Chapter 3

Histopathological and Molecular Characteristics of Wilms Tumor

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Abstract

Diagnosis of malignant renal tumors does not mostly create difficulties. Although micrometastases may be encountered during postmortem examination, kidney is not a preferred organ for clinically detected metastases of malignant tumors. Therefore, almost all renal tumors in adults and children are primary tumors. When primary renal tumors are encountered, most of the cases pose a diagnostic simplicity. Indeed, diagnosis of malignant kidney tumors in children is Wilms tumor (WT) in 80–90% of the cases, while it is renal cell carcinoma in adults. In fact, a typical WT contains tissue components in three different morphologies. These are mesenchymal component resembling primitive fetal mesenchyme, epithelial component that reminds us fetal renal tubules and glomeruli, and blastomatous component consisting of clusters of blast

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cells that contributed to the coinage of the term "nephroblastoma." However, not all WTs are triphasic, and different tissue components in very restricted areas may be overlooked. Besides, immunohistochemical staining methods helpful in the differential diagnosis of other tumors are not much useful in WT. Embryonic development of kidney is a complex process in which different transcription factors, proto-oncogenes, and various types of growth factors are effective. WT can be considered a failure of this transition. A number of genes are involved in nephrogenesis, as well as in Wilms tumorigenesis. Recently, some of these genes are believed to be regulated by *HACE1*, *glypican 3* (GPC3), and six *WT genes*. The incidence of WT is 1:10,000 worldwide. Currently, high cure rates can be achieved, and multimodality treatment has resulted in a significant improvement in outcomes. In this chapter, histopathological features of WT, genetic and molecular modifications related to WT, the effects of these genetic abnormalities on prognosis, and clues for differential diagnosis were evaluated.

Key words: Anaplastic; Blastemal type; Differential diagnosis; Favorable histology

Introduction

Diagnosis of malignant renal tumors does not mostly create difficulties. Although micrometastases may be encountered during autopsy, kidney is not a preferred organ for clinically detected metastases of malignant tumors. Therefore, almost all renal tumors in adults and children are primary tumors (1–3). When primary renal tumors are encountered, most of the cases pose a diagnostic simplicity. Indeed, diagnosis of malignant kidney tumors in children is Wilms tumor (WT) in 80–90% of the cases, while it is renal cell carcinoma in most adults. In fact, a typical WT contains tissue components in three different morphologies. These components are mesenchymal component resembling primitive fetal mesenchyme, epithelial component that reminds us fetal renal tubules and glomeruli, and blastomatous component consisting of clusters of blast cells that contributed to the coinage of the term "nephroblastoma." However, not all WTs are triphasic, and different tissue components in very restricted areas may be overlooked. Immunohistochemical staining methods helpful in the differential diagnosis of other tumors are not much use in WT, such as clear cell sarcoma or even renal cell carcinoma subtypes or other even more rare renal tumors (1, 3, 4).

Embryonic development of kidney is a complex process in which different transcription factors, proto-oncogenes, and various types of growth factors are effective. WT can be considered a failure of this transition. A number of genes are involved in nephrogenesis, as well as in Wilms tumorigenesis. Recently, some of these genes are believed to be regulated by *HACE1*, *glypican 3* (GPC3), and six *WT genes*. In addition, several studies have demonstrated that Cav-1 interacts with multiple members of the EGF-R/RAS/ERK and PI3/AKT pathways to modify signaling activity (5, 6).

The incidence of WT, the most common primary malignant renal tumor of childhood, is 1:10,000 worldwide. Currently, high cure rates can be achieved, and multimodality treatment has resulted in a significant improvement in outcomes. Recent studies have revealed that several genetic abnormalities are associated with a worse prognosis in WT, even in those with localized stage and favorable histology (7, 8). In this chapter, histopathological features of WT, genetic and molecular modifications related to WT, the effects of these genetic abnormalities on prognosis, and clues for differential diagnosis were analyzed.

Pathogenesis of WT

Kidney development is a complex process, consisting of two distinct embryological origins, the nephrogenic (mesenchymal) and the ductogenic (ureteric) (9). Both development pathways are regulated by transcription factors, proto-oncogenes, polypeptide growth factors that act as signaling molecules, and their receptors (10, 11). WT is the direct result of maldevelopment of the embryonic kidney and has led to many fundamental insights such as the link between normal development and tumorigenesis. Understanding the normal kidney development has helped in our understanding and treatment of WT. The metanephric kidney develops from the intermediate mesoderm, and this structure gives rise to three cell types that will form the kidney. In conclusion, this structure consists of the epithelial nephric or Wolffian duct, Six2-positive mesenchymal cells that will form the nephrons, and Foxd1-positive cells that will give rise to the stromal cells (6). WT can be considered a failure of this transition. It arises from pluripotent renal precursors that undergo excessive proliferation resulting in undifferentiated stromal components, blastemal cells similar to the condensing mesenchyme, and primitive epithelial structures resembling comma and S-shaped bodies and glomeruli. The presence of associated nephrogenic rests consisting of foci of persistent embryonic remnant tissues that failed to mature to normal renal parenchyma further points toward impaired differentiation in early renal development (6, 9, 11–14). WT was one of the three types of cancer in which Knudson and Strong (15) based his two-hit model for tumor suppressor genes, and the loss of WT1 in a subset of WT cases remains an archetypal example of a classic tumor suppressor gene, as originally proposed (6). Since then, many variations in classifications and the genetics and mechanics of tumor suppressor genes have been found (16), and the biological basis of the multiple tumors that arise in genetically predisposed individuals may clearly involve genes other than WT1. A number of genes involved in nephrogenesis, especially in the mesenchymal to epithelial transition, have also been implicated in Wilms tumorigenesis (9, 17).

Common genetic abnormalities in WT

WT, or nephroblastoma which is currently the preferred term, is the most common pediatric renal cancer (6). The biology of WT illustrates some important aspects of childhood

neoplasms such as the relationship between malformation and neoplasia, the histological similarities between the organogenesis and oncogenesis, and the two-hit theory of recessive tumor suppressor genes (7). WT morphologically resemble embryonic kidneys with a disrupted architecture, associated with undifferentiated metanephric precursors (6–8). Previous studies demonstrated that the risk of WT is increased in at least three groups of congenital malformations associated with distinct chromosomal loci. Although WT arising in these malformations accounts for no more than 10% of cases, these syndromic tumors have provided important insight into the biology in this neoplasm (7).

The first disorder that is associated with WT is WAGR syndrome, characterized by WT, aniridia, genital anomalies, and mental retardation. Lifetime risk of developing WT in these patients is approximately 33%. Patients with WAGR syndrome carry germ line deletion of chromosome 11p13, and the first identified WT-associate gene, WT1, is located on this chromosome. WT1 deletion in WAGR syndrome represents a "first hit"; the development of WT in these individuals frequently correlates with the occurrence of the mutation in the second WT1 allele as the "second hit" (6–9). The second disorder, Denys–Drash syndrome (DDS), is characterized by gonadal dysgenesis and early-onset nephropathy based on glomerulosclerosis leading to renal failure. Lifetime risk of WT in patients with DDS is approximately 90%. These patients demonstrated germ line point mutations in the zinc finger region of the WT1 protein that affects its DNA-binding properties (7). However, bi-allelic inactivation of WT1 must be required for the development of the WT phenotype in DDS (13–17). The third disorder, Beckwith–Wiedemann syndrome (BWS), is characterized by organomegaly, macroglossia, hemihypertrophy, and omphalocele. BWS has served as a model for tumorigenesis by genomic imprinting. Genetic locus of BWS or WT2 gene is on the 11p15.5. Unlike WAGR syndrome or DDS, the genetic basis for BWS is considerably more heterogeneous, in that no single genetic region is involved in all cases. Recent genetic studies have also elucidated the role of beta-catenin in WT. Beta-catenin belongs to the WNT (wingless) signaling pathway. Gain-of-function mutations have been demonstrated in 10% of sporadic WT. Similarly, mutations and deletions of WT1 gene are less common in sporadic WT cases (7, 8, 17, 18).

Histopathological features of WT

WT recapitulates normal nephrogenic differentiation, but while normal developing nephrons are beautifully structured, nephrogenic structures in WTs are disorganized (6). Most WTs show triphasic patterns such as blastemal, epithelial, and stromal (Figure 1). Clinical investigations reveal that the outcome of children with WT is dependent on histology. The cure rate in these cases is close to 90% (12). Favorable histology is characterized by the presence of all three histological elements and the absence of diffuse anaplasia (12, 14, 19–22). In

WT cases that had been pretreated with chemotherapy before surgery, the blastemal type also has been well recognized now as an adverse prognostic subtype (23, 24). However, the histological features are not sufficient to predict the prognosis of WT, and some chromosomal mutations may play a role as adverse biological markers, even in those with localized (stage I and II) favorable histology WT (25–33).

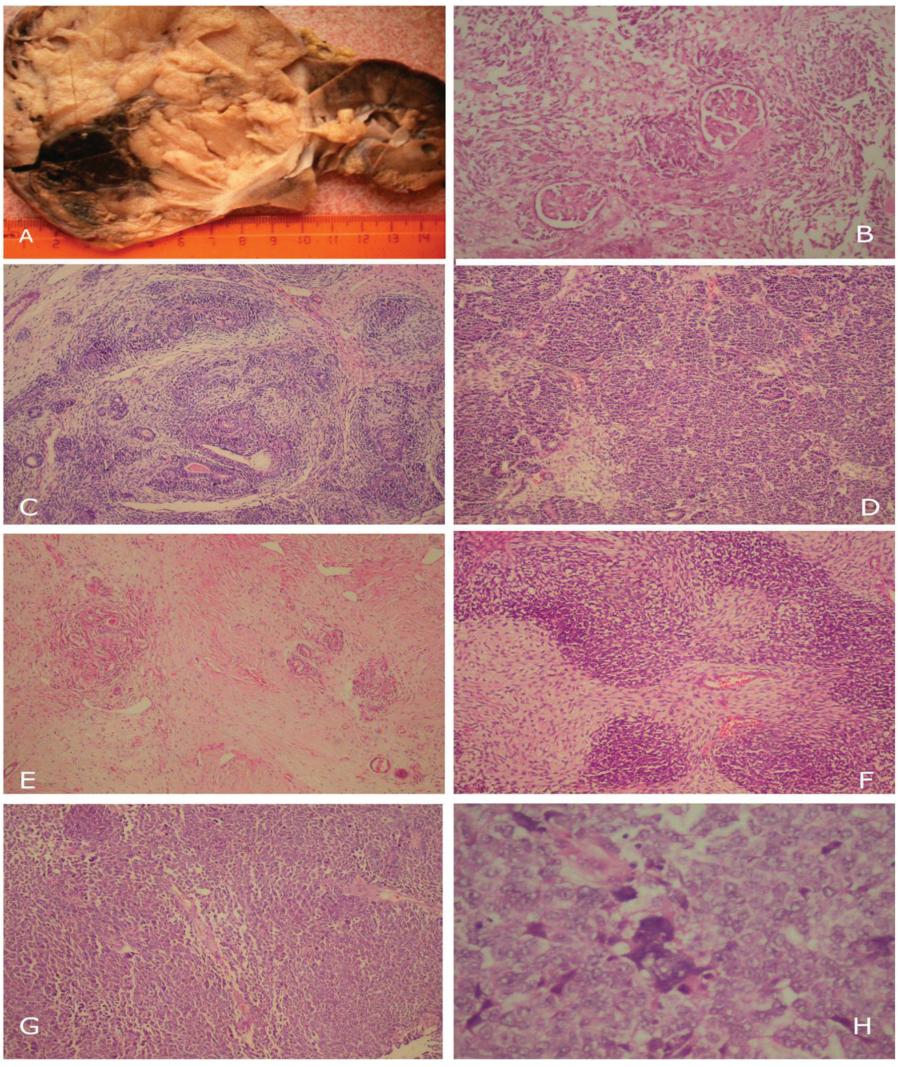

Figure 1. (A) Gross pathology of WT, (B) entrapped two normal glomeruli in a WT, (C, D) typical triphasic WTs, (E) differences after therapy, (F) blastemal and stromal areas in a WT, and (G, H) anaplastic WT.

The three histological components of WT have different proliferation potentials and different responses to therapy. In most reports, the lowest proliferation index was determined in the stromal component, and this component generally was not affected by chemotherapy. There were different results for the highest Ki-67 index in the literature. For example, the blastemal component had the highest proliferative activity in three studies, and the authors demonstrated that the surviving blastemal component after chemotherapy was a highly significant indicator of metastases and adverse outcome in WT (14, 30, 31). However, in two other studies, the highest Ki-67 index was determined in the epithelial component (22, 32). A fundamental difference in the behavior of normal versus tumor cells in culture is that normal cells divide for a limited number of times and exhibit cellular senescence, whereas tumor cells usually have the limitless proliferative capacity (14). The most prominent hypothesis is that the maintenance of telomere stability is required for the long-term proliferation of tumors. The tumor cells may escape from cellular senescence and become immortal by telomere maintenance. The simplest way of this maintenance is the activation of telomerase. Telomerase activity has been found in almost all tumors but not in adjacent normal cells (34–37). This activity was mainly evaluated with molecular studies, but it was also determined that the immunohistochemical staining pattern of TERT was correlated with telomerase activity (14, 34–38). The high telomerase reverse transcriptase (hTERT) staining was restricted to the nucleus in both normal telomerase-positive cells and cancer cells. The immunolocalization of hTERT in specimens of adult cancers revealed that the levels of telomerase activity mainly depended on the number of tumor cells with telomerase activity (14). Telomere maintenance is evident in virtually all types of malignant cells where either a telomerase-dependent or alternative lengthening of telomeres (ALT) mechanism exists. For this reason, effective strategies targeting telomere maintenance in cancer cells require telomerase inhibitors or ALT inhibitors (14, 34–38). The importance of telomerase activity is novel and potentially relevant in WT biology and progression because WT1 has been identified as a repressor of telomerase protein catalytic subunit promoter (36). In addition, functions of TERT other than telomere lengthening such as oncogenic transcriptional activation were reported (14). Although several genes such as HACE1, GPC3, and six WTs have been reported to involve in the pathogenesis of WT, they are not associated with specific histological features of WT (39–45).

Clues for differential diagnosis of WT

If a WT shows triphasic patterns, the diagnostic procedure is often not difficult. Wherein the case is of a monophasic pattern, differential diagnosis may be tiresome. In this condition, the main differential diagnosis of WT includes the so-called non-WT renal tumors,

that is, clear cell sarcomas of kidney, congenital mesoblastic nephroma, renal malignant rhabdoid tumors, neuroblastoma, and primitive neuroectodermal tumors (PNETs). In a pure epithelial tumor, metanephric adenoma should be considered for differential diagnosis. Especially, positivity of WT1 in metanephric adenoma creates the diagnostic difficulty in this tumor (1, 8, 17). Pure stromal WT is also rare, and in those cases, differential diagnosis includes the congenital mesoblastic nephroma. The age of cases is helpful for differential diagnosis, as most cases with mesoblastic nephroma occur in children younger than 6 months. In addition, WT with purely blastemal appearance after chemotherapy can be too hard to differentiate from neuroblastomas and PNETs (23, 24). Immunohistochemical stains provide limited benefit in the differential diagnosis of WT subtypes. Immunohistochemical profile of the various components of WT mirrors that of their counterparts in the developing kidney. For example, the blastematous elements show focal positivity for vimentin, the epithelial elements react for keratin and epithelial membrane antigen (EMA), and the mesenchymal elements show a heterogeneous reactivity according to the morphological appearances. Immunoreactivity for WT1 antigen is determined in the 90% of WTs, and it is the most useful marker for differential diagnosis. By contrary, positive immunoreactivity for TTF-1 is determined in 17% of WTs, and it represents a potential source of misdiagnosis (6, 7, 17). However, IHC can be very helpful in the conformation of non-WT subtypes.

Conclusion

In conclusion, WT that demonstrates monophasic appearance can be too hard to discriminate from other primary renal tumors, such as neuroblastoma, clear cell sarcoma, rhabdoid tumor, mesoblastic nephroma, or even sarcomatoid-type renal cell carcinoma (1, 3, 4). Apart from histology, genetic risk factors may aid in stratifying patients for future treatment.

Conflict of Interests

The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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