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Wilms' tumours: about tumour suppressor genes, an oncogene and a chameleon gene

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Abstract

Genes identified as being mutated in Wilms' tumour include *TP53*, a classic tumour suppressor gene (TSG); *CTNNB1* (encoding β -catenin), a classic oncogene; *WTX*, which accumulating data indicate is a TSG; and *WT1*, which is inactivated in some Wilms' tumours, similar to a TSG. However, *WT1* does not always conform to the TSG label, and some data indicate that *WT1* enhances cell survival and proliferation, like an oncogene. Is *WT1* a chameleon, functioning as either a TSG or an oncogene, depending on cellular context? Are these labels even appropriate for describing and understanding the function of *WT1*?

Wilms' tumour, a childhood embryonic tumour of the kidney, has long fascinated biomedical investigators, partly owing to its unique histology, which is suggestive of disorganized and incomplete kidney development, and partly owing to the early recognition that only a few rate-limiting alterations have key roles in tumorigenesis. Although this observation held the promise of a possible quick identification and functional understanding of these crucial gene alterations, this has not been the case. Mutations in Wilms tumour 1 (*WT1*), Wilms tumour gene on the X chromosome (*WTX*; also known as *FAM123B* and *AMER1*), β -catenin (*CTNNB1*) and *TP53* have been identified in tumours^{1–4}, although they occur, either singly or in combination, in only one-third of tumours⁵. *CTNNB1* and *TP53* are a well-established oncogene and a tumour suppressor gene (TSG), respectively, and accumulating data (reviewed below) suggest that *WTX* also functions as a TSG^{4–7}. The observed tumour-specific ablation of *WT1* led to its labelling as a TSG with the subsequent assumption that its normal cellular function is to inhibit cell proliferation. However, some observational and experimental data, and studies of haematopoiesis and acute myeloid leukaemia (AML), are suggestive of a role for *WT1* as a survival and/or a growth-enhancing factor^{8–14}. Furthermore, in some studies on certain cancers (for example, AML) *WT1* expression has been associated with poor clinical outcome^{15–18}, and neutralizing antibodies against *WT1* have shown great promise in clinical trials^{19–21}. Thus, the TSG label may mask a far more complex role for *WT1* during development and tumorigenesis. In this Review, I present data that suggest an oncogenic role for *WT1* during leukaemogenesis, I compare

these data with data from studies on Wilms' tumours and I discuss the effect of differentiation status and the presence of other gene mutations on the functional importance of *WT1* mutation. I also present recent data on *WTX* that further support its tumour suppressor designation.

Wilms' tumour genetics

Wilms' tumour occurs at a frequency of ~1 in 10,000 live births. The mean age at diagnosis is 43–48 months, depending on gender, and 95% of cases are diagnosed by 10 years of age. Tumours occur unilaterally (in a single kidney) in 90–95% of cases, but can also occur bilaterally; bilateral cases present ~12 months earlier than unilateral cases²². Approximately 2% of cases have an affected relative, and Wilms' tumour predisposition segregates in families as an autosomal dominant trait^{22–24}. Familial Wilms' tumour cases are generally associated with an increased frequency of bilateral disease and an earlier age of diagnosis, although variability exists between Wilms' tumour families^{22–25}. Similarly, patients with Wilms' tumour with aniridia, genitourinary anomalies and mental retardation (known as WAGR syndrome) are more likely to develop bilateral disease and at an earlier age²². Statistical analysis of the age and frequency of bilateral tumours in both sporadic and familial patients with Wilms' tumour suggested that, like retinoblastoma (another embryonic tumour occurring in childhood), the genetic aetiology of Wilms' tumour fits a model in which two genetic alterations are rate-limiting (although not necessarily sufficient) for tumour development²³. Patients with bilateral tumours, congenital anomalies and/or a family history were thought to inherit the first alteration and acquire the second event somatically. Patients with unilateral tumours and no family history were hypothesized to acquire both rate-limiting alterations somatically²³.

Molecular analyses of patients and their tumours are consistent with this initial model. The presence of heterozygous, cytogenetically detectable deletions encompassing chromosomal band 11p13 in most patients with WAGR immediately localized a Wilms' tumour gene to this genomic region, and this was key to the identification of *WT1*, which encodes a transcription factor (described below). Tumours from patients with WAGR who are germline hemizygotes for *WT1*, that is, they have only one allele of *WT1*, invariably have somatic mutations in the remaining *WT1* allele. Tumours from patients heterozygous for an intragenic *WT1* mutation are reduced to homozygosity for the germline mutation. Additionally, tumours from patients with no germline *WT1* mutation are sometimes homozygous for somatic *WT1* mutations or can carry compound heterozygous mutations^{2,26}. In all these cases, the mutations are predicted to abolish *WT1* function^{2,26}. These observations strongly suggest that *WT1* is a TSG, the homozygous inactivation of which is a crucial step in the development of Wilms' tumour.

Wilms' tumour is known to be genetically heterogeneous; *WT1* mutations are present in only ~20% of tumours^{2,5}. Other genes mutated in Wilms' tumour include *WTX* (~20%), a gene identified through high-resolution comparative genome hybridization (CGH) analysis and which encodes a protein reported to negatively regulate the Wnt pathway; *CTNNB1* (~15%), which encodes β -catenin and upregulates the Wnt pathway; and the well-known TSG, *TP53* (5%)^{1,3–5,27} (TABLE 1). Loss of heterozygosity (LOH) studies and CGH studies have

implicated other regions of the genome in the development of Wilms' tumour, but the corresponding Wilms' tumour gene has generally not yet been identified. Although *WT1* germline mutations are observed in some patients with Wilms' tumour, familial predisposition to Wilms' tumour is rarely due to *WT1* mutation, and predisposition in some families has been linked to loci on 19q and 17q^{28,29}.

Additional molecular alterations are observed in Wilms' tumours, most notably LOH or loss of imprinting (LOI) on 11p15, which occurs in up to 70% of tumours^{30,31}. This region is known to harbour genes for the somatic overgrowth syndrome, Beckwith–Wiedemann syndrome (BWS), one feature of which is a predisposition to embryonal tumours, including Wilms' tumour, embryonal rhabdomyosarcoma and hepatoblastoma^{32,33}. Tumour range differs between BWS families, and those with cases of Wilms' tumour carry germline mutations that alter the imprinting (methylation) of the insulin-like growth factor 2 (*IGF2*)–*H19* gene cluster and result in biallelic expression of *IGF2*, which is normally expressed solely from the paternally derived allele^{34,35}. *IGF2* upregulation is also observed in Wilms' tumours with 11p15 LOH or LOI^{30,31,36}. These data strongly suggest that epigenetic upregulation of *IGF2* can be an important, but not sufficient, alteration for tumorigenesis.

In summary, the known genetic alterations that occur in Wilms' tumours are mutations of tumour-suppressor genes (*WT1*, *WTX* and *TP53*) and an oncogene (*CTNNB1*)^{1–4} (BOX 1). But are these labels appropriate? In some malignancies does *WT1* function as an oncogene? Is *WTX* clearly a TSG? Before exploring this issue, it is important to establish what is known about the function, expression, biological role and mutational consequence of these genes.

WT1

The *WT1* gene consists of ten exons and encodes a zinc finger transcription factor containing an amino terminal proline glutamine-rich protein-interaction domain and four carboxy-terminal zinc finger domains^{37,38} (FIG. 1). Two different alternative splice sites result in four isoforms: proteins with and without exon 5, which encodes 17 amino acids, and proteins with and without three amino acids (lysine, threonine and serine (KTS)) between the third and fourth zinc finger domains encoded by exons 9 and 10, respectively. Mice homozygous for a *Wt1* allele lacking exon 5 have no salient phenotype³⁹. By contrast, a variety of data point to a profound functional importance of the alternative KTS splicing. For example, mice carrying either a KTS-positive (KTS⁺) or a KTS-negative (KTS[−]) *Wt1* transgene display distinct phenotypes⁴⁰, and X-ray crystallographic and structural studies along with DNA binding studies indicate that the KTS insertion destabilizes the interaction of WT1 with DNA^{41,42}. A role for the KTS⁺ isoform in binding to RNA has also been reported⁴³.

In addition to splice variants, a non-AUG translation start site 5' to the traditional AUG start site associated with the Kozak consensus sequence has been reported and results in an additional 68 amino acids at the N terminus. The functional importance of this variant is unknown; mice homozygous for an allele unable to encode this 68 amino acid extension display no phenotype⁴⁴. An alternative exon 1 (exon 1a) containing an alternative AUG has also been reported, but the functional importance of this is unknown⁴⁵. Editing of the *WT1*

transcript at an exon 6 codon has been proposed, based on sequence data from a rat testes cDNA library⁴⁶. However, no evidence of such editing has been detected in Wilms' tumours or in a genome-wide assessment of RNA editing^{47,48}. WT1 has been reported to be post-translationally modified by phosphorylation, ubiquitylation and sumoylation^{49–51}, but the effect of these modifications on protein function is still unclear.

Although WT1 has been reported to have a role in RNA processing⁴³, the most well-documented function for WT1 is that of a transcription factor, and many genes have been identified by various approaches as being regulated, either positively or negatively, by *WT1*. Studies using *Wt1* conditional knockout mice have identified *Sox9*, *Snail* and *Cdh1* (encoding E-cadherin) as being deregulated *in vivo* following *Wt1* ablation in embryonic testes or cardiovascular progenitor cells^{52,53}, although evidence for a direct role for WT1 in transcriptional regulation is only available for *Snail* and *Cdh1* (REF. 53).

WT1 in nephrogenesis

The histology of Wilms' tumour suggests that tumorigenesis involves, at least in part, a perturbation of normal kidney development (BOX 2). Consistent with this, *Wt1* is expressed early in nephrogenesis^{54,55}. In the mouse, low levels of *Wt1* expression are first detected at ~embryonic day (E) 9 in the intermediate mesoderm and later in the metanephric mesenchyme from which the nephrons and stroma of adult kidneys are derived. *WT1* expression is widespread in the metanephric mesenchyme and is upregulated upon metanephric mesenchyme condensation. Subsequently, *WT1* expression becomes increasingly restricted until, in the mature kidney, it is expressed in the highly differentiated glomerular podocytes.

Observations of Wilms' tumour and, as described below, early-onset renal failure in some patients with germline *WT1* mutations first implicated *WT1* as having important roles in kidney development and function. The manipulation of *Wt1* in mouse embryos and the generation of *Wt1* mutant mouse models have greatly elucidated these roles. In *Wt1*^{-/-} mouse embryos metanephric blastema is present at E10.5, but quickly becomes apoptotic. By E12 no blastemal cells are detectable and embryos display complete renal agenesis⁵⁶. Therefore, *Wt1* is crucial, either directly or indirectly, for the survival of these early progenitor cells. However, somatic ablation of *Wt1* at ~E13.5 results not in apoptosis but in a profound disruption of metanephric mesenchyme differentiation resulting in an almost complete absence of nephrons at birth⁵⁷. A similar result was observed following downregulation of *Wt1* using small interfering RNA (siRNA) in E11.5 kidney rudiment explant culture⁵⁸. Thus, the cellular phenotype resulting from *Wt1* knockdown at this later stage of nephrogenesis is compatible with its deduced role as a TSG in Wilms' tumour.

WT1 alterations, genitourinary anomalies and Wilms' tumour

Consistent with its expression and crucial role in the development of the kidney and gonad, in addition to predisposition to Wilms' tumour, individuals who are heterozygous for *WT1* germline mutations can also display genitourinary tract anomalies and glomerulosclerosis, leading to renal failure⁵⁹. As depicted in FIG. 1, there are strong (but not inviolable) genotype–phenotype associations such that germline *WT1* deletion or truncation mutations

are associated with Wilms' tumour and genitourinary anomalies (more frequently observed in males), and *WT1* missense mutations at specific, highly conserved codons result in Denys–Drash syndrome^{60,61}. Genitourinary anomalies and renal failure (albeit at a later age of onset) are also observed, along with gonadoblastoma, in Frasier syndrome⁶². Interestingly, patients with Frasier syndrome are typically heterozygous for IvS9 germline mutations that affect KTS splicing and reduce the KTS⁺ isoform/KTS⁻ isoform ratio⁶³. However, as patients with Frasier syndrome can present with *WT1* mutations that are commonly seen in patients with Denys–Drash syndrome — and vice versa — this has led to the suggestion that Denys–Drash syndrome and Frasier syndrome represent two ends of a phenotypic range⁶². Mutations identified in these *WT1*-associated syndromes are also observed as homozygous somatic mutations in tumours from patients with Wilms' tumour who do not have a germline mutation. Interestingly, given that *WT1* ablation is considered to be a rate-limiting step in Wilms' tumour development, those tumours that do not carry a *WT1* mutation often express *WT1* at substantially higher levels than cells from the adjacent normal kidneys resected with the tumours¹¹⁰, consistent with tumours arising from mesenchyme that robustly expresses *WT1*.

WT1 in haematopoiesis and leukaemogenesis

A role for *WT1* during haematopoiesis was first suggested by the observation of *WT1* expression in thymus and spleen⁵⁵. Although no *WT1* expression is observed in long-term haematopoietic stem cells from bone marrow^{14,15,64–66}, it is expressed in a small (~1%) subfraction of CD34⁺ multi-potent progenitor cells, both in the uncommitted, quiescent CD38⁻ fraction, where its expression levels are comparable to those seen in K562 leukaemic cells⁶⁶, and in the committed, proliferating CD38⁺ fractions^{14,15,64–66}. *WT1* expression is upregulated during early myeloid differentiation, and during this stage ~4% of common myeloid progenitors (CMPs), ~2% of granulocyte-monocyte progenitors (GMPs) and ~17% of megakaryocyte/erythroid progenitors (MEPs) express *WT1*. *WT1* expression is downregulated at later stages of differentiation, and only a small proportion (<1%) of fully differentiated cells are positive for WT1 (REF. 14).

Fetal liver cells from *Wt1*^{-/-} mouse embryos can reconstitute the haematopoietic system in lethally irradiated recipients⁶⁷. However, in *Wt1*^{-/-}; *Wt1*^{+/+} adult chimaeras, *Wt1*^{-/-} blood cells are not detectable. These data in aggregate suggest that, although *Wt1* is not essential for haematopoietic progenitor cell viability, its loss results in impaired haematopoiesis⁶⁷. Ectopic expression of *Wt1* in long-term mouse haematopoietic stem cells (HSCs) leads to a reduction in the numbers of HSCs and in HSC progeny, including CMPs, GMPs and MEPs, which normally express *WT1* (REF. 14). Exogenous expression of *Wt1* in non-committed progenitors results in quiescence, not proliferation, and exogenous *Wt1* expression in committed progenitors induces proliferation^{9–11,13}. This changing role for WT1 during haematopoietic differentiation is similar to that observed during nephrogenesis. WT1 in early undifferentiated cells is important for the viability or the quiescence of uncommitted cells (intermediate mesoderm and CD34⁺CD38⁻ cells), but in committed cells (metanephric mesenchyme and CD34⁺CD38⁺ cells), WT1 is important for differentiation.

WT1 expression and mutation in leukaemias

Data regarding *WT1* mutation and expression in leukaemia and the subsequent inferences about its role in leukaemogenesis have been contradictory. The observation of *WT1* expression in immature myeloid leukaemias and during chronic myelogenous leukaemia (CML) blast crisis suggested a tumour-promoting or oncogenic role for *WT1* in leukaemogenesis⁸. However, the early identification of a somatic *WT1* mutation in the leukaemic cells of a patient with WAGR who later developed AML suggested that, as in Wilms' tumour, *WT1* functioned as a TSG in leukaemia⁶⁸.

Subsequent data from patients with leukaemia continue to be seemingly contradictory. *WT1* is expressed in most acute leukaemias and is a useful marker for the detection of residual disease^{15,69,70}. Additionally, but controversially, the level of its expression has been reported to be of prognostic importance with higher levels associated, variably, with decreased attainment of remission, poor disease-free survival and/or poor overall survival^{15–18}. Other studies have found that there is no prognostic relevance to the presence or absence of *WT1* expression or when *WT1* expression is coded as low versus high^{71,72}. In one study of paediatric AML, higher levels of *WT1* correlated with better overall survival⁷³. These results and conclusions are confounded by technical differences between studies in quantifying *WT1* expression and in the patient populations examined, with differences in patient age and histological and cytogenetic subtypes of leukaemia being notable confounding factors. Furthermore, data indicating that WT1 protein stability is modulated through interactions with HSP90 in myeloid leukaemia xenografts⁷⁴ further complicates the interpretation of *WT1* gene expression data.

Additionally, and importantly, *WT1* is mutated in a considerable proportion of both adult and childhood AML^{75–77}. The vast majority of alterations are insertions or deletions occurring in exon 7 or exon 9 and, to a lesser extent, missense mutations in exon 9 like those observed in patients with Denys–Drash syndrome (FIG. 1c). *WT1* heterozygous, homozygous and compound heterozygous mutations have been reported at different frequencies in AML. Interestingly, *WT1* mutation is mainly observed in cytogenetically normal AML or in those with mutations in the kinase FLT3 (REFS 75–77). In a study of childhood AML, *WT1* mutation (either heterozygous or homozygous) was significantly and independently associated with poor prognosis, and most patients with a relapse carried the same *WT1* mutation as that observed at diagnosis⁷⁸. In ~10% of cases, relapsed patients had *WT1* mutations even though at initial diagnosis leukaemic cells had none⁷⁷.

Initial data suggested that transgenic overexpression of *Wt1* was required for leukaemogenesis in mice, and mice transplanted with bone marrow cells expressing the *AML1–ETO* fusion gene did not develop leukaemia, whereas those with bone marrow cells expressing both *AML1–ETO* and *WT1* did develop leukaemia¹³. Subsequent work using two different mouse models of leukaemia (mice expressing the oncogenic fusion proteins TEL–PDGFBR and AML1–ETO or BCR–ABL1 alone) found that *Wt1* expression was not required for disease development. Although an increased frequency of *Wt1*⁺ cells, especially immature leukaemic cells, was observed in both these models, *Wt1*[–] leukaemic cells were also present. Additionally, *Wt1*[–] and *Wt1*⁺ subfractions were equally leukaemogenic when

transplanted into recipient animals. These contrasting data may be due to the different mutational contexts in which the leukaemic potential of *Wt1* expression was tested.

Interestingly, one of the oncogenes used in these studies, TEL–PDGFBR, is reported to activate the Ras–ERK pathway⁷⁹, and contrasting roles for WT1 in RAS-mediated cell proliferation have been observed, with WT1 expression either inhibiting HRAS-dependent transformation or being required for KRAS-mediated cell proliferation^{80,81}. WT1 has also been reported to negatively regulate inhibitors of the Ras–ERK pathway^{82,83}.

WTX

WTX expression has been observed in 22 adult and six fetal tissues assessed, although the level of expression varies^{4,84}. The gene contains two exons and is transcribed as a ~7.5 kb mRNA, which encodes a 1,135 amino acid protein (FIG. 2). Use of alternative splice donor and splice acceptor sites, both located within exon 2, results in the production of an isoform lacking 277 amino acids in the N-terminal portion of the protein²⁷. The full-length WTX protein resides at the plasma membrane and cytoplasm and possesses three adenomatous polyposis coli (APC) binding domains. These facilitate its interaction with APC at the plasma membrane^{84,85}. The short isoform localizes primarily to the nucleus^{85,86}. Nuclear WTX forms a complex with β -catenin and the destruction complex (AXIN1, β -transducin repeat-containing protein 2 and APC) and promotes ubiquitylation and degradation of β -catenin²⁷. Therefore, it is the short WTX isoform that negatively regulates the Wnt-signalling pathway. A C-terminal region of both WTX isoforms has been reported to interact with the WT1 protein *in vitro*, and, interestingly, enhancement of WT1 function as a transcriptional activator is observed, but only in the case of the short isoform⁸⁶.

WTX alterations in Wilms' tumour

The *WTX* gene is altered in 7–29% of Wilms' tumours, with most of these tumours (about two-thirds) carrying deletions of the entire *WTX* gene^{4–6,87,88}. The remaining one-third of *WTX*-mutated Wilms' tumours carry truncating mutations (nonsense mutations, and insertions and deletions that cause frameshifts, resulting in termination codons) or missense mutations. The functional importance of the missense alterations is unclear, as the missense alteration can be present in normal tissue from the same patient, whereas the deletion and truncation mutations are always specific to the tumour. The truncation mutations result in loss of most or all of the β -catenin-binding region (FIG. 2). Truncated *WTX* results in an increase in nuclear β -catenin *in vitro*²⁷, and these data suggest that the *WTX* mutations observed in Wilms' tumour have a similar *in vivo* role and stabilize β -catenin. A correlation between *WTX* mutations and increased expression of Wnt pathway target genes is observed in Wilms' tumours displaying a predominantly blastemal histology, but not in epithelial-predominant tumours⁸⁷. These data suggest that, as noted above with respect to different phenotypic effects of *WT1* mutations at different stages of kidney development, differentiation status may also have an important effect on the functional importance of *WTX* mutations.

WTX germline mutations are observed in both the familial and sporadic forms of the rare sclerosing bone dysplasia, osteopathia striata congenita with cranial sclerosis (OSCS) (BOX

3). Interestingly, individuals with OSCS display no increased risk for Wilms' tumour or any cancer⁸⁵, and this observation led to the questioning of the labelling of *WTX* as a TSG. However, nephrogenic rests, potential Wilms' tumour precursor lesions, have recently been reported in the kidney of one male germline *WTX* mutation carrier⁷, suggesting that such mutations can predispose to Wilms' tumour. The lack of overt Wilms' tumours in patients with OSCS might be due to the generally infrequent progression of the precursors to tumours and the rarity of *WTX*-mutated OSCS cases. Another possibility is that *WTX* mutation, in the absence of other genetic alterations, is not tumorigenic. This is similar to what is observed with *WT1*. Although *WT1*-ablated nephrogenic rests have been reported in some patients⁸⁹, in mice *Wt1* ablation alone does not result in tumours⁵⁷.

Mutation associations

In the absence of any associations — positive or negative — between gene mutations, the frequency of the co-occurrence of two mutations in Wilms' tumours will be the product of their independent frequencies. Interestingly, some combinations of mutations occur more frequently than is predicted by chance.

A strong positive association was initially observed between the presence of *WT1* and *CTNNB1* mutations⁹⁰ and has been confirmed in subsequent studies^{4,5,87,91–96}. Compiled data from these studies (a few of which enriched their samples for *WT1*-mutant tumours) show that of 154 tumours with *CTNNB1* mutations, 121 (79%) also have mutations in *WT1*, a significant co-occurrence considering the frequency of *WT1* mutations (~20%) in Wilms' tumours overall. Interestingly, assessment of tumours and nephrogenic rests from two patients with Wilms' tumour revealed that, although tumours carried both *WT1* and *CTNNB1* mutations, the autologous nephrogenic rests carried only *WT1* mutations; *CTNNB1* mutations occurred at a later step in tumour development⁹⁷ (BOX 4).

WTX mutations in Wilms' tumours were initially reported to be negatively correlated with mutations in *WT1* (REF. 4), but subsequent studies showed *WTX* mutations to be approximately equally frequent in tumours with and without mutations in *WT1* (REFS 5,87). Thus, although *WTX* enhancement of *WT1* function has been reported⁸⁶, existing mutation data do not consistently support a model whereby *WTX* and *WT1* mutations are functionally redundant.

By contrast, inactivating mutations in *WTX* (deletions and truncations or frameshift mutations) are reported to be negatively correlated with activating mutations in exon 3 of *CTNNB1* in Wilms' tumours, according to two studies^{5,87}. Compiled data from four studies^{4,5,87,96} indicate that of the 60 tumours with *CTNNB1* exon 3 mutations studied, only two (3%) also carried *WTX* mutations, far fewer than the ~20% expected on the basis of the frequency of *WTX* in Wilms' tumours overall. These observations suggest a functional redundancy in which ablation of *WTX*, which normally destabilizes β -catenin, has a similar effect to directly stabilizing β -catenin through mutation of the exon 3-encoded phosphorylation sites. However, the lack of an association between *WTX* and *WT1* mutations when such a strong association exists between *CTNNB1* and *WT1* mutations suggests that, although *WTX* and *CTNNB1* mutations may share some functional effect, mutation of

CTNNB1 has an additional consequence that relates to its co-occurrence with *WT1* ablation that *WTX* mutation does not. Plausible explanations for this possible phenotypic difference include differing roles of β -catenin and *WTX* in Wnt signalling in adherens junctions and/or in fetal kidney development.

***WTX*: a TSG?**

Current data support the TSG label for *WTX*. Deletion and resulting loss of expression is observed in tumours, consistent with a TSG function. Although the absence of Wilms' tumour in patients with OSCS initially raised the question of whether *WTX* germline mutation acted as the classic first hit for tumorigenesis, as would be expected for a TSG, the recent case report⁷ of Wilms' tumour precursor lesions in an OSCS male fetus suggests that *WTX* mutation does predispose to Wilms' tumour, as the TSG name implies.

***WT1*: a TSG, an oncogene, both or neither?**

Data from human malignancies seem to suggest a contradictory role for *WT1*: that of a TSG in Wilms' tumour and that of an oncogene in leukaemia. Can these roles be reconciled? Could potential tissue-specific differences in post-translational modification of *WT1* alter its regulation of downstream targets? As a transcription factor, does *WT1* positively regulate growth-enhancing genes at one stage of development and then positively regulate growth-suppressing genes at a later stage of differentiation? Or does *WT1* regulate a similar set of target genes, but the consequence of their expression differs depending on the differentiation status of the cell? Or is the role of *WT1* more related to regulating differentiation, and loss of its function at different stages of differentiation results in varying responses by cells to a disruption in that process? Similarly, is the phenotypic consequence of *WT1* mutation affected by the presence of other gene alterations? Are there parallels in the role of *WT1* in kidney and haematopoietic development and malignancy that can potentially help in understanding the seemingly contradictory data regarding its tumour suppressor role in Wilms' tumour and its oncogenic role in leukaemia? Are there alternative interpretations of the data?

A simple depiction of *WT1* expression during nephrogenesis and haematopoiesis and the effect of its loss or upregulation, based on observational and experimental data from *in vivo* or *ex vivo* studies, is shown in FIG. 3. Although the two developmental processes are not strictly analogous, there are many similarities. In both tissue types *WT1* is expressed in lineage-committed precursor cells, upregulated during differentiation and then downregulated in all but a subset of the resulting fully differentiated cells. In both, loss of *WT1* has been observed to result in a block in, or severe retardation of, further differentiation. In both, *WT1* mutation is observed in malignancies, and in these malignancies *WT1* expression is often increased relative to fully differentiated cell types. This observation has not led to the moniker of oncogene for *WT1* in Wilms' tumour for various reasons: because of patient data that implicated *WT1* as a TSG even before it was cloned, the subsequent molecular data that confirmed this tumour suppressor role and the knowledge that *WT1* was expressed in the developing kidney. Additionally, the realization that Wilms' tumour is genetically heterogeneous provided a reasonable explanation for the

observation of *WT1* overexpression in some tumours compared with mature kidney tissue: the genetic aetiology of these tumours was due to the alteration of other Wilms' tumour genes, and tumours displayed robust *WT1* expression owing to their origin from a cell type in which *WT1* was robustly expressed.

Can the same reasoning be applied to the role of *WT1* in leukaemia? As in Wilms' tumours, *WT1*-inactivating mutations are observed in a subset of AMLs, notably those that are cytogenetically normal and those that carry *FLT3* mutations^{75–77}. Interestingly, although in most *WT1*-mutant Wilms' tumours the mutations are homozygous, in leukaemias *WT1* mutations are often heterozygous, implying a functional importance for reduced, but not absent, WT1 function. Alternatively, factors that reduce WT1 protein stability in leukaemias⁷⁴ might result in a net loss of WT1 function, despite the presence of one wild-type *WT1* allele.

Similar to the higher level of *WT1* expression in Wilms' tumours compared with normal kidney, AMLs express higher levels of *WT1* than normal bone marrow. However, *WT1* is expressed and is upregulated and downregulated in specific subsets of cells during haematopoiesis. These data are consistent with and support previous suggestions that the *WT1* overexpression observed in some human leukaemias may be a reflection of cancer ontogeny^{11,65,66,98}. As in Wilms' tumour, malignant cells have arisen from undifferentiated cells that express *WT1* at higher levels than the normal, differentiated cells with which leukaemic cells are being compared. Together, the observations of *WT1* mutation and expression in AML suggest that, as in Wilms' tumour, loss of WT1 function, not *WT1* overexpression, is important, although not sufficient, for the development of a subset of AMLs.

Do the data and the above discussion imply that *WT1* is a TSG in the sense that its role is to negatively regulate cell growth? or could the role of *WT1* in malignancy relate more to its effect on differentiation? As presented in FIG. 3, *WT1* mutation or induced expression *in vivo* or *ex vivo* variably result in apoptosis, quiescence, enhanced differentiation and a block in differentiation, depending on the differentiation status of the affected cell. In both nephrogenesis and haematopoiesis, loss of expression of *WT1* in committed precursors results in disrupted or reduced differentiation and has an important role in tumorigenesis in some Wilms' tumours and leukaemias. In more differentiated haematopoietic cells, exogenous *WT1* expression results in enhanced proliferation. These combined data indicate that developmental stage has a profound effect on the phenotypic consequence of *WT1* ablation or overexpression and suggest a model in which alteration of *WT1* directly affects the differentiation status of the cell and this, in turn, results indirectly in a change in cell viability and/or proliferative status. Additional to the effect of differentiation status — and its unique gene expression profile — on the phenotypic result of *WT1* mutations is the potential effect of other gene mutations. As discussed (BOX 4) in Wilms' tumours, *CTNNB1* mutation and/or *IGF2* upregulation are sometimes observed in *WT1*-mutant tumours, and data from both humans and mice indicate that these alterations have a direct effect on the phenotypic effect of *WT1* mutations. Conversely, *WT1* mutation may also have a strong effect on the phenotypic consequence of cells carrying mutations in other genes (as recently

described for Kras-mutated cells⁸¹) and it is likely that this effect is variable, depending on what other gene is mutated.

Therefore, the labels of TSG or oncogene may not be appropriate for describing the function of *WT1*. Doing so may misleadingly put *WT1* in the same category as, for example, receptor tyrosine kinases that have a direct role in stimulating cell proliferation, or p53 that has a direct role in regulating the cell cycle and apoptosis. The role for *WT1* for which the strongest data exist to date is that of a gene that regulates key differentiation genes. The oncogenic or tumour suppressive effect of *WT1* alteration is likely to be a result of how a cell at a particular stage of development responds to perturbations in the expression of those genes. Perhaps the TSG and oncogene labels should be retired for *WT1*. They are a convenient shorthand notation, but, rather than describing the cellular function of *WT1*, they more accurately describe the effect of *WT1* alteration in the varying contexts of cell type, differentiation status, presence of other gene alterations and microenvironment.

Conclusion

Genes mutated in Wilms' tumours include TSGs (*WTX* and *TP53*), an oncogene (*CTNNB1*) and a chameleon gene, *WT1*. Observational data from Wilms' tumours and AML along with an improving knowledge of the temporal and spatial expression of *WT1* during nephrogenesis and haematopoiesis indicate that loss of *WT1* function has an important role in a subset of these malignancies. However, *in vivo* and *ex vivo* experimental data indicate that loss of *WT1* has differing phenotypic consequences — apoptosis, quiescence or proliferation — depending on the differentiation status of the cell. The presence of other gene mutations and the microenvironment of the mutant cells are likely to be additional confounding factors that change the effect of *WT1* alteration. Teasing out the direct downstream effect of *WT1* loss versus the subsequent cellular response to that initial effect, all in the context of varying stages of differentiation and the presence of other gene mutations and differing microenvironments, will be challenging. However, accomplishing this will be a major step in understanding the role of *WT1* during development and how its loss, in addition to other molecular alterations, results in malignancy.

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Glossary

Autosomal dominant trait	A trait that is the result of being heterozygous for an allele for a gene that is present on any non-sex (not the X or Y) chromosome.
Aniridia	Lack of development of the iris of the eye.
Rhabdomyosarcoma	A tumour of skeletal muscle.

Intermediate mesoderm	Region of the embryonic mesoderm from which the kidneys and gonads arise.
Metanephric blastema	A subpopulation of the intermediate mesoderm that induces the outgrowth of the ureteric bud and from which the nephrons and stroma of the mature kidney arise.
Renal agenesis	Lack of development of the kidney.
Kidney rudiment explant culture	Embryonic kidneys as early as the stage at which the ureteric bud begins to invade the metanephric mesenchyme can be grown in culture chambers on filters. The reciprocal inductive interactions between the ureteric bud and the metanephric mesenchyme occur in culture, resulting in a three-dimensional structure of nephrons and collecting ducts like a normal kidney but without capillary invasion.
Ureteric bud	A developmental structure that buds off the mesonephric (Wolffian) duct, invades the metanephric mesenchyme and develops into the collecting duct system of the kidney.
Glomerulosclerosis	A general term to describe scarring of the glomerulus, the primary filtration structure in the kidney predominantly composed of capillaries and podocytes.
Denys–Drash syndrome	A phenotypic triad of Wilms’ tumour, congenital genitourinary anomalies and early-onset renal failure.
Gonadoblastoma	A tumour arising in the developing ovary or testes.
TEL–PDGFBR and AML1–ETO	Two different fusion proteins resulting from chromosomal translocations and observed in some myeloid leukaemias. In TEL–PDGFBR the N-terminal region of TEL is fused with the domain of transmembrane and cytoplasmic domains of the receptor kinase protein, PDGFBR. In AML1–ETO the DNA binding domain of AML1 is fused with the ETO co-repressor protein.
Comma-shaped and S-shaped bodies	Two morphological stages observed as induced metanephric mesenchyme differentiates and epithelializes to form mature nephrons.
Osteopathia striata congenita with cranial sclerosis	(OSCS). X-linked dominant condition in which increased bone density and aberrant development of the skull is commonly observed. Fetal or perinatal lethality is often observed in males.

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Box 1***CTNNB1* in nephrogenesis and Wilms' tumour**

CTNNB1, which encodes β -catenin, has crucial roles during development and tumorigenesis, functioning both as a component of membrane adherens junctions and as an effector in the canonical Wnt signalling pathway^{99–101}. In the developing kidney, *CTNNB1* gene expression and signalling is detected in the ureteric bud, metanephric mesenchyme and condensed mesenchyme. Its expression is downregulated during differentiation and no expression is detected in adult kidney¹⁰². Consistent with this expression pattern, overexpression of a constitutively stable β -catenin mutant in tubule epithelium in transgenic mice results in renal cysts with associated increased cell proliferation in the tubular epithelium¹⁰³. Global stabilization of β -catenin using pharmacological inactivation of glycogen synthase kinase 3 (GSK3) results in the induction of nephron development and also ectopic nephrogenesis in kidney organ culture¹⁰⁴. Stabilization of β -catenin specifically in nephron progenitors in the condensed mesenchyme results in a similar phenotype of ectopic nephron induction *in vivo* in mice¹⁰⁵. Interestingly, mature nephrons are not observed in these mice. Moreover, knock out of *Ctnnb1* in the same nephron progenitor cells results in an almost complete loss of mature nephrons¹⁰⁵. These data suggest a role for β -catenin in maintaining a balance between progenitor cell renewal and the initiation of nephron differentiation¹⁰⁵. Although the role of β -catenin in normal development — and the effect of abnormal stabilization of β -catenin — is still not well understood, these data suggest that activation of β -catenin must be tightly regulated temporally and spatially for normal nephrogenesis. *CTNNB1* mutations with known functional importance are observed in ~15% of Wilms' tumours, and the majority are three nucleotide deletions or missense mutations that delete or mutate Ser45, one of the key targets for the phosphorylation and degradation of β -catenin^{3,90}. *CTNNB1* mutations are invariably somatic and are heterozygous (TABLE 1).

Box 2**Kidney development and Wilms' tumour histology**

The peculiar pathology of Wilms' tumours first suggested a link between tumorigenesis and abnormal kidney development. The adult kidney arises during embryogenesis from intermediate mesoderm (reviewed in REF. 106). Its formation entails complex reciprocal interactions between the ureteric bud and the metanephric mesenchyme with the ureteric bud inducing mesenchyme condensation, which in turn induces ureteric bud growth and branching, to form the collecting duct system of the kidney. Condensed mesenchyme forms, in succession, distinctive structures (for example, renal vesicles, comma-shaped bodies and S-shaped bodies) that ultimately become the glomerulus and proximal and distal tubules of the nephron. During this morphogenesis, cells undergo a mesenchymal to epithelial transition and differentiate into the many specialized cells of the mature nephron. This process is asynchronous, with the ureteric bud continuously branching and invading new metanephric mesenchyme at the periphery of the developing organ. In humans, nephrogenesis is normally complete at birth; in mice it continues a few days post-natally.

Wilms' tumours are thought to arise from metanephric mesenchyme and typically display a fascinating triphasic pathology in which histologically distinctive cell types derived from fetal mesenchyme (blastema, stroma and epithelial tubules) are observed (reviewed in REF. 10). These cells, however, are not normally differentiated and are aberrantly organized. In some tumours, heterologous elements, such as smooth muscle and cartilage, are observed. Thus, Wilms' tumour is thought to be a malignancy of a relatively early mesenchymal progenitor cell that is able to differentiate, albeit aberrantly, and does not respond to normal growth control signals.

Box 3***WTX* mutations in osteopathia striata congenita with cranial sclerosis (OSCS)**

Mutations in *WTX* have been observed in both the familial and sporadic forms of osteopathia striata congenita with cranial sclerosis (OSCS)⁸⁵. Similar skeletal phenotypes are observed when the Wnt signalling pathway is upregulated via other gene alterations⁸⁵; that *WTX* mutations result in a similar phenotype is consistent with both the experimental and observational data above. Whole-gene deletions constitute the major class of *WTX* mutations in Wilms' tumours^{4-6,87,88}, whereas truncating mutations are more common in OSCS. However, the range of *WTX* truncating mutations that is observed in tumours is identical to that observed in OSCS (FIG. 2). Some of these mutant alleles affect only the full-length protein; the normal *WTX* isoform 2 protein would be generated in carriers of these alleles. However, the presence of the normal smaller isoform is not sufficient to rescue the lethal effect of the *WTX* mutation in affected males with OSCS⁸⁵, suggesting that the function of the smaller *WTX* isoform 2 protein may be distinct from that of the larger isoform, consistent with its localization to the nucleus rather than to the plasma membrane²⁷. The smaller *WTX* isoform does retain the β -catenin binding region and so may have a role in binding to β -catenin in the nucleus and regulating its activity⁸⁵.

Box 4**Other genetic alterations modulate *WT1*-mutant phenotype**

Wilms' tumours with *WT1* mutations often display aberrant biallelic expression of the imprinted gene insulin-like growth factor 2 (*IGF2*) and/or mutation of *CTNNB1* (REFS 90,108). Do these epigenetic and genetic changes alter the phenotypic effect of *WT1* mutations? The observation of *CTNNB1* mutations occurring in tumours, but not in *WT1*-mutant precursor lesions (nephrogenic rests), from the same patient⁹⁷ suggests a model whereby developmentally arrested mesenchyme becomes fully malignant as a result of *CTNNB1* activation. Although it is tempting to speculate that *IGF2* upregulation or β -catenin stabilization confers a proliferative capacity to *WT1*-mutant mesenchyme, the differentiation of which has been disrupted, the observation of variability in mitotic activity both in prelesions and tumours¹⁰⁹ suggests that the role of these alterations with respect to *WT1*-associated tumorigenesis is far more complex and may alter differentiation programmes that result in the unique, heterogeneous type of histology typical of Wilms' tumours. What is implicit in this model is that in *WT1*-mutant Wilms' tumours, *WT1* mutations alone are not sufficient for tumorigenesis; that the rate-limiting two hits proposed in the original genetic model for Wilms' tumour are not the mutation of one *WT1* allele and then the mutation and loss of the remaining allele, but the functional loss of *WT1* and the alteration of a second locus. This is strongly supported by the recent generation of a mouse model for Wilms' tumour in which somatic, mosaic ablation of *Wt1* results in endogenous tumours in the context of constitutional upregulation of *Igf2*, but not in the absence of *Igf2* upregulation⁵⁷.

At a glance

- *WT1* and *WTX* seem to function as tumour suppressor genes (TSGs) in Wilms' tumours, but questions have arisen about these labels.
- The lack of an increased frequency of Wilms' tumours or other malignancies in patients with osteopathia striata congenita with cranial sclerosis (OSCS) with *WTX* germline mutations initially challenged its designation as a TSG, but a recent observation of Wilms' tumour precursor lesions in a patient with OSCS supports this label.
- In Wilms' tumours, *WT1* conforms to a TSG label: patients heterozygous for *WT1* germline mutations are predisposed to Wilms' tumour and *WT1* is inactivated in tumours. These data link loss of *WT1* function with enhanced cell viability and/or proliferation.
- By contrast, ablation of *WT1* at the initial stages of kidney development results in apoptosis and renal agenesis, indicating that it has a crucial role in maintaining cell viability.
- In some leukaemias, the increased expression of *WT1* compared with normal bone marrow cells, along with some reports of *WT1* expression being a marker of poor prognosis, suggest that *WT1* functions as an oncogene. By contrast, observations of *WT1* inactivating mutations in leukaemias suggest it functions as a TSG.
- *WT1* has important roles in regulating normal differentiation in various organs and cell types. During both nephrogenesis and haematopoiesis, loss of *WT1* or overexpression of *WT1* is associated with differing phenotypic consequences, depending on the differentiation status of the cell.
- The oncogenic or tumour suppressive effect of *WT1* alteration is likely to be a result of how a cell at a particular stage of development responds to perturbations in normal differentiation. In short, either label may be misleading and/or inadequate when used to describe the function of *WT1*.

Figure 1. WT1 mutations observed in Wilms' tumour and acute myeloid leukaemia (AML)

a | *WT1* gene and protein structure. Blue shading indicates alternatively spliced domains. Four isoforms result from exon 5 and KTS alternative splicing. **b** | Germline mutations observed in patients with Wilms' tumour-associated phenotypes. Similar mutations are observed somatically in tumours. **c** | Somatic mutations observed in AML. Location of gene mutations are indicated by blue (insertion or deletions and frameshift mutations), purple (nonsense mutations), green (missense mutations) and orange (splice site mutations) ovals. Predicted mutant proteins are shown for deletion mutations. Asterisk indicates reduced KTS⁺/KTS⁻ isoform ratio as a result of IVS9 splice site mutations. ZNF, zinc finger domain. Data from REFS 61,76–78.

Figure 2. WTX mutations in Wilms' tumour and osteopathia striata congenita with cranial sclerosis (OSCS)

The full-length WTX protein possesses two phosphatidylinositol(4,5)-bisphosphate (PtdIns(4,5)P₂) binding domains that mediate the localization of the protein to the plasma membrane, whereas the smaller isoform lacks these domains and localizes primarily to the nucleus^{84–86}. Both WTX isoforms contain a β-catenin binding domain. Additionally, three adenomatous polyposis coli (APC) binding domains are in the full-length protein⁸⁴; one of these is truncated in the shorter WTX isoform^{85,86}. As denoted, mutations of known functional importance include whole-gene deletions and mutations (blue ovals) resulting in truncated protein products (lines). Blue shading denotes a region of the protein present only in the larger isoform owing to alternative intra-exonic splicing. aa, amino acid.

Figure 3. WT1 expression during kidney and myeloid differentiation, and phenotypic consequences of altered expression or gene ablation

a | *WT1* loss of function in the developing kidney results in cell death when ablated in early metanephric mesenchyme⁵⁶ or results in a disruption of differentiation if ablated in committed condensed mesenchyme^{57,58}. The observation of *WT1* mutations in Wilms' tumours implies that its loss — in conjunction with other alterations — ultimately results in proliferation (tumours). **b** | During haematopoiesis, loss of *WT1* retards cell proliferation and/or differentiation⁶⁷. Exogenous expression of *WT1* in the CD34⁺CD38⁺ committed precursor population results in quiescence, whereas such expression in the more differentiated common myeloid progenitors results in proliferation^{9–13}). Ectopic expression of *WT1* in long-term haematopoietic stem cells results in reduced haematopoiesis¹⁴. As with Wilms' tumour, the observation of *WT1* mutations in some leukaemias links its loss at some stage of haematopoiesis with a proliferative phenotype.

Table 1

Commonly occurring gene mutations in Wilms' tumour

Gene	Type of mutation	Frequency	Germline or somatic?	Tumour zygosity	Predicted effect	Refs
<i>WT1</i>	Whole or partial gene deletion, insertion, nonsense and missense	~20%	Both	Predominantly homozygous	Inactivation of protein	2,5
<i>WTX</i>	Whole or partial gene deletion and nonsense	~20%	Somatic only	Heterozygous and hemizygous	Inactivation of protein	4–7
<i>CTNNB1</i>	Inframe deletions and missense	~15%	Somatic only	Heterozygous	Stabilization of protein	3,90
<i>TP53</i> *	Missense	~5%	Both	Heterozygous and homozygous	Inactivation of protein	1

* Associated with anaplastic Wilms' tumours.