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Clinically Relevant Subsets Identified by Gene Expression Patterns Support a Revised Ontogenic Model of Wilms Tumor: A Children's Oncology Group Study[1,2]

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Abstract

Wilms tumors (WT) have provided broad insights into the interface between development and tumorigenesis. Further understanding is confounded by their genetic, histologic, and clinical heterogeneity, the basis of which remains largely unknown. We evaluated 224 WT for global gene expression patterns; *WT1*, *CTNNB1*, and *WTX* mutation; and 11p15 copy number and methylation patterns. Five subsets were identified showing distinct differences in their pathologic and clinical features: these findings were validated in 100 additional WT. The gene expression pattern of each subset was compared with published gene expression profiles during normal renal development. A novel subset of epithelial WT in infants lacked *WT1*, *CTNNB1*, and *WTX* mutations and nephrogenic rests and displayed a gene expression pattern of the postinduction nephron, and none recurred. Three subsets were characterized by a low expression of *WT1* and intralobar nephrogenic rests. These differed in their frequency of *WT1* and *CTNNB1* mutations, in their age, in their relapse rate, and in their expression similarities with the intermediate mesoderm *versus* the metanephric mesenchyme. The largest subset was characterized by biallelic methylation of the imprint control region 1, a gene expression profile of the metanephric mesenchyme, and both interlunar and perilobar

Abbreviations: WT, Wilms tumor; ICR1, imprint control region 1; ICR2, imprint control region 2; MM, metanephric mesenchyme; LOI, loss of imprinting; LOH, loss of heterozygosity; ROI, retention of imprinting; UPD, uniparental disomy

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[2]This article refers to supplementary materials, which are designated by Tables W1 and W2 and are available online at www.neoplasia.com.

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nephrogenic rests. These data provide a biologic explanation for the clinical and pathologic heterogeneity seen within WT and enable the future development of subset-specific therapeutic strategies. Further, these data support a revision of the current model of WT ontogeny, which allows for an interplay between the type of initiating event and the developmental stage in which it occurs.

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Introduction

The initiation and progression of the most common adult cancers result from a stepwise accumulation of multiple genetic events within a finite number of pathways occurring over many years. In contrast, the initiation of neoplasia in children results from one to two genetic events that occur over the course of months rather than years. These events usually involve genes responsible for normal development and result in tumors that closely resemble cells within the developing embryo. They are also often the same genetic events that participate in the development of adult tumors. Therefore, pediatric embryonal neoplasms provide invaluable insights into normal development and into both adult and childhood neoplasia. The investigation of Wilms tumor (WT), one of the most common tumors of childhood, is a remarkable illustration of this. This unique success is due in part to the fact that WT is the only embryonal neoplasm that arises within precursor lesions known as nephrogenic rests, of which there are two predominant types: perilobar and intralobar [1]. WTs are also capable of showing a striking spectrum of appearances ranging from undifferentiated "blastemal" tumors to "teratoid" tumors composed of a mixture of differentiated skeletal muscle, chondroid, and a variety of epithelial cell types. This heterogeneity implies a complexity to the underlying causes of WT that has fascinated investigators for decades.

Two genetic loci have consistently been associated with the pathogenesis of WT, the *WT1* gene at 11p13, and the WT2 locus at 11p15. *WT1* encodes a transcription factor important in multiple phases of normal renal, gonadal, and cardiac development [2,3]. Germline mutations of *WT1* result in syndromes, including Denys-Drash and Wilms tumor-aniridia-genitourinary malformation-mental retardation: both are characterized by an increased risk of WT and abnormal genitourinary development [4–6]. Somatic mutations of *WT1* are seen in 10% to 20% of sporadic WT [7–9]. Frequently accompanying *WT1* mutation is canonical Wnt activation, most commonly due to activating mutation of β-catenin (*CTNNB1*) [10,11]. Inactivating mutations of *WTX*, a protein that contributes to β-catenin degradation, may also occur in 15% to 20% of patients with WT, regardless of their *WT1* mutation status [12–15]. While canonical Wnt- activating mutations likely occur subsequent to *WT1* mutation [16,17], whether or not Wnt activation is required for tumor development after *WT1* mutation is not clear. Nor is the role of Wnt activation in WT that lack *WT1* mutation known.

The WT2 chromosomal region came to scientific attention with the observations of 11p15 loss of heterozygosity (LOH) or loss of imprinting (LOI) in a large proportion of sporadic WT [18], and 11p15 uniparental disomy (UPD) or duplication in patients with Beckwith-Wiedemann syndrome (BWS), which carries an increased risk of WT and developmental abnormalities including organ and limb overgrowth [19–22]. 11p15 methylation abnormalities resulting in WT are accompanied by aberrant methylation at imprint control region 1 (ICR1), resulting in biallelic expression of *IGF2*, a gene normally expressed only from the paternally inherited allele [21,23]. Although 11p15 clearly plays a critical role in the pathogenesis of WT, the observation of 11p15 LOH in normal tissue from some WT patients [24] and the lack of tumors arising in mutant mice with ICR1 LOI [25] imply that biallelic expression of *IGF2* alone is insufficient for tumor development.

Other loci implicated in WT infrequently are the familial predisposition loci FWT1 at 17q12-q21 and FWT2 at 19q13.4 [26,27]. The documented association between relapse and LOH for 1p and 16q [28] is being used to stratify patients within the current Children's Oncology Group therapeutic protocols. The critical genes within these regions are not known.

In summary, despite the wealth of knowledge that the investigation of WT has provided, much remains unknown and further progress is made difficult by the genetic, histologic, and clinical heterogeneity that characterizes WT. The goal of this study was to investigate patterns of global gene expression and known genetic and epigenetic changes in a large number of prospectively identified WTs to identify and characterize distinctive subsets that may merit therapeutic stratification or respond to specific therapies. In addition, the recent availability of large data sets of gene expression patterns identified in microdissected samples of different embryologic stages and in different cell types during normal renal development [29,30] offers a unique opportunity to place each of these WT subsets within their developmental context. We have accomplished this, and here we provide a revised ontogenic model for the development of WT.

Materials and Methods

Clinical Samples

Samples were taken from a case-cohort sampling previously described [31]. Briefly, all patients with Favorable Histology Wilms Tumor (FHWT) registered on the National Wilms Tumor Study 5 for whom pretreatment tumor tissues were available were identified. From the resulting 1451 patients, all those known to have relapsed and a random sample including approximately 30% of the remaining were identified. The resulting 600 patients were randomly divided into two groups of 300 patients each. The use of case-cohort sampling allows for the resource-efficient investigation of clinical outcome in a tumor characterized by a low relapse rate. Institutional review board approval and informed consent were obtained for all tumor specimens. Frozen sections of each sample confirmed more than 80% viable cellular tumor. Pathologic features (diagnosis, local stage, histologic pattern, skeletal muscle quantification, presence, and type of nephrogenic rests) were recorded prospectively at the time of central pathology review. Skeletal muscle quantification represents the estimated proportion of the tumor volume containing cells with cross striations.