillary urothelial carcinomas.[23–30] In general, the frequency of *FGFR3* gene mutations appears to correlate inversely with increasing tumor grade and stage.[23] As such, in high-grade and muscle-invasive bladder cancers, *FGFR3* gene mutation has been noted in as little as 20–22%. Conversely, the rate of mutation of the *TP53* gene appears to increase from 7 to 11% in the lowest grade (previously considered grade 1) and noninvasive tumors to as high as ~51–58% in high-grade and invasive tumors.[23] Some overlap between these two molecular pathways has also been reported, with a smaller subgroup of tumors harboring mutation of both genes.[46–48] In addition to *TP53* and *FGFR3*, the roles of a wide array of other genes have begun to be elucidated in bladder cancer pathogenesis, including *RB1*, *ERBB2*, *FGFR1*, *HRAS*, *KRAS*, *NRAS*, *PI3KCA*, *AKT1*, *CCND1*, *CDKN2A*, *CTNNB1*, *MDM2*, *PTEN*, *TERT*, and *TSC1* with potential therapeutic targets.[23,40,49–52] the chromosomal level, the UroVysion probe set was designed to target common chromosomal abnormalities in urothelial carcinomas, one of which includes deletion of *CDKN2A* (also known as p16), located at chromosome 9p21.[53] Simultaneous utilization of four multicolored probes for other common chromosomal abnormalities (chromosomes 3, 7, and 17) has been found to improve the sensitivity of the assay, while remaining technically feasible for separate evaluation.[19] The *TP53* gene in particular is located on chromosome 17p13.1. Although the exact mechanisms by which these chromosomal gains and losses contribute to urothelial carcinogenesis are not entirely elaborated, other genes located at these sites that have been implicated in tumorigenesis include *PIK3CA* (chromosome 3q26.3), *ERBB2* (17q11.2–q12), and *CTNNB1* (3p21).[49,52,54]

Only a few studies have attempted to address the molecular pathogenesis of urothelial carcinomas of young patients. Linn *et al*[12] examined a series of tumors from patients under age 30 years. Overall, numerical abnormalities of chromosomes 9 and 17 by *in situ* hybridization were uncommon. As the majority of the tumors were low stage and low grade, the lack of chromosome 9 abnormalities was surprising and contrasted with low-grade tumors of older adults. Aneuploidy of chromosome 17 was found in a smaller group of tumors, representing a comparatively large fraction of the invasive urothelial carcinomas and urothelial carcinoma *in situ*. Nuclear labeling for TP53 by immunohistochemistry was surprisingly common, including low-grade, low-stage tumors that might not otherwise be expected to have *TP53* gene mutation. However, mutation status of the *TP53* gene itself was not evaluated.[12]

More recently, Wild *et al*[10] evaluated immunohistochemical and molecular characteristics in a series of urothelial tumors from patients 4–19 years of age. Using a wide array of molecular tests, relatively few abnormalities were identified.[10] *FGFR3* gene mutation and 9p deletion in particular were absent, despite the predominant composition of the study set by low-grade tumors, similar to the findings of Linn *et al*.[10,12] Arguing against the possibility that the high rate of positivity for TP53 by immunohistochemistry observed by Linn *et al* is the result of frequent mutation of the gene, only 1 of 14 tumors showed nonsense mutation in the *TP53* gene and positive immunohistochemical labeling for TP53.[10,12] Overall, the authors concluded that urothelial neoplasms in this age group are infrequently

associated with the typical genetic alterations of urothelial carcinoma in older patients.[10]

A recent study by Owen *et al*[11] examined epigenetic alterations in urothelial carcinomas stratified by age. The authors examined the same group of patients under age 19 years as analyzed by Wild *et al*.[10] This youngest group had the lowest rates of promoter methylation for eight tumor suppressor genes involved in urothelial carcinogenesis.[11] Interestingly, patients over 20 years of age had similar epigenetic profiles to those of the oldest patient group (>46 years, median 78), suggesting that an age of ~19–20 years separates two genetically distinct groups of urothelial tumors.[11]

Supporting the hypothesis that urothelial carcinoma in young patients has a distinct molecular pathogenesis from that of older adults, we did not identify mutations of *FGFR3* or *TP53* in this study, despite the key roles of these gene mutations in urothelial carcinomas in general. In particular, the absence of *FGFR3* gene mutation in the group of low-grade, noninvasive papillary urothelial carcinomas contrasts strikingly with the typical profile of these tumors from older adults, in which rates of *FGFR3* gene mutation have been noted to be >75%.[23] Although a relatively small number of tumors were considered high grade, complete absence of *TP53* gene mutation in this subgroup also contrasts with high-grade tumors of older adults. We did, however, observe chromosome 9p21 loss as a sole abnormality in a subgroup of low-grade papillary urothelial carcinomas (2 of 17 tumors overall and 2 of 10 low-grade papillary urothelial carcinomas). In adults, loss of heterozygosity and deletion of this region are common events in urothelial carcinoma, thought to be an early event in carcinogenesis and associated with tumor recurrence.[23,32,40,55–58] This region contains the gene *CDKN2A* (also referred to as p16 or INK4a) that is thought to play an important role in the development of bladder cancer.[59,60] Similar to the limited existing data in this population,[10,12] our results suggest that this alteration is less common in young patients than in older adults, although our results do indicate that this aberration is occasionally present, particularly in patients above age 19 years. The UroVysion fluorescence *in situ* hybridization technique also includes chromosome enumeration probes directed to chromosomes 3, 7, and 17, selected for the frequent alterations of these chromosomes in urothelial carcinomas.[61,62] In contrast to loss of 9p21 as a sole abnormality in some low-grade urothelial carcinomas, abnormalities of one or more of these other chromosomes were detected by UroVysion fluorescence *in situ* hybridization in exclusively high-grade tumors (3 of 17 tumors overall and 3 of 5 high-grade tumors). Similar to aberrations of 9p21, these abnormalities of chromosomes 3, 7, and 17 in high-grade tumors were also observed only in patients ≥19 years of age. Interestingly, only one of these high-grade tumors exhibited a polysomic pattern (gain of two or more of the chromosomes in the probe set) that in adults is a common pattern for high-grade urothelial carcinoma.[19] Further expanding the understanding of molecular pathogenesis of urothelial carcinoma in young patients and linking the results of Linn *et al* with those of Wild *et al* and Owen *et al*, our results support the idea that an age of ~19–20 years may separate two distinct pathways of urothelial carcinogenesis at the molecular level ('pediatric' patients and young adults).[10–12] However, absence of *FGFR3* and *TP53* gene mutations, even in tumors from young adults, represents a point of contrast. Further investigation of tumors in these settings will be useful to better elucidate these differences, and whether other alterations, such as translocations,[63] play a role in the development of urothelial carcinomas of pediatric patients.

In conclusion, urothelial neoplasms are rare in pediatric and young adult patients. When they do occur, it appears that their biologic behavior and molecular pathogenesis may be distinct from those of older patients. However, the molecular features of these tumors have previously been examined in only a few series. In this study, we analyzed molecular abnormalities in urothelial neoplasms from young patients below 30 years of age. We observed no mutations of the *FGFR3* or *TP53* genes, supporting the hypothesis that these abnormalities are rare in urothelial neoplasms of young patients. Although chromosomal abnormalities detected by UroVysion fluorescence *in situ* hybridization have been generally regarded as uncommon in urothelial neoplasms of young patients, we detected such abnormalities in a subgroup of cases, exclusively in patients ≥19 years of age. Loss of chromosome 9p21 as a sole abnormality occurred in low-grade papillary noninvasive tumors, whereas gains or losses of other chromosomes were observed only in high-grade or invasive tumors. Our findings support the idea that distinct pathogenetic mechanisms may be involved in urothelial carcinogenesis particularly for patients under age 19 years, in whose tumors molecular abnormalities appear to be the most rare. In patients older than 19 years of age, chromosomal abnormalities detected by fluorescence *in situ* hybridization occasionally overlap with those of older adults.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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