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Original contribution

BRAF mutations in pediatric metanephric tumors☆,☆☆

Rose Chami MD[a,b], Minzhi Yin MD[c], Paula Marrano MLT[a], Chinachote Teerapakpinyo PhD[d], Shanop Shuangshoti MD[d,e], Paul Scott Thorner MD, PhD[a,b,e,*]

[a]*Division of Pathology, The Hospital for Sick Children, Toronto, Canada M5G 1X8*
[b]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada M5S1A1*
[c]*Department of Pathology, Shanghai Children's Medical Center, School of Medicine, Shanghai Jiaotong University, Shanghai 200127, China*
[d]*Chula GenePRO Center, Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*
[e]*Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*

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SummaryMetanephric neoplasms of the kidney are uncommon, and some cases are associated with papillary carcinoma. Most cases of metanephric adenoma occur in adults, with fewer than 25 cases reported in children, and metanephric adenofibroma is even less common. The few metanephric tumors studied at the genetic level have not shown the gains of chromosomes 7 and 17 commonly seen in renal cell carcinoma, suggesting that the carcinoma arising in this setting has a separate genetic origin from the adenoma. However, the assumption that this carcinoma has the same chromosome gains as sporadic renal cell carcinoma has never been validated. We studied 4 cases of metanephric tumors in children, including 1 metanephric adenofibroma with papillary carcinoma. The composite tumor was studied by single nucleotide polymorphism array and fluorescence in situ hybridization, with the adenoma and carcinoma components analyzed separately. No copy number alterations were detected in either component. A BRAF V600E mutation has been reported in most cases of metanephric adenoma in adults. We performed BRAF V600E immunostaining and sequencing in our 4 pediatric cases. Three cases had a BRAF V600E mutation including the composite tumor, with both the adenoma and carcinoma components showing the same mutation. This finding provides the first genetic evidence that these 2 tumors are biologically linked. Ten cases each of pediatric renal cell carcinoma and Wilms tumor were immunonegative. Thus, BRAF V600E immunostaining is a helpful marker for pediatric metanephric adenoma, and additional research is required on the possible role of this mutation in the development of renal carcinoma.

1. Introduction

Metanephric neoplasms of the kidney are rare tumors, composed of epithelial and/or stromal cells, showing diverse histopathologic features. This group of tumors includes metanephric adenoma, metanephric adenofibroma, and metanephric stromal tumor. Metanephric adenoma is the most common of the metanephric neoplasms but still only accounts for 0.2% of

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* Corresponding author at: Division of Pathology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.
E-mail address: paul.thorner@sickkids.ca (P. S. Thorner).

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adult renal epithelial neoplasms [1]. It occurs mostly at 50 to 60 years of age, with a mean age at presentation of 41 years and a female-to-male ratio of 2:1 [2,3]. Approximately 50% of metanephric adenomas are clinically asymptomatic; other patients present with flank pain, palpable mass, and/or hematuria. Curiously, 10% of patients have polycythemia, and rare cases have hypercalcemia and chyluria [2,4,5]. Resection is curative. Fewer than 25 cases in the pediatric age group have been reported, generally in older children and teenagers [2,3,5–12], with the youngest case at 15 months of age [13].

Metanephric adenofibroma is a less common, biphasic tumor, in which the epithelial elements are associated with a spindle cell stroma [14–16]. The epithelial component is the same as in metanephric adenoma, and the stromal part is the same as in metanephric stromal tumor [17]. Fewer than 40 cases have been reported for all ages [14–16,18–23]. This tumor generally occurs in younger patients than does metanephric adenoma, with ~50% of cases occurring in the pediatric age group, at ages ranging from 5 months to 36 years and a mean age of 6 years. There is a male-to-female ratio of 2:1. Patients generally follow a benign clinical course but can present with polycythemia, hematuria, or hypertension. In the 2 largest series, metanephric adenofibroma was associated with epithelial predominant Wilms tumor (28% of cases) or papillary renal cell carcinoma (8%-20% of cases) [2,14]. If restricted to the pediatric age group, fewer than 10 cases of metanephric adenoma associated with carcinoma have been reported [7,14,16], including one report of metanephric adenofibroma in combination with Wilms tumor and papillary carcinoma [20].

There are only a small number of studies examining metanephric adenoma at the genetic level, mainly in an attempt to relate this tumor to renal cell carcinoma, and most studies support the concept that metanephric adenoma and papillary renal cell carcinoma are distinct [7,24–26]. Recent studies have reported that more than 85% of metanephric adenomata have a *BRAF* V600E mutation; however, these series include only 1 patient younger than 25 years (age 16 years) [27–29]. In the present study, we examined 4 pediatric cases of metanephric tumors at the genetic level, including 1 associated with a papillary carcinoma with 2 purposes in mind: (1) investigate whether any genetic link could be demonstrated between the metanephric tumor and concomitant carcinoma, or whether such a tumor is more closely related to sporadic renal cell carcinoma or instead Wilms tumor, and (2) explore the usefulness of BRAF V600E mutation detection in the diagnosis of pediatric metanephric tumors.

2. Materials and methods

The pathology archives at the Hospital for Sick Children, Toronto, Canada, were searched for metanephric tumors since 1987, and 3 cases with blocks available were identified. An additional case was contributed by Shanghai Children's Medical Centre, Shanghai, China.

2.1. Single nucleotide polymorphism array

DNA was extracted from the metanephric adenoma component only of case 4 using histology from a stained section of the block as a guide and coring out the area of interest. The formalin-fixed, paraffin-embedded core was dewaxed in xylene and rehydrated in a series of ethanol washes. Tissue was then placed in proteinase K buffer overnight at 37°C, and DNA was then extracted using phenol-chloroform, air-dried, and reconstituted in water. A minimum of 750 ng of DNA per sample were hybridized to the Affymetrix (Santa Clara, CA) Human Single Nucleotide Polymorphism (SNP) 6.0 arrays, at The Centre for Applied Genomics at The Hospital for Sick Children. DNA was digested with *Nsp*I and *Sty*I restriction enzymes, ligated to adaptors followed by a polymerase chain reaction (PCR) using primers that recognizes the adaptor. PCR conditions were optimized to preferentially amplify fragments in the 200- to 1100-base-pair (bp) size range. Two extra PCR cycles were done for the paraffin-embedded samples. The amplified DNA was then labeled and hybridized to the Genome-Wide Affymetrix Human SNP 6.0 array. The SNP array data were analyzed and integrated using the Partek Genomic Suite 6.6 Software (Partek, St Louis, MO). The normal reference used was the Human HapMap data set (www.hapmap.ncbi.nlm.nih.gov), which is derived from 270 individuals of different ethnic origins. Following the manufacturer's recommendations for copy number analysis, the minimum number of adjacent genomic markers on the array with a copy number change that would be viewed as a valid change was set at 10 (genomic segmentation parameter). Fragment lengths were restricted to less than 700 bp for the formalin-fixed, paraffin-embedded samples, as previously recommended [30]. The genomic segmentation and hidden Markov model algorithms were used for detection of copy number variations.

2.2. In situ hybridization

For fluorescence in situ hybridization (FISH), Spectrum orange– or Spectrum green–labeled bacterial artificial chromosome (BAC) DNA was acquired from The Applied Centre for Genomics, Toronto, Canada (http://www.tcag.ca/) and selected according to the UCSC Genome Bioinformatics Browser (http://genome.ucsc.edu/, March 2006 Build). Each labeled BAC DNA was tested on normal human lymphocyte metaphases to ensure that the BAC mapped to the correct chromosomal location. The following BAC probes were used: RP11-659I1 (1q21.1), RP11-197N17 (1q25.2), RP11-909L14 (2p11.2), RP11-89K7 (4p15.33), RP11-831P11 (4q13.2-q13.3), RP11-974I24 (6p21.32), RP11-344A19 (7q34), RP11-76I20 (9q21.11), RP11-46G16 (17q21.1-q21.2), and RP11-76I20 (19q13) along with Vysis centromere 11 probe (Abbott Molecular, Des Plaines, IL, #06J37-011). These probes were selected based on reports in the literature of changes in metanephric adenoma [15,25,31–33] or changes in copy number suggested by SNP array. Slides