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INVITED REVIEW

WT1 in disease: shifting the epithelial–mesenchymal balance

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Abstract

WT1 **is a versatile gene that controls transitions between the mesenchymal and epithelial state of cells in a tissue-context dependent manner. As such,** ***WT1*** **is indispensable for normal development of many organs and tissues. Uncontrolled epithelial to mesenchymal transition (EMT) is a hallmark of a diverse array of pathologies and disturbance of mesenchymal to epithelial transition (MET) has been associated with a number of developmental abnormalities. It is therefore not surprising that** ***WT1*** **has been linked to many of these. Here we review the role of** ***WT1*** **in proper control of the mesenchymal–epithelial balance of cells and discuss how far these roles can explain the role of** ***WT1*** **in a variety of disease states.**

Keywords: WT1; EMT; MET; kidney; podocyte; cancer

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Introduction

The oldest preparation of what is now known as a 'Wilms' tumour', or nephroblastoma, can be found in the collection of John Hunter from the second half of the 18th century. However it was not until 1899 that Max Wilms, in his monograph 'Die Misch Geschwülste der Niere', realized that the different cell types in a variety of renal childhood tumours were all derived from the mesodermal layer during embryonic development. These tumours became the archetypal example of disturbance of normal development leading to tumour formation [1]. Seven decades later, it became one of the childhood cancers based on which Alfred Knudson developed the two-hit model for tumour suppressor genes [2]. In 1990, the *WT1* gene, whose inactivation is responsible for 15–20% of Wilms' tumour cases, was identified on 11p13 [3–5]. Early work on the expression pattern of the gene in humans [6] and mice [7] immediately gave important clues on the biology of the disease and led to important hypotheses on the developmental functions of the gene. Since then, *WT1* has been confirmed to be involved in a variety of developmental processes (see later) and diseases (see Table 1). An emerging theme in the analysis of WT1 function in normal development and tissue homeostasis, as well as in the pathological situations in which *WT1* is mutated or misexpressed, is the balance between the epithelial and mesenchymal state of cells. In this review, we will discuss this aspect of WT1 biology in normal situations and in disease.

WT1 and the mesenchymal–epithelial balance

As several extended and up-to-date reviews are available on WT1 functions [8,9], here we will only give a limited overview of this, such as is needed to appreciate the role of WT1 in MET and EMT in development and disease. The *WT1* gene encodes proteins carrying four C-terminal zinc-fingers and is characterized by multiple alternative isoforms. Combinations of alternative exons (selective use of exon 5 and an alternative exon 1a with its own promoter), alternative start codons (an upstream CTG start codon and an in-frame downstream ATG start within exon 1), alternative splice sites, and RNA editing can theoretically give rise to 36 different proteins [8]. The physiological relevance of most variations remains to be confirmed, with the exception of the KTS isoform. In this, three amino acids (lysine–threonine–serine or KTS) are present or not between zinc fingers 3 and 4. This variation is conserved throughout vertebrate evolution, and targeted mouse models specifically removing the +KTS or −KTS isoforms show different phenotypes in the homozygous state [10,11], confirming at least partially differing functions for these variations. The zinc fingers have been found to function in the sequence-specific binding of nucleic acids. The +KTS isoform's functions appear to be biased towards post-transcriptional functions, as they localize to splicing speckles and directly bind splicing factors [9,11–14]. The *IGF2* transcript was shown to be bound by WT1 soon after the first publication of an RNA role for the +KTS

Table 1. Summary of *WT1*-associated pathologies

Disease	Phenotype	Wt1 abnormality
Wilms' tumour	Developmental renal tumour	WT1 mutations found in 15–20% of cases [5]
WAGR syndrome	'Wilms' tumour–aniridia–genitourinary abnormalities–mental retardation' syndrome	Deletions of 11p13, to include both *WT1* and *PAX6* [3]
Denys–Drash syndrome	Wilms' tumour; diffuse mesangial sclerosis and rapidly progressive renal failure; ambiguous genitalia	Usually point (missense) mutations in exons 8 or 9 (zinc-finger regions) [59, 60]
Frasier syndrome	Male pseudo-hermaphroditism; gonadoblastoma; progressive renal disease (usually focal segmental glomerulosclerosis)	Point mutations in intron 9 resulting in loss of the +KTS WT1 isoform [71]
Isolated diffuse mesangial sclerosis and focal segmental glomerulosclerosis	Renal disease without other features of Denys–Drash or Frasier syndrome	Both missense mutations in exons 8 and 9 and intron 9 mutations identified, with marked phenotypic variability [82]
Meacham syndrome	Multiple malformation syndrome characterized by male pseudo-hermaphroditism; abnormal internal female genitalia; complex congenital heart defect and diaphragmatic abnormalities	Point mutations in zinc-finger regions [101]
Paroxysmal nocturnal haemoglobinuria	Acquired clonal haematological disorder resulting in complement-mediated haemolysis, venous thrombosis, and bone marrow failure	Associated with increased WT1 expression—although mechanisms of pathogenesis unclear [109]
Alzheimer's disease	Progressive neurodegenerative disease	WT1 expression found to co-localize with brain neurofibrillary tangles [99]
Cirrhosis		Re-expressed in cirrhotic liver and associated with disease progression [86]
Cancer	Many types	Heterozygous mutations (AML) or ectopic activation (others) [8,33]

isoforms [15], but since then, no new RNA targets for the +KTS isoforms have been described. As a result, the transcriptional regulation functions of WT1 −KTS have received much more attention, and many putative targets have been described [8,9]. WT1 −KTS can, at least *in vitro*, act as an activator or a repressor of target genes in a cell type-dependent context. Finally, it was found that both +KTS and −KTS isoforms can shuttle from the nucleus to the cytoplasm, where they bind to the polysomes [16,17]. This polysomal localization and nuclear–cytoplasmic shuttling are dependent on Wt1 interacting with the cytoskeleton via direct interaction with β-actin [18].

The expression of *WT1* after birth appears to be mainly restricted to the podocytes in the kidney, whereas during development *WT1* expression is found in many developing tissues often, if not always, in cells that are going through an epithelial–mesenchymal transition (EMT) or the reverse mesenchymal–epithelial transition (MET) [19]. In fact, WT1 is often found in cells that express epithelial as well as mesenchymal markers, such as podocytes, which may suggest that they maintain the potential to transition in either direction. Here we will argue that this role in EMT and MET processes is essential to understand the role of WT1 in normal biology and disease.

WT1 in development

Wt1 is indispensible for normal mouse embryonic development. The conventional knockout of *Wt1* that was created by Kreidberg *et al* [20] was found to be embryonic lethal half-way through gestation, with phenotypes in several of the tissues known to express Wt1. The initial description of this model noted a complete absence of kidneys and gonads, as well as disturbed heart development and diaphragmatic hernia [20]. Later studies identified additional phenotypes in adrenal glands [21], retina [22], liver [23], and spleen [24]. Most, if not all, of these phenotypes can be linked to a role for Wt1 in the control of EMT or MET. The likely cause of death in Wt1-deficient animals is a disturbance of the developing heart, where thinning of the epicardium was observed [20]. The epicardium consists of a layer of progenitor cells that gives rise to several cell types in the heart, including the cells of the coronary vasculature, via a process of EMT. Our laboratory has recently shown that *Wt1* controls epicardial EMT and the subsequent generation of vasculature progenitors by directly activating the expression of *Snai1* (Snail), an essential pro-EMT gene, and repression of *Cdh1* (E-cadherin), a gene that needs to be inactivated for EMT [25]. Wt1-expressing cells originating from the coelomic epithelium contribute, via an EMT, to the stellate cells of the liver, and the liver phenotype in Wt1-deficient embryos is believed to be linked to a disturbance of these stellate cells. The diaphragmatic hernia phenotype might be caused by a defect in the septum transversum mesenchyme, another cell population derived from the coelomic epithelium via EMT [23]. A similar liver phenotype was found in *RXR*α knockout mice [23], and indeed Wt1 is an

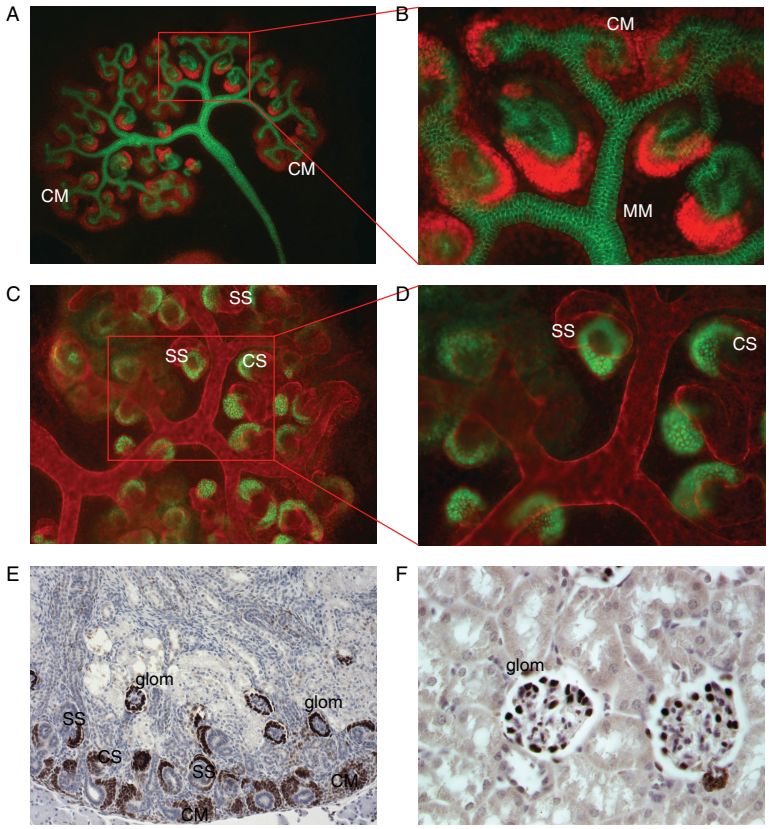

Figure 1. *Wt1* expression in different stages of nephron development. (A–D) Six-day kidney organ cultures from E11.5 mouse embryonic kidneys. (A, B) Red: Wt1; green: E-cadherin. (C, D) Red: laminin; green: Wt1. (E, F) Sections of E18.5 embryonic mouse kidneys stained for Wt1. CM = cap mesenchyme; MM = metanephric mesenchyme; CS = comma-shaped body; SS = S-shaped body; glom = glomerulus. Original magnifications: (A) 5×; (B) 20×; (C) 10×; (D) 20×; (E) 10×; (F) 40×.

important regulator of retinoic acid metabolism through direct activation of Radlh2 expression [26].

The role of *Wt1* in the developing kidney appears to be more diverse. Low expression of *Wt1* is found in the intermediate mesoderm, mesenchymal cells that line the Wolffian duct and that will give rise to the metanephric kidney. This mesenchyme is induced by invasion of the ureteric bud, an epithelial duct that arises from the Wolffian duct. Subsequently, the mesenchyme signals towards the bud, which in response will form the first two branches. The newly formed bud tips signal back to the mesenchyme, which responds by forming a condensate of mesenchymal cells around the tips. The cells in this condensate go through an MET to form epithelialized structures (the renal vesicle, comma-shaped bodies, and S-shaped bodies) that finally form the mature nephron, consisting of proximal and distal tubules and the glomerulus. For this MET, *Wnt4* is known to be essential [27] and sufficient [28]. Wt1 expression is increased in the condensed mesenchyme and stays high in the comma- and S-shaped bodies, but becomes restricted to the proximal half of the developing nephron (see Figure 1). In the mature nephron, Wt1 expression is found only in the podocytes of the glomerulus (see below). Although the renal vesicle has previously attached to the ureteric bud [29], which will then form the collecting duct system, both the ureteric bud and the collecting duct are negative for Wt1. In homozygous *Wt1* knockout embryos, the intermediate mesoderm (the earliest cells in the renal linage to express Wt1) becomes apoptotic before the stage of nephron MET is reached [20]. The role of Wt1 in later stages of nephron development, including the nephron MET, has therefore been harder to study. Using an RNAi in organ culture approach, we have been able to show that Wt1 is essential for nephron development [30]. Knockdown of Wt1 in kidney rudiments *in vitro* after invasion of the ureteric bud, but before the first condensed mesenchymes form the renal vesicle via an MET, results in an almost complete absence of nephrons. The phenotype that we observed was highly reminiscent of the *Wnt4* knockout model, and indeed was found to be identical to *ex vivo* Wnt4 knockdown [30]. This clearly showed that Wt1 is essential for the nephron MET. Wt1 has previously been shown to lie upstream of *Wnt4* [31], and we have recently been able to prove that Wt1 is directly controlling *Wnt4* expression in the kidney mesenchyme. [32] Using our conditional *Wt1* mouse model and different Cre lines, we have also been able to confirm the role of Wt1 in this MET, as well as in the later stages of nephron development (Berry *et al*, personal communication, 2011). Therefore, although the renal agenesis in *Wt1* knockout mice has not been directly attributed to an EMT/MET defect, its later role in nephron development clearly is.

WT1 in cancer

As a recent review on the roles of *WT1* in cancer is available [33], here we will focus on the potential importance of MET and EMT processes controlled by *WT1* in tumourigenesis.

WT1 as a tumour suppressor gene

Wilms' tumours are paediatric kidney tumours found in 1 : 10 000 children, usually before the age of 5 years. The tumours are characterized by the presence of different cell types, epithelial, blastemal, and stromal (which leads to the tumours being referred to as 'triphasic'), and sometimes by ectopic tissues such as bone, muscle, cartilage, and fat, suggesting that they arise due to a developmental problem. Wilms' tumours are often associated with nephrogenic rests, which are defined as embryonal kidney structures that remain in a postnatal kidney. Wilms' tumour classification is generally based on the type of nephrogenic rests found associated with the tumour. Based on their location, they are categorized as perilobar or intralobar nephrogenic rests. The two types are associated with different prognoses and responses to therapy, with the intralobar rest-associated tumours showing the better outcome [34].

WT1 was originally identified through its role in the origins of Wilms' tumours, in which it behaves as a classic tumour suppressor gene [8]. Familial cases, mainly caused by large multi-gene deletions including *WT1* in WAGR syndrome patients (see below), show loss of the wild-type allele in the tumours, whereas non-familial cases show bi-allelic somatic loss of the gene. *WT1* mutations are usually found in intralobar nephrogenic rest (ILNR)-associated tumours [35], and loss of WT1 can already be observed in the rests [36]. It is this *WT1* mutant subset of Wilms' tumours that shows ectopic tissue development [37,38].

There is also a large overlap in the occurrence of activating mutations in the β-catenin oncogene and *WT1* loss in Wilms' tumours [39–41]. In one study, nephrogenic rests were found to have already lost *WT1* but not to have activated β-catenin [42], whereas in another study, different β-catenin mutations were found in independent tumours from the same patient with a germline *WT1* mutation [43]. Both studies indicate that loss of *WT1* is the rate-limiting initiating event with activation of the β-catenin oncogene being a later event during tumour progression.

Whether *WT1*-linked tumours are defined by *WT1* loss, the occurrence of β-catenin mutations or the association with ILNRs, all data supports the view that an important event in the origins of this subset of tumours is the MET stage during nephron development. A strong increase in the expression of genes normally found in the condensed mesenchyme before this MET would fit with a block of this particular step. However, the recent generation and analysis of several new Wilms' tumour lines has suggested a functional overlap with mesenchymal stem cells from the paraxial mesoderm [44], although whether this is pointing to a paraxial mesodermal origin or transdifferentation towards this remains to be determined. As discussed above, RNAi-mediated knockdown of Wt1 at the MET stage results in a block of epithelialisation, showing a direct role for WT1 in this process [30]. The same embryonic phenotype was recently observed in the first reproducible mouse model for Wilms' tumour, in which a conditional *Wt1* allele was combined with increased *Igf2* expression through loss of the *H19* epigenetic regulator of the *Igf2* locus [45]. This model was driven by a ubiquitously expressed inducible *Cre* allele, and can therefore not distinguish between a condensed mesenchymal or paraxial mesodermal origin of the tumours. In a previous study, a single chimaeric mouse carrying a Denys–Drash-type mutation in *Wt1* (see below) was found to have developed a Wilms' tumour at 8 months of age [46], but this finding could not be replicated. Therefore, the *Wt1/H19*-driven Wilms' tumour model developed by Vicki Huff and coworkers presents a major breakthrough in the analysis of the role of *Wt1* in Wilms' tumours.

WT1 as an oncogene

Whereas *WT1* behaves as a classic tumour suppressor gene in Wilms' tumours, increasing data on activation of the gene in other types of cancer, both leukaemia and solid tumours, suggest additional roles as an oncogene. As *WT1* is expressed at some stages of haematopoietic development, but not others, its expression in leukaemia could reflect the cell type of origin of the malignancy. In certain subsets of acute myeloid leukaemia (AML) however (carrying *FLT3* mutation), heterozygous mutations are found in *WT1* [33]. In solid tumours, the situation appears to be different, as here *WT1* activation (but not mutations) has been found in tumours originating from tissues that do not express *WT1* in adults. Interestingly, WT1 is not known to be expressed in these tissues during development either [8]. Here the role of WT1 in controlling the balance between the mesenchymal and epithelial state of the cells might provide an important rationale for the activation of the gene. EMT processes are essential for the metastasis of carcinomas. Epithelial cells need to acquire mesenchymal characteristics to disseminate from the primary tumour and migrate to different sites of the body [47]. As expected, the expression of genes essential for EMT is associated with poor prognosis, as is activation of *WT1* [8]. In addition, an ectopic EMT can provide epithelial cells with the characteristics of cancer stem cells [48]. This was experimentally achieved by forced expression of *SNAI1* (amongst other methods), which is a known physiologically relevant target for Wt1 [25]. A WT1-driven EMT via SNAI1 or other EMT-related targets such as *SNAI2* (*SLUG*) [49] or WT1-controlled down-regulation of *CDH1* [25,50] would therefore provide

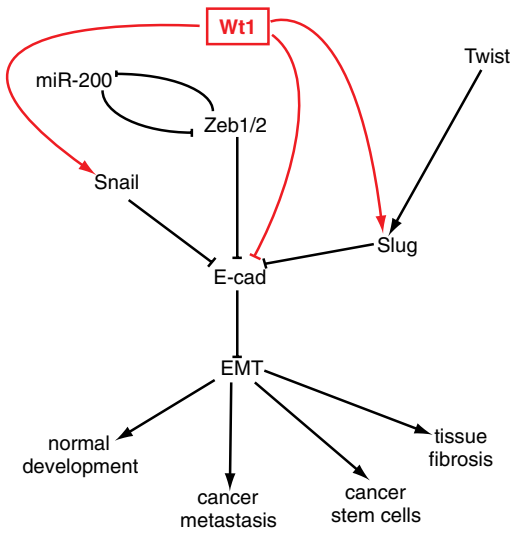

Figure 2. Simplified scheme for the control of EMT in development and disease, and the possible role of Wt1 in this process.

a very strong selective pressure for tumours to activate *WT1* expression (see Figure 2). This, however, remains to be validated experimentally. Finally, recent data support a role for WT1 in overcoming senescence downstream of oncogenically activated *RAS* [51]. Previously, the same has been shown for activation of an EMT via expression of Twist [52], so this finding provides another way in which a WT1-controlled EMT could be advantageous for a developing tumour. Finally, *WT1* has been implicated in the apoptotic response to cytotoxic stress in chemotherapy through processing by the HtrA2 protease [53,54].

Irrespective of the mechanism through which *WT1* could be involved in adult cancer, the fact that in adult healthy individuals its expression is mainly restricted to the podocytes could make it a useful target in therapy. Much attention has been given so far to the use of *WT1* peptides to elicit an immune response, both in leukaemias and in solid tumours. Phase I and II clinical studies have shown very promising results, including cases of complete remission in AML, regression in tumour masses in breast and lung cancer patients, and tumour growth suppression in renal cell carcinoma [55]. Further studies are needed to provide a complete view of the role of *WT1* aberrations in different cancer types. Obviously, its role as a putative tumour suppressor gene would be limited to tissues where the gene is expressed, and therefore is likely restricted to childhood cancers. Its role as a potential oncogene, if mediated by ectopic activation of *WT1*, might extend beyond current knowledge. Additionally, so far no distinction can be made between *WT1* activation being necessary or sufficient for tumourigenesis.

WT1 and renal disease

Given that *WT1* was discovered through its role in developmental kidney cancer, it is not surprising that

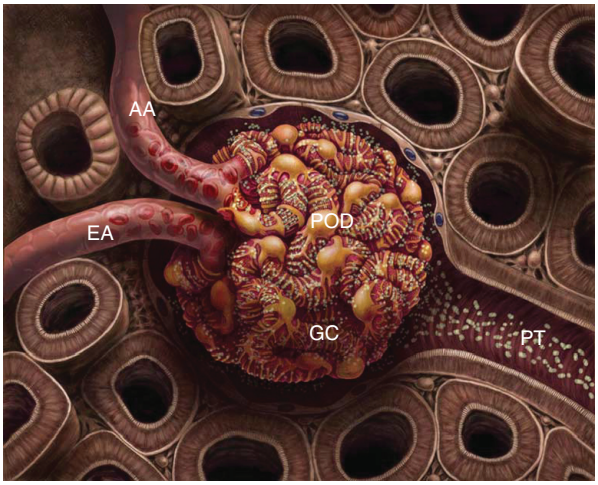

Figure 3. The glomerulus: the filtering unit of the kidney. Podocytes wrap around the glomerular capillaries, interlinking via their elongated foot processes, to form part of the glomerular filtration barrier. AA = afferent arteriole (bringing blood into the glomerulus); EA = efferent arteriole (carrying filtered blood out of the glomerulus); GC = glomerular capillaries; PT = proximal tubule (containing glomerular filtrate); POD = glomerular podocyte. Artwork reproduced with permission of Jim Stanis (http://www.jimstanis.com).

research into the role of *WT1* in the kidney has dominated the field. *WT1* mutations have been found to be associated with a number of developmental syndromes resulting in renal and genitourinary abnormalities, as well as an increased risk of Wilms' tumour. As previously discussed, WT1 is expressed throughout renal development, so such *WT1* mutations have profound effects. In the adult, WT1 expression is thought to be limited to the renal podocyte (see Figures 1F and 3). These podocytes are complex, terminally differentiated cells that play a pivotal role in the renal glomerulus, the filtering unit of the kidney. Although traditionally thought of as an epithelial cell, the podocyte maintains some mesenchymal characteristics including motility and expression of vimentin [56–58].

Developmental renal disease

Denys–Drash syndrome (DDS) constitutes a triad of Wilms' tumour, rapidly progressive renal disease, and ambiguous genitalia or pseudo-hermaphroditism in males [59,60]. It is caused by point mutations in the zinc fingers of *WT1*, usually missense mutations located within exons 8 or 9, coding for zinc fingers 2 or 3. As would be expected, abnormalities of the zinc fingers result in altered DNA binding properties. The mutant WT1 protein in DDS is thought to act in a dominant-negative manner, as dimerization of mutant and wild-type protein prevents zinc-finger-mediated DNA-binding [61,62]. *In vivo* animal studies support this theory, as a mouse model of DDS, in which zinc finger 3 is truncated at codon 396, expresses only low levels of mutant protein (5% of total) but still results in diffuse mesangial sclerosis and male urogenital abnormalities in heterozygotes [46]. This dominant-negative effect may explain the extremely high risk (90% or

more) of Wilms' tumour in DDS patients [63]. Patients with WAGR syndrome (discussed below), which have a complete deletion of *WT1* (so do not express a mutant protein), exhibit less severe renal failure and genitourinary abnormalities, and a much reduced risk of Wilms' tumour (30% in WAGR, 90% in DDS [63]).

The characteristic glomerular lesion of DDS is diffuse mesangial sclerosis, rapidly progressing to end-stage renal failure in the early years of life [59]. The main histological lesion is within the mesangium, although increasing evidence demonstrates an abnormal podocyte phenotype in DDS, with podocyte hypertrophy and foot process effacement. WT1 expression is severely reduced in DDS podocytes, with increased levels of proliferation where WT1 levels are low, and overexpression of PDGFα and TGFβ, known to be potent initiators of EMT [64,65]. In conditionally immortalized podocyte cell lines generated from DDS patients, cells fail to develop the arborized structures found in differentiated podocytes *in vitro* and maintain a fibroblast-like appearance and express α-smooth muscle actin [66]. This would be in keeping with a failure to proceed fully through the MET process.

More recently, evidence also suggests that DDS podocytes actually resemble a fetal or developmental form. VEGF165 is almost exclusively expressed by podocytes and precursors from the S-shaped body stage, and stimulates endothelial cell proliferation, migration, and differentiation [67]. An inhibitory isoform, VEGF165b is also found in mature differentiated podocytes [68]. In DDS, this inhibitory isoform is missing, with ongoing high expression levels of the stimulatory VEGF165 form. Constituents of the glomerular basement membrane in DDS also resemble those of the S-shaped body stage and not those of differentiated adult glomeruli. The authors have interpreted this to mean that glomerular maturation is delayed in DDS, again in keeping with a failure to proceed fully through MET [69]. Whether this defect can be linked to disturbances of the RNA metabolism roles (+KTS isoforms) or transcriptional targets (−KTS isoforms) of WT1 has yet to be elucidated.

The major histological lesion in DDS is within the mesangial cells, and *WT1* mutations have also occasionally been found in cases of isolated diffuse mesangial sclerosis [70]. Mesangial cells, in adult life, are not thought to express WT1, although these cells do originate from WT1-expressing tissues. However, given the increasing evidence for the dynamic nature of cellular interactions within the glomerulus, it may be that the abnormal podocyte phenotype has a major influence on other cells within the glomerulus, including the mesangium. Of note, *WT1* mutations in isolated diffuse mesangial sclerosis have included both exon 8 and exon 9 mutations, most commonly associated with DDS, and also intron 9 mutations, most commonly associated with Frasier syndrome [70].

Frasier syndrome almost always results from mutations in intron 9, resulting in the loss of the +KTS WT1 isoform [71]. Frasier syndrome describes the combination of male pseudo-hermaphroditism, predisposition to gonadoblastoma, and progressive glomerulopathy, most commonly a focal segmental glomerulosclerosis (FSGS). End-stage renal disease is usually reached by adolescence or early adulthood. The risk of Wilms' tumour is much less than in DDS [72,73]. As discussed before, the WT1 KTS splice variation is conserved across all vertebrates [74], and transgenic mice unable to express the different isoforms exhibit differing renal phenotypes, with homozygous animals dying shortly after birth [10]. Mice carrying a splice donor site mutation in intron 9 are unable to express the +KTS isoform (mirroring human Frasier syndrome) and develop albuminuria and renal failure at a few months of age, secondary to a combination of focal segmental glomerulosclerosis and diffuse mesangial sclerosis. The podocytes of homozygotes are abnormal, exhibiting impaired foot process formation and reduced expression of synaptopodin. Homozygous mice unable to express the −KTS isoform demonstrate severe developmental abnormalities, with small kidneys containing few and shrunken glomeruli and streak gonads [10]. The differing phenotypes of the isoform mutants confirm the distinct roles of these isoforms in genitourinary development, but also indicate a degree of redundancy in other developmental processes, given that they are both less severe than the *Wt1*-null mouse [11].

However, it is interesting to note that these genotype–phenotype correlations are not clear-cut and there appears to be a degree of overlap between the mutations associated with both DDS and Frasier syndrome. Intron 9 mutations have been identified in DDS patients, without Wilms' tumour [75], and exon 9 mutations, which should not affect the KTS splice isoform ratio, have also been found in two cases with clinical Frasier syndrome [76].

WAGR syndrome (Wilms' tumour–aniridia–genitourinary–mental retardation syndrome) was the first *WT1*-associated disease and is found in approximately 0.8% of individuals with Wilms' tumour. It is caused by deletions of chromosome 11p13 that encompass both *WT1* and *Pax6*. Renal failure occurs in 40% of individuals [77]. FSGS is once again the most common pathology. This had been postulated to be a secondary phenomenon after nephrectomy for Wilms' tumour, but rates of renal failure are much higher in those with WAGR syndrome post-nephrectomy than in patients with isolated Wilms' tumour, who have a very low risk [78]. Abnormal small and sclerosed glomeruli, similar to those found in DDS, have been identified in patients with WAGR syndrome [79], suggesting that loss of one *WT1* allele may explain the resultant nephropathy. Certainly, the *Wt1*-heterozygous mouse develops albuminuria, glomerulosclerosis, and renal failure that would mirror this situation [80,81].

WT1 mutations have also been identified in a number of isolated cases of FSGS [82]. TGFβ is increased in podocytes in FSGS and it has been suggested that this may induce podocyte injury via

EMT, among other mechanisms, to form the glomerular scar [83,84]. Recent data demonstrate that *WT1* is down-regulated by TGFβ1 in both cultured podocytes and transgenic mice overexpressing *TGFβ1*, prior to podocyte loss [85]. This is in contrast to the findings in hepatocyte cultures, in which incubation with TGFβ caused a significant increase in *WT1* expression [86]. However, a tissue-specific role for *WT1* in maintenance of the epithelial–mesenchymal balance, as previously described in development, would not be unexpected.

WT1 and adult kidney

There has been increasing focus on the importance of the role of the podocyte in renal function and disease over recent years. However, the role of *WT1* in adult podocytes, where it is highly expressed, remains poorly understood. Studies looking at *WT1* expression are confounded by the progressive podocyte loss that occurs with many glomerular diseases, and thus far, all information gleaned from animal models is influenced by the co-existing developmental effects. The use of conditional models affecting *Wt1* only in the adult will add much to this field.

It is, however, clear that WT1 is required for maintaining glomerular health [80]. Podocyte dysfunction is thought to underlie the development of many glomerular disorders, and a number of potential mechanisms, including podocyte detachment, apoptosis, EMT, and dedifferentiation, have been proposed to explain the progressive nature of glomerular disease. The concept of podocyte EMT remains controversial, especially given that podocytes themselves maintain some mesenchymal elements, but a number of recent papers indicate that this may be a potential mechanism leading to podocyte dysfunction [87]. Podocytes cultured with TGFβ down-regulate epithelial markers such as ZO-1 and begin to express mesenchymal markers such as snail, desmin, and fibronectin [88]. Evidence of such changes is also found in proteinuric kidney diseases such as diabetic nephropathy and FSGS [88,89].

It is already known that WT1 interacts with a number of key renal genes including renin, podocalyxin, and nephrin. Co-localization of renin and WT1 protein has been demonstrated in rat kidney, and in transfected human embryonic kidney cell lines, overexpression of WT1 −KTS down-regulates renin expression via transcriptional repression of an upstream regulatory region. This repression effect was lost with the expression of a mutant WT1 protein [90]. Nephrin, a transmembrane receptor molecule and a constituent of the podocyte slit diaphragm, has been shown to be transcriptionally regulated by WT1, which directly binds and activates the *NPHS1* promoter [91]. The expression of podocalyxin, a podocyte sialoglycoprotein that functions to maintain the structural architecture of the podocyte foot process, is also positively regulated by WT1 via direct activation of the *PODXL* promoter [92]. These interactions strongly suggest that WT1 plays a specific role in the specialized functions of the podocyte, as well as its established role in renal development.

Unlike many other tissue situations, there does exist a strong developmental rationale for podocyte EMT, although it must be emphasized that this remains highly controversial and *in vivo* evidence for such a phenomenon remains limited, and mainly observational. However, many proteinuric diseases result in the loss of expression of key podocyte genes, known to be regulated by WT1, with up-regulation of mesenchymal markers. Early evidence would also suggest that *WT1* appears to be influenced by TGFβ, a key mediator of EMT [86,93]. So far, there are only limited data on the role of WT1 in adult podocytes, especially *in vivo*, given the confounding effects of *WT1* mutations in development. Therefore, the exact mechanisms of how WT1 may be influencing the epithelial–mesenchymal balance in podocytes remain to be elucidated.

WT1 and renal regeneration?

Nephrogenesis is completed by birth in humans, and the regeneration of nephrons in response to damage or surgical loss does not appear to occur in mammals. The hallmark of glomerular disease is its progressive nature, with ongoing loss of podocytes at the individual nephron level and of whole nephrons at the organ level. There does appear to be limited reparative potential of individual nephrons, but this is mainly limited to the tubular epithelial cells. Podocytes were not thought to be able to regenerate. However, recent key papers have demonstrated the existence of both potential renal precursors at the glomerular tuft [94] and the potential of parietal epithelial cells, which line Bowman's capsule, to migrate onto the glomerular tuft and acquire podocyte characteristics [95]. The authors demonstrate that during migration onto the glomerular tuft, these cells gain expression of Wt1. This has also been shown in models of proteinuric kidney diseases, where parietal epithelial cells have been shown to express podocyte markers in response to injury [96]. Whether the expression of Wt1 occurs in order to initiate this process of epithelial cell migration and re-differentiation in the kidney, or further downstream in this pathway, is yet to be discovered.

WT1 and non-renal disease

Until recently, it was believed that despite its extensive developmental expression, the only site of Wt1 expression in the adult was in the renal podocyte. However, increasing evidence suggests a more widespread role for *Wt1*, both in development and in adult tissue maintenance. Novel roles for *Wt1* in other organ systems have been identified, including the cardiovascular system, brain, eye, bone marrow, and liver [22,23,97–100].

WT1 and the heart/cardiovascular system

As discussed previously, in cardiac development *WT1* plays a key role in maintaining the mesenchymal state of cardiac progenitors via an EMT process. There is also early evidence to suggest a more general role for *Wt1* in vasculogenesis. Novel WT1 expression has been demonstrated in the vascular endothelium of various tumours [97]. *In vitro* experiments in osteosarcoma cell lines demonstrated the up-regulation of various genes involved in blood vessel formation, particularly VE-cadherin, with expression of WT1 −KTS. Novel WT1 expression has also been demonstrated in rats after myocardial infarction, in the vascular endothelial and vascular smooth muscle cells of the border zone of infarcted tissue. It has been suggested that this may represent an EMT of vascular cells to enable proliferation and promote neovascularization [100]. However, it must be noted that WT1 expression in the vasculature of most organs has not been described, so its role in angiogenesis may well be context-specific and perhaps linked to the role of EMT in cardiac vascularization.

Meacham syndrome

Meacham syndrome is a rare congenital syndrome of male pseudo-hermaphroditism; congenital heart disease, most commonly hypoplastic left heart; and diaphragmatic hernia, with some cases showing adrenal and spleen phenotypes. The overlap in symptoms with the phenotypes observed in the *Wt1* knockout mouse is obvious, and several cases have been shown to be caused by heterozygous mutations in *WT1* [101]. Just as was argued for the *Wt1*-deficient mouse model, the symptoms found in Meacham syndrome can be traced back to EMT processes in coelomic epithelium and septum transversum that are likely to be controlled by WT1. Interestingly, the *WT1* mutations identified in patients with Meacham syndrome (Arg366His and Arg394Tryp) have also previously been identified in patients with DDS and/or isolated Wilms' tumour, demonstrating marked genotype–phenotype variability within a single mutation, and suggesting the influence of other modifying factors [101].

WT1 and tissue fibrosis

Tissue fibrosis is the universal outcome for chronic injury in a variety of organs. For the last 15 years, numerous researchers have investigated the role of EMT as a potential cause of tissue fibrosis, with resident epithelial cells undergoing an EMT to acquire a fibroblast (mesenchymal) phenotype and deposit matrix. Although a large amount of the data are based on *in vitro* experiments, Iwano *et al* demonstrated, via lineage tracing experiments, that proximal tubular epithelial cells were the source of up to 30% of kidney myofibroblasts in models of renal fibrosis [102]. In the kidney, both tubular epithelial cells and podocytes have been proposed by some researchers to undergo EMT in response to injury, leading to dedifferentiation and scarring [87]. However, more recent *in vivo* studies question the role of EMT in tissue fibrosis, advocating the perivascular smooth muscle cell or pericyte as an alternative source of scar-producing cell. These recent fate-mapping studies, using labelled kidney epithelial and stromal cells, have failed to identify any epithelial-derived myofibroblasts, demonstrating instead their pericyte origin [103]. Wt1-positive mesothelial cells form perivascular cells, or pericytes, within the lung [104] and liver [23], and although this has not yet been demonstrated in renal tissue, the podocyte itself has been proposed as a specialized type of pericyte [105,106]. Endothelial to mesenchymal transition has also been proposed as another potential source of tissue myofibroblasts. Using fluorescently labelled endothelial cells, Zeisberg *et al* demonstrated the presence of fluorescent protein expression in activated fibroblasts following kidney injury in three different disease models, suggesting that they originated from labelled endothelial cells [107]. A specific role for WT1 has not yet been studied, but WT1 expression has been found in coronary vascular endothelium in response to ischaemia [22], and it has been proposed that this may allow phenotypic alteration of endothelial cells to a motile and proliferative phenotype [108], as proposed in endothelial to mesenchymal transition. Further work in this area is needed. Whether tissue fibrosis is the result of EMT of epithelial cells, or endothelial cells, or originates from pericytes, a potential role for *WT1* seems likely.

Conclusions and future directions

For much of the 21 years that have elapsed since the identification of *WT1*, its functional implications have remained fragmentary. Ever since the first publications of its expression pattern during development, a potential role in EMT and MET processes has been likely, but until recently it has not been possible to explain this role in molecular terms. The role of Wt1 in MET during nephron formation [30] via control of *Wnt4* expression (Essafi *et al*, personal communication, 2011) and the epicardial EMT essential for vascular progenitor formation via control of *Snai1* and *Cdh1* [25] are examples of molecular targets of Wt1 with physiological relevance in development. They illustrate the importance of Wt1 for both MET and EMT processes in a tissue-dependent manner. The biochemical mechanism behind this, however, remains an enigma. Several laboratories, including ours, are actively pursuing this using different techniques, and we expect the next few years to be enlightening.

Here we have tried to interpret the role of *WT1* in controlling the mesenchymal–epithelial balance development into an explanation for the role of *WT1* in a variety of diseases. We have discussed how many diseases in which the gene has been implicated can be

explained by a direct MET/EMT-linked role for Wt1 during development, as in Wilms' tumour or Meacham syndrome, or in a more general hypothesized role in these processes, such as its presumed roles as an oncogene and in tissue fibrosis. We propose that in disease situations where a role for *WT1* has been shown but not explained, this mesenchymal–epithelial balance will be a strong candidate for further elucidation of the disease process. Vice versa, for diseases that are known to involve disturbances in EMT or MET regulation but in which no genetic smoking gun has been identified, *WT1*, or other genes associated with it, could provide promising leads.

There are many unresolved issues that deserve further analysis. For instance, the renal agenesis in *Wt1* knockout mice cannot be explained by a role in MET or EMT. Identification of physiologically relevant targets in this phenotype might lead to the identification of WT1 involvement in other diseases, such as Alzheimer's [99]. Second, the transcriptional regulation functions of WT1 have so far received most attention. Identification of targets for its putative roles in RNA metabolism/splicing and translation might give new directions to explain the role of *WT1* in disease, as well as the importance of these processes for EMT and MET. Finally, the possible effects of the different WT1 isoforms in disease have not been analysed. As many of the non-standard start codon isoforms (such as the upstream CTG and AWT1 isoforms) have been suggested to act as dominant negatives, their (over)expression in disease states might change our current thinking on the mechanisms of *WT1* involvement in disease. In any case, enough questions remain for at least another 21 years of research.

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Author contribution statement

This manuscript is the equal work of both co-authors and both approve it.

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