# The Molecular Biology of Wilms' Tumor

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Wilms' tumor is the most common malignant neoplasm of the urinary tract in children accounting for 8 % of all childhood solid tumors, and better than 80 % of all genitourinary malignancies in children younger than 15 years [68]. The incidence of Wilms' tumor has remained relatively constant at 7 cases per million children per year [67] regardless of race or country [4]. It has a bimodal incidence with sporadic cases presenting at a mean patient age of 3.5 years, and inherited and bilateral cases occurring at a mean age of 2.5 years [4].

Wilms' tumor is thought to be the result of the abnormal proliferation of metanephric blastema without normal differentiation into tubules and glomeruli. It may consist of a classical form, composed of embryonic blastema cells, or a differentiated form that contains striated muscle, squamous epithelial, cartilaginous and other cell types [56].

Improvements in surgery and anesthesia, as well as advances in radiation therapy and chemotherapy have produced remarkable improvements in survival for children with Wilms' tumor over the past several decades. Today, Wilms' tumor is one of the most favorable of all childhood cancers, with a 5-year survival rate approaching 90 % [14].

While national and international clinical treatment protocols have been undergoing refinements, significant advances have been made in the molecular biology and cytogenetics of this disease. Techniques of molecular biology, available only since 1970's have allowed scientists to probe the human genome in depth. Knowledge has thus accumulated at remarkable speed with enormous consequences. Our understanding of Wilms' tumor in the past two decades has progressed from a simple model of carcinogenesis to multi-interrelated hypotheses incorporating genetics, tumorigenesis and normal development.

The purpose of this review is to look at recent advances in the molecular biology and cytogenetics of Wilms' tumor in an attempt to providing a better understanding of the complex genetics of Wilms' tumor as well as for understanding the molecular mechanisms of pathogenesis of other tumors.

## Tumor suppressor genes

Two classes of genes are implicated in causing or helping to cause cancer. Dominant **oncogenes** are altered genes whose presence leads to neoplasia. **Tumor suppressor genes** are genes whose absence allows the malignancy to manifest. Every gene has a matching copy on the paired homologous chromosome. The gene and its copy are each called an **allele**. Dominant oncogenes function in a genetically dominant manner; the presence of an alteration in just one allele is tumorigenic. In the recessively acting tumor suppressor genes, both alleles must be inactivated to cause loss of growth control [37].

The evidence for existence of tumor suppressor genes has converged from several distinct lines of work. Early evidence came from somatic cell hybridization studies [63]. Geneticists took cultures of two cell lines, one a tumor cell line and one normal, and fused them to form cell hybrids that retained the genomes of both parents. If the tumorigenic phenotype is dominant, as oncogene researchers expected, the hybrids should all be tumorigenic because they carry the oncogenes from the tumorigenic parent. But in fact the hybrids were all normal. Therefore, the normal cells must possess genes that were capable of suppressing the neoplastic phenotype of their tumor cell partners.

Evidence that there were specific tumor suppressor genes in normal cells came from examining revertants of these hybrid cells that regained the tumorigenic phenotype. These hybrid cells had unstable karyotypes and frequently shed chromosomes.

By examining hybrids that spontaneously regained the transformed phenotype, investigators could identify the normal chromosome that was always lost from such cells. In human cells, this was commonly chromosome 11 or 13. These studies led to the concept that many tumors arise through loss of genetic material and provided the first clue that cancer cells often lose critical growth-regulating information during their progression toward full malignancy.

Human genetics provided a second clue that suggested the existence of tumor suppressor genes. The hereditary and sporadic forms of retinoblastoma led Knudson [26] in 1971 to develop the "two-hit" hypothesis. He postulated that the development of retinoblastoma required two rare mutations. In the sporadic form of the tumor, both mutations would have to occur within a single retinoblast cell, an exceedingly infrequent circumstance. In the inherited form, however, he suggested that one of the mutations is already present (inherited) in all retinal cells. Therefore, only a single additional mutation is required for tumorigenesis.

A decade later, analysis of the chromosomes in tumor cells and normal tissues of retinoblastoma patients resoundingly confirmed Knudson's hypothesis. Many retinoblastoma patients carried deletions in chromosome 13q14. And the mutated gene inherited by afflicted children, termed RB, was mapped to this same chromosome. Thus, development of retinoblastoma appeared to require that both copies of the RB gene be mutated. The RB gene must therefore be a tumor suppressor gene that normally functions to arrest the growth of retinal precursor cells. Even one copy is sufficient to keep growth in check. But loss of both copies of RB eliminates the block, and a tumor develops [61].

A third clue for discovering tumor suppressor genes was suggested by the genetic mechanisms used by evolving tumor cells to eliminate both copies of genes like RB. The first copy of a suppressor gene is inactivated by a somatic (or a germline) mutation. The chromosomal region carrying the surviving wild-type allele may then be replaced by a duplicated copy of the homologous chromosome region that carries the mutant allele. Most tumors that lack functional copies of a suppressor gene (like RB) display two identically mutated (homozygous) alleles while the unaffected tissues could be shown to carry one mutant RB allele and one normal one. Therefore, the **loss of heterozygosity (LOH)** at particular chromosomal regions in tumor cells (compared with somatic cells from same individual) is generally regarded as evidence for unmasking of mutations in tumor suppressor genes located in these regions.

The finding of loss of genetic information at the RB locus associated with retinoblastoma formation spurred investigators to examine other malignancies for LOH at RB and other chromosomal locations. Utilizing polymorphic DNA markers to survey systemically tumor cell genomes, numerous malignancies show LOH; these include urological neoplasms such as Wilms' tumor, von Hippel-Lindau disease, renal cell cancer and bladder cancer [63].

## Mapping the genetic loci associated with Wilms' tumor

The pattern of familial and sporadic cases of Wilms' tumor is strikingly parallel to that seen for retinoblastoma. This led early workers to describe its underlying genetic mechanisms using a model identical to that of retinoblastoma. As with retinoblastoma, this tumor occurs in infants and young children and arises from embryonal precursors. However, the Wilms' tumor syndrome diverges from retinoblastoma in several important respects:
First, the Wilms' tumor genetic deficit is not associated with a variety of tumors in other tissues. Second, Wilms' tumors are histopathologically heterogeneous. Last, the genetic basis of Wilms' tumor is complex; the locus most intensively studied to date (11p13) may represent only one of two or three loci that are involved in Wilms' tumor pathogenesis (Fig. 1) [22].

### Chromosome 11p13

The mapping of genetic loci associated with Wilms' tumor resulted from a combination of clinical observations, karyotype analyses, and molecular genetic studies. Children with the very rare WAGR syndrome [34] (Wilms' tumor, Aniridia, Genitourinary abnormalities and mental Retardation) were found to have gross deletions involving chromosome band 11p13 in one of the two germline chromosomes [17,51]. In these individuals, inactivation of a tumor suppressor gene at the locus by a

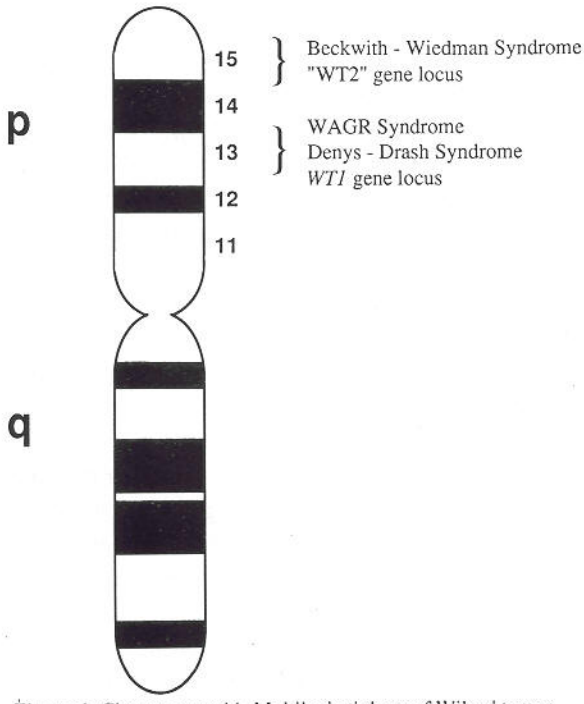


**Figure 1.** Chromosome 11. Multiloci etiology of Wilms' tumor.

major deletion represented the first of the two hits predicted by Knudson and Strong (Fig. 2) [27].

Whereas karyotype evidence first showed that the 11p13 locus was involved in germline predisposition to Wilms' tumor, molecular genetic studies demonstrated that inactivation of a gene at the locus could also constitute the second "somatic hit" leading to tumorigenesis. With the use of polymorphic DNA probes, a number of groups showed that sporadic Wilms' tumors had lost heterozygosity on the short arm of chromosome 11 [15,28,39]; moreover these tumors included some with DNA losses restricted to 11p13 itself [19,30,32].

Patients with Denys-Drash syndrome [11,12] develop Wilms' tumor in association with pseudohermaphroditism and renal failure. Large deletions on 11p13 are not observed but there are specific point mutations in the Wilms' tumor gene which then affect the DNA-binding properties of the gene [43]. These observations implied that an initial small mutation at that locus was followed in these tumors by gross chromosomal rearrangements or DNA loss, either of which resulted in loss of the second normal allele.

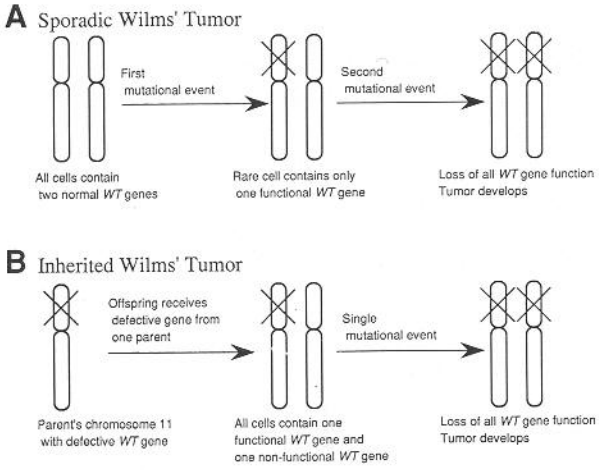


**Figure 2.** Simplified model of the development of sporadic and inherited Wilms' tumor. In fact, formation of Wilms' tumor is likely to involve additional loci, as discussed in the text, and possibly dominant negative effects of altered Wilms' tumor (WT1) gene products.

## Chromosome 11p15

The short arm of chromosome 11, however, carries more than one potential Wilms' tumor suppressor gene. Indeed, a second genetic locus at 11p15, closer to the telomere, has been identified by a combination of karyotype and molecular studies. Beckwith-Wiedemann syndrome is a collection of congenital abnormalities that includes enlargement of the tongue and visceral organs, often asymmetrically, an umbilical hernia, and neonatal hypoglycemia. The syndrome confers an increased susceptibility to a number of pediatric cancers, including Wilms' tumor, adrenocortical carcinoma, and hepatoblastoma [1,65]. In patients with familial Beckwith-Wiedemann syndrome, genetic linkage studies implicated chromosome band 11p15 as the disease locus [29,45]. Some individuals with sporadic Beckwith-Wiedemann syndrome have also been shown to have germline chromosomal abnormalities involving 11p15 [59,62].

More importantly, LOH studies of sporadic Wilms' tumors have shown that 11p15 is the most commonly deleted chromosomal region - in fact, more commonly deleted than the 11p13 area [49]. The presence of two Wilms' tumor loci on the short arm of chromosome 11 makes such genetic analyses somewhat complex. A single chromosomal recombination event that renders the 11p13 locus homozygous will also affect 11p15 and all other loci between p13 and the telomere. Thus, only a chro-

mosomal deletion (such as is seen in WAGR syndrome) or a double recombination event can demonstrate 11p13 loss of heterozygosity without affecting 11p15.

The 11p15 locus, in contrast, is closer to the telomere of chromosome 11, and loss of heterozygosity at that locus can be readily demonstrated in Wilms' tumor that have retained heterozygosity at the more proximal 11p13 locus. Henry et al [23] have suggested that genes located at both the 11p15 region and 11p13 region may be involved in the etiology of Wilms' tumor by cooperating with one another. However, data from in vivo systems suggest these two loci are separate and discrete key elements of different negative growth regulatory pathways [22].

### Familial Wilms' tumor

In addition to the 11 p13 and 11p15 Wilms' tumor loci, a third potential locus has been suggested on the basis of studies of familial Wilms' tumor. As mentioned above, familial pedigrees showing susceptibility to Wilms' tumor are rare. To date, three large families have been reported in which linkage analysis has excluded 11p13 and 11p15 from transmission of disease susceptibility [20,25,53]. These studies suggested the existence of the third Wilms' tumor gene, whose chromosomal location has yet to be determined. It is of interest, however, that in one of these families, a Wilms' tumor was shown to have loss of heterozygosity at 11p15 [20]. This would be consistent with an inherited mutation at the putative third Wilms' tumor locus, which is followed by a somatic mutation and homozygosity at the 11p15 locus.

## Identification of the WT1 gene

Cloning the retinoblastoma (RB) gene was deceptively simple because one of the earliest anonymous chromosome 13 DNA probes isolated was found to map within the region of the RB locus. The reality of the arduous nature of physical cloning methods became more evident with attempts to clone the Wilms' tumor (WT1) gene.

Using a combination of genetic strategies, including the use of somatic cell hybrids containing only an intact human chromosome 11, a large number of probes were identified that mapped to 11p13. Eventually, the technique of pulsed-field gel electrophoresis (PFGE), which provides a means of separating large DNA fragments and constructing long-range restriction maps of chromosomal regions, proved successful in isolating a candidate WT1 gene [56]. Two groups independently used PFGE to isolate a DNA fragment with the following properties: it mapped to 11p13; it was partially deleted in Wilms' tumors that had homozygous deletions in 11p13; and it recognized a 3.0-kb transcript in fetal kidney and not in other tissues [6,18].

cDNA clones were obtained and sequence analysis indicates a protein with four zinc finger domains and a region rich in proline and glutamine residues, features common to transactivation domains of transcription factors [35]. The amino acid sequence of the predicted polypeptide also shows significant homology (60 %) with a member of the immediate-early family of genes, EGR1 [7,8,57]. Transcription of EGR1 is rapidly induced by cell-surface stimuli such as growth factor-receptor interaction. The induced EGR1 protein activates transcription of genes that may be necessary for mitogenic and/or cellular differentiation.

The observations described above suggest that the protein encoded by WT1 will exhibit sequence-specific DNA-binding activity and function to regulate transcription of specific target genes. Indeed, the WT1 protein recognizes the EGR consensus sequence: 5'-CGCCCCCGC-3' [47]. Furthermore, a mutation in WT1 originally identified in a patient with Wilms' tumor abolishes the DNA-binding activity of the WT1 protein [47]. These results suggest that the WT1 protein binds DNA at the same site as a growth factor-inducible, immediate-early gene product (EGR1) and that loss of WT1 DNA-binding activity contributes to tumor formation [36]. Recently it has been shown that the WT1 protein functions as a repressor of transcription when bound to the EGR site in the promoter regions of target genes [31]. However, an alternatively spliced version of the WT1 transcript gives rise to a variant protein which does not recognize the EGR1 site but instead is thought to bind to as yet uncharacterized DNA motifs [3].

## Normal developmental expression of WT1

The embryonal tumors of childhood, with their resemblance to the corresponding fetal tissue, have long suggested that relationship exists between cancer and developmental processes. Nowhere is this more true than in the case of Wilms' tumor. Here, the primitive malignant cells may resemble the undifferentiated metanephric mesenchyme of the fetal kidney or may show varying degrees of differentiation which mimic the pathways taken during normal development to quite a remarkable extent.

Whereas RB is expressed ubiquitously [2], WT1 is expressed in a narrow range of tissues including the kidney and urogenital precursors [46]. In humans, levels of WT1 mRNA are elevated in developing kidney, including the blastemal cells thought to give rise to Wilms' tumor, but are very low in adult kidney [21,46]. In the mouse, where the time course of development can be followed, WT1 mRNA levels are detectable at the earliest stages of fetal development, rise during gestational growth, peak at about the time of birth, and then rapidly decline to low adult levels by day 17 post birth [5].

With the use of RNA in situ hybridization, WT1 expression in the developing kidney was shown to be restricted to very specific cells: the condensed mesenchyme, renal vesicle, and glomerular epithelium [46]. Wilms' tumor are classically composed of different histologic components, and it is of interest that the epithelial and blastemal components appear to express high levels of WT1, while the stromal elements express very low levels. These different histologic elements within Wilms' tumor may reflect different pathways of kidney differentiation that are derived from the primitive malignant stem cell.

In trying to understand the role of WT1 in normal kidney development and tumorigenesis, it may be possible that the expression of WT1 in certain embryonal kidney precursors may interrupt the growth program in these cells by antagonizing EGR1 protein function thereby allowing these cells to undertake, as an alternative, a commitment to end stage differentiation. Wilms' tumors with WT1 mutations are frozen in time, dividing according to an uncontrolled developmental plan. Wilms' tumor without WT1 mutations, on the other hand, are blocked at another step in the developmental pathway, and their expression of wild-type WT1 is insufficient to surmount this other alteration in growth control [22].

## Identification of putative WT2 gene

"WT2", the Wilms' tumor suppressor gene at 11p15, has not been cloned, but there is nevertheless some interesting experimental evidence as to what some of its characteristics might be. Whereas the study of WAGR syndrome eventually led to the isolation of WT1 gene, understanding the cytogenetics of Beckwith-Wiedemann syndrome and the characteristics of LOH at 11p15 may help lead to isolation of putative WT2 gene.

It has recently been observed that some patients with Beckwith-Wiedemann syndrome have inherited paternal chromosomes 11 and no maternal copy [24]; such an abnormality is called **uniparental isodisomy**. In several studies [32,49,52] it has been shown that the maternally rather than the paternally derived allele was invariably lost. This is in contrast to Knudson's model where the maternal and paternal gene would be expected to have an equal chance of carrying the somatic mutation. This phenomenon of differential genetic contribution of parents to their offsping is called **genomic imprinting** [16].

The allele which is imprinted is silenced in terms of its mRNA expression by an unknown mechanism in passage through one particular parental germline (maternal or paternal). Preferential loss of the maternally-derived alleles in Beckwith-Wiedemann syndrome is particularly interesting in view of previous studies in sporadic Wilms' tumors. LOH studies of a total of 24 cases have now been reported and in 23 of these the maternal alleles of 11p 15 markers were lost [42]. The observations clearly indicate selection against the maternally derived chromosome during tumorigenesis and strongly suggest paternal imprinting of a tumor suppressor gene (WT2) at 11p15. As a result of imprinting, the activity of this gene (or genes) is suppressed on the paternally derived chromosome 11 (Fig. 3).

The important point is that imprinting (silencing) of one allele results in a situation in which only a single mutational/deletional "hit" is required to produce bi-allelic inactivation, and thereby cause a Wilms' tumor. These findings suggest that gene dosage and imprinting may be crucial aspects in both Beckwith-Wiedemann syndrome and in genesis of

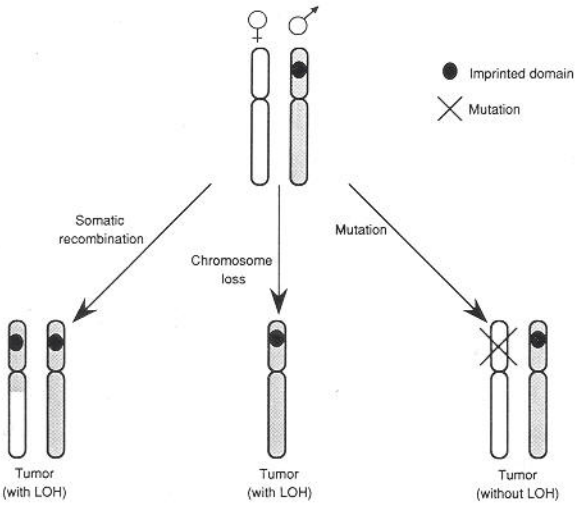


**Figure 3.** Inactivation of paternal allele of suppressor by imprinting. Somatic recombination, chromosome loss, or mutation of the maternal chromosome would result in loss of heterozygosity (LOH) with retention of the paternal allele in the tumor, except in the case of the maternal chromosomal mutation.

Wilms' tumors.

Evidence is accumulating to suggest a role for genomic imprinting in the pathogenesis of other tumors. Toguchida et al [58] showed that maternal alleles are frequently lost in osteosarcomas. In addition evidence for genomic imprinting has been suggested in the pathogenesis of Prader-Willi syndrome [38], Huntington's disease [50], hereditary glomus tumor syndrome [60] and neurofibromatosis type II [13]. In fact, the phenomenon of genomic imprinting, resulting in monoallelic gene expression, has recently been demonstrated directly at the molecular level for a human gene, H19, mapping to 11p15 [69]. H19 is expressed in a number of organs during a restricted period of fetal development and in embryonal carcinoma cells after induction of differentiation [40,41,66]. This gene is also activated when human fetal kidney blastema cells differentiated into glomeruli and tubules (Tycko, unpublished observations). Therefore, H19 itself is a candidate WT2 gene. However, no mutations in H19 have yet been reported in Wilms' tumors. Most importantly, monoallelic expression will be a useful criterion in evaluating additional WT2 candidate genes, since any bona fide WT2 gene must show this phenomenon.

In this context, another candidate WT2 gene may be the gene encoding the insulin-like growth factor II (IGF II) which is located at 11p15. This gene is imprinted in the mouse, and appears to be expressed only in the paternally derived gene. Mouse chromosome 7 is homologous to human chromosome 11p15. Introduction of an inactivated IGF II gene into mouse germline results in abnormally small-sized offspring only if the inactive gene is transmitted as the paternal allele. An inactive IGF II gene transmitted by the mother is phenotypically silent which is consistent with the absence of expression of that allele [9,10].

In addition Reeve [48] and Scott [54] and their associates demonstrated in Wilms' tumor tissue, elevated levels of IGF II transcript, indicating inappropriate activity of the gene. Although IGF II is present in several fetal tissues, including the kidney, increased expression in Wilms' tumor tissue may reflect an etiological role since IGF II is a known embryonic mitogen [33,35].

## Conclusion

The loss or inactivation of both normal alleles at a locus thought to encode for tumor-suppressing activities may represent an event common to many childhood neoplasms. The paradigm for such controlling locus is 13q14, the site of the retinoblastoma gene. Based on recent studies in familial and sporadic Wilms' tumor which suggests etiological heterogeneity, theoretical modifications of the carcinogenesis model which has been central to understanding retinoblastoma may soon be forthcoming to explain molecular mechanisms operative in other cancers.

A candidate Wilms' tumor-suppressor gene (WT1) has recently been cloned [6,18], however the ability of this gene by itself to mediate the suppressed phenotype has not yet been demonstrated. Introduction of the cloned WT1 gene into a Wilms' tumor cell line known to have an inactivated endogenous gene will be required to demonstrate the tumor suppressing potential of this gene. Studies are also in progress to isolate "WT2" gene at the 11p15 locus by utilizing the candidate gene approach and by physical mapping. The role of genomic imprinting in Wilms' tumor and carcinogenesis is only recently being explored, but evidence already suggests an important role for this phenomenon in "one hit tumorigenesis."

With further advances in molecular biology, we may also see new types of cancer therapy arise

through manipulaton of suppressor genes. By inserting wild-type genes into tumor cells that lack them, one may be able to reinstate a semblance of normal growth control and cause reversion of tumor cells to more normal growth pattern. In fact, insertion of an entire chromosome 11 causes Wilms' tumor cells to lose tumorigenicity [64]. Perhaps more practically, an understanding of the normal modes of action of tumor suppressor genes may suggest new types of chemotherapeutic agents with anti-tumor activity.


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