Human Molecular Genetics, 2006, Vol. 15, Review Issue No. 2 **R196–R201**
doi:10.1093/hmg/ddl196

The many facets of the Wilms' tumour gene, *WT1*

Peter Hohenstein and Nicholas D. Hastie*

MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

Received July 14, 2006; Revised and Accepted July 26, 2006

Over the years, many apparently contradictory findings and functions have been ascribed to the protein product of the *WT1* tumour suppressor gene. These include being a transcriptional activator or repressor, a function in transcription versus RNA metabolism, and these days even a function as oncogene or tumour suppressor gene. To fully understand the role of WT1 in different diseases and normal development, we will need to understand these contradictions. In this review, we will discuss the present state of knowledge and suggest that a role for WT1 in influencing the mesenchymal–epithelial state of cells might be a common function that could explain many of the previously described findings.

INTRODUCTION

The *WT1* tumour suppressor gene, involved in the development of Wilms' tumour of the kidney, was among the first tumour suppressor genes to be cloned (1). It encodes a protein with four C-terminal Zn-fingers characteristically found in transcription factors. Besides a role in the development of Wilms' tumours, specific mutations in the Zn-finger region are found in Denys-Drash syndrome and Frasier syndrome, both characterized by urogenital abnormalities, sometimes in combination with Wilms' tumours. Creation of mouse mutants has shown that WT1 is essential for the development of certain mesodermally derived tissues, including the kidneys, gonads, cardiac vasculature and spleen, and also for the proliferation of certain neuronal progenitors.

Research in the 16 years since its cloning has not yet revealed the mechanisms by which *WT1* mutation leads to the development of Wilms' tumours and the other syndromes. This is at least partially due to the complex nature of the gene and its products. A combination of many different protein isoforms, different and apparently opposite molecular and biochemical functions, a mouse model that lacks kidneys instead of developing tumours—and nowadays even accumulating data on a potential oncogenic role in adult cancer—has sometimes led to a confusing collection of publications on many aspects of the gene. Here, we will summarize what is known about the different biochemical and developmental functions of WT1. We hypothesize that WT1 may have either pro- or anti-differentiation functions during development, depending on the tissue and context, and speculate how this provides a framework for understanding its role as a tumour suppressor in some cancers but a potential oncogene in others.

WT1 ISOFORMS

The identification of an almost disappointingly small number of genes in the human genome has led to the realization that alternative protein isoforms will form an essential ingredient of the complexity of higher organisms. The *WT1* gene is no exception to this (Fig. 1). A total of 24 different isoforms resulting from combinations of alternative splicing events, alternative startcodons and RNA editing were described soon after cloning of the gene. Best studied are the exon 5 variants, resulting from the use or skipping of this 51 nt exon, and the KTS isoforms, which either include or exclude three amino acids (KTS) between Zn fingers 3 and 4 of the protein through the use of a cryptic splice donor site. Other alternative isoforms are derived from the use of an upstream and in-frame CTG start codon, an internal ATG start codon at the end of exon 1 and a residue in exon 6 subject to RNA editing. Lately, two extra variants have been described. Dallosso *et al*. (2) identified the *AWT1* transcript, resulting from an alternative exon 1 with its own promoter sequences. In contrast to the normal *WT1* promoter, this alternative promoter is imprinted, with the expression confined to the paternal allele. All exon 5 and KTS variants were identified in AWT1 transcripts. An additional isoform starting at the end of intron 5 has been identified, so far only in prostate, breast and leukaemia cancer cells (3). This brings the theoretical total number of isoforms to 36 (Fig. 1). As it has been shown in some cases that specific isoforms can have specific functions (discussed subsequently), this makes the use of cDNA constructs in overexpression experiments of limited use in deciphering the function of WT1.

The conservation of WT1 isoforms through evolution can provide insights in their function and their importance.

*To whom correspondence should be addressed. Email: nick.hastie@hgu.mrc.ac.uk

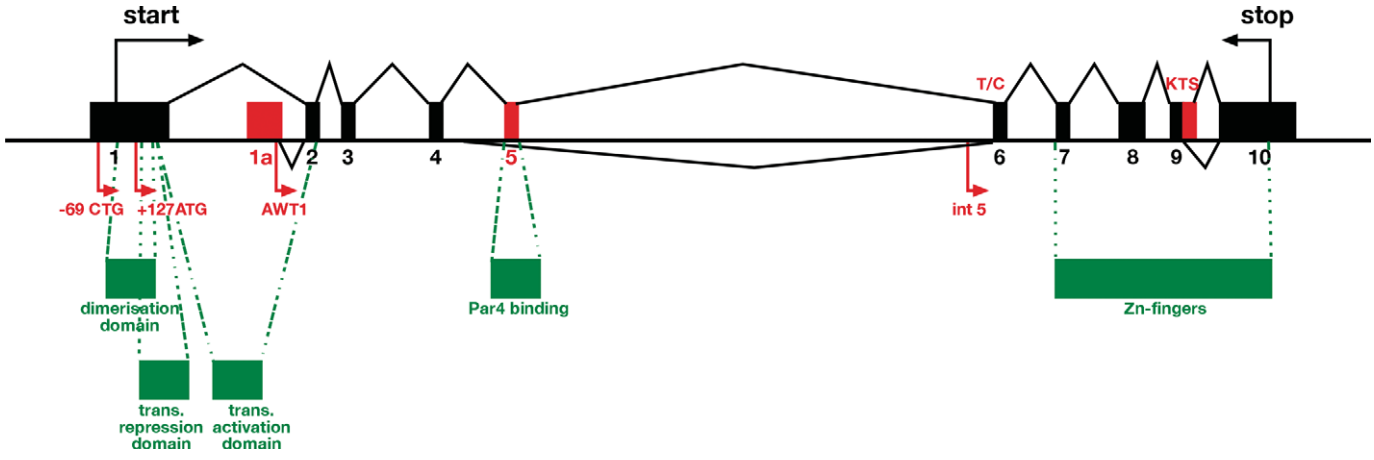

Figure 1. The WT1 locus encodes at least a theoretical maximum of 36 isoforms. Alternative start codons, exons and splices and RNA editing are depicted in red and functional domains affected by these alternative events are depicted in green.

For instance, the RNA editing of WT1 has only been described in mouse and human samples, whereas the exon 5 variants and alternative start codons are conserved throughout mammals. As of yet, there are no data published on the conservation of alternative start isoforms. The only variation that is known to be conserved throughout all vertebrates is ±KTS.

On the basis of this conservation, one could argue that the different KTS isoforms comprise the most important variation. However, this would neglect the possibility of mammalian-specific functions for the novel features such as exon 5 and upstream start site. An additional way of studying the role of different isoforms that does acknowledge this is by generating mouse models using gene targeting to delete specific isoforms rather than overexpression experiments. Several of these mouse models have now been described. The first were mice that express either only the +KTS or only the −KTS isoforms (4). Both +KTS only and −KTS only mice were found to die soon after birth because of kidney defects, and the exact phenotypes were different, supporting the notion that they have different biological roles as suggested by biochemical data (discussed subsequently) (5). Subsequently, it has been shown that lack of +KTS isoforms specifically leads to defects in the development of olfactory neurons (6). Mouse models addressing other isoforms have been less informative. Mice lacking exon 5 are viable, normal and fertile (7). In our laboratory, we deleted the isoform resulting from the upstream CTG start codon with the same result (8). Although in these cases, the opposite experiments, generating mice expressing only the +exon 5 or CTG isoforms, have not been performed, they do support the idea that the KTS variants might indeed be the most important variants to study.

WT1 AS A REGULATOR OF TRANSCRIPTION AND POST-TRANSCRIPTIONAL PROCESSES

The first function described for WT1 was a role in transcriptional regulation. As a recent review on this aspect of WT1 is available (9), we can here be brief about this. Again, paradoxical findings have been described on the exact role of WT1 in this process, where it appears to function as either a transcriptional activator or repressor, depending on the cellular and experimental context. For many years, the identification of WT1 downstream targets solely relied on *in vitro* overexpression and reporter assay data, complicated by the fact that no clear and unique WT1-binding element has been found. Recent years have seen the publication of data on transcriptional WT1 targets that passed more tests for *in vivo* relevance, such as differential expression in *Wt1* mutant mouse models, interaction of endogenous WT1 with DNA detected via chromatin immunoprecipitation or overlapping expression patterns in mouse embryos. Maybe surprisingly, so far all these confirmed target genes such as *Amphiregulin* (10), *Sprouty1* (11), *TrkB* (12), *nephrin* (13), *nestin* (14) and *Pou4f2* (15) appear to be activated rather than repressed by WT1. Yet, several cofactors such as BASP1 (16) and WTIP (17) have now been described, which specifically act as transcriptional co-suppressors for WT1, confirming this is a physiological relevant function for WT1. It is important to add that only the −KTS isoforms, constituting around one-third of total WT1 protein, bind DNA with high affinity and function efficiently in transcriptional regulation.

In addition to a role in transcriptional regulation, there is a wealth of circumstantial evidence pointing to a role for WT1 in RNA metabolism, possibly splicing, mediated via Zn-finger 1 and with some specificity for the +KTS isoform (18–20). However, we await demonstration of a specific role of WT1 in RNA metabolism and its physiological relevance. In this regard, it will be important to identify whether WT1 binds to specific RNA molecules *in vivo* and whether mutation of WT1 leads to altered processing of these RNAs.

Recent data suggest the role of WT1 might not be limited to transcriptional regulation and RNA metabolism. Anecdotal evidence from many labs showed that some endogenous Wt1 protein could be found in the cytoplasm, but for a long time, this was dismissed as an antibody-staining artefact. It has now been found that, in fact, ∼10–25% of endogenous Wt1 in murine kidney and differentiated ES cells is indeed

cytoplasmic and shuttles actively between the nucleus and the cytoplasm (21). Even more surprising, fractionation of cytoplasmic protein showed that WT1 could be found at the actively translating polysomes. Although so far highly speculative, it does open up the possibility that WT1 might play a role in translation as well. If this is proven to be the case, it will be interesting to see whether the same genes that are transcriptional targets are also bound as RNA and during translation, in which case the function of WT1 might be rather different from what is believed at the moment. Recently, the first study to report a specific post-transcriptional function for WT1 was published. The authors showed that +KTS but not −KTS WT1 isoforms can stimulate polysome binding and translation of an RNA retaining an intron (22). Although the physiological relevance of these findings remains to be confirmed, they demonstrate a new potential function for WT1.

PRO- VERSUS ANTI-DIFFERENTIATION: MESENCHYME-EPITHELIAL TRANSITION VERSUS EPITHELIAL-MESENCHYME TRANSITION

Over the past few years, several findings have started to provide more insight into the, again apparently contradictory, biological roles of WT1. The complete absence of kidneys in *Wt1*-deficient mice (23) has for a long time hampered the study of the role of WT1 in kidney development. Using a combination of *in vitro* kidney organ culture and siRNA treatment, we have now been able to show that *Wt1* is needed to induce the mesenchymal–epithelial transition (MET), leading to the formation of nephrons (24), widely accepted to be the cause of Wilms' tumours when disturbed (25). This finding finally coupled *WT1* to the process involved in the formation of these tumours. It has been known for some time that Wnt4 is involved in the same process, and *in vitro* data have suggested that Wnt4 is a downstream Wt1 target (26). Preliminary rescue data using ectopic re-introduction of Wnt4 into Wt1 knockdown kidneys seem to confirm the physiological relevance of this pathway (Anna Webb, Nicholas D. Hastie and Peter Hohenstein, unpublished data). Just as found in $Wt1^{-/-}$ kidneys, some parts of the siRNA-treated kidneys showed an increase in apoptosis, supporting a role here as a survival factor. A similar function was described elsewhere in the urogenital system in transgenic mice using a tissue-specific RNAi approach in Sertoli cells, where it was found that Wt1 expression protects the germ cells from apoptosis (27). In contrast, regions of our siRNA-treated kidneys where nephrons would normally have formed showed an increase in proliferation (24). On the basis of this, we believe that in the developing kidney, *Wt1* functions by forcing the cells out of cycle and stimulating differentiation. The opposite has been found in other organs. Mice lacking the +KTS isoform show decreased cell proliferation in neuronal progenitor cells (6). Most strikingly, our published (28) and unpublished data (Perez-Pomares *et al*., submitted for publication) have shown that in the developing heart, WT1 is required for proliferation of vascular progenitors arising from the epicardium. Our data suggest that WT1 is required for maintenance of the undifferentiated state in these progenitors. These observations support a model where Wt1 expression in the kidney induces differentiation and forces exit from the cell cycle but, in other tissue types, maintains cell proliferation and preserves a progenitor state. These processes are also linked to an opposite shift in the mesenchymal–epithelial state of the cells. In the developing kidneys, the uninduced mesenchymal cells will undergo a WT1-driven MET during the onset of nephron formation. In contrast, in the developing heart, WT1 appears to be required for an epithelial–mesenchymal transition (EMT). Previously, reporter mice had suggested that regulation of the mesenchymal–epithelial balance could be an important aspect of the function of Wt1 (28,29). Together, these data suggest that understanding the epithelial–mesenchymal balance and the role WT1 plays in different tissues might be pivotal to explain several paradoxical observations on the biological function(s) of WT1.

TUMOUR SUPPRESSOR GENE VERSUS ONCOGENE

WT1 is widely accepted to function as a tumour suppressor gene in the formation of Wilms' tumours. However, over the past few years, data have accumulated on the expression of WT1 in adult tumours from different origin, including colorectal (30), breast (31), desmoid (32) and brain tumours (33). As these tumours arise in tissues that normally do not express WT1 but no mutations in the gene have been identified, it has been suggested that expression of WT1 might play an oncogenic role in these tumours. In fact, evidence using antisense oligonucleotides shows that WT1 is required for proliferation while inhibiting apoptosis of tumour cells in culture (34). *WT1* expression in the adult appears to be limited to the kidney podocytes; therefore, oncogenic *WT1* expression might be a relatively tumour-specific target for therapeutic intervention. Indeed, trials using peptide vaccines against WT1 in patients with leukaemia, breast or lung cancer were promising (35). If WT1 is functionally active in the tumourigenic process in these tumours, additional therapeutic schemes can be envisioned.

These observations will need further study. First of all, so far there are no clear data on the isoforms expressed in these tumours. As *in vitro* data on the isoforms that result from the alternative start sites (up- or downstream) suggest dominant-negative effects for these isoforms, this might be an important aspect of the role of WT1 in these tumours. Second, all publications on WT1 expression in adult cancers show mainly, if not only, cytoplasmic localization of the protein. This too might be part of an oncogenic role for WT1, as normally only 10–25% of the protein is found in the cytoplasm (21). Third and finally, it is not known whether *WT1* is expressed during development of the tissues where the tumours are found. If so, the expression found in the tumours might reflect either de-differentiation of cells or the cancer stem cell origin of the tumour. New gain and loss-of-function mouse models will need to be developed to fully analyse the oncogenic potential of WT1 isoforms as oncogenes.

The apparent contrasting roles of WT1 in inducing differentiation versus inhibiting differentiation and the

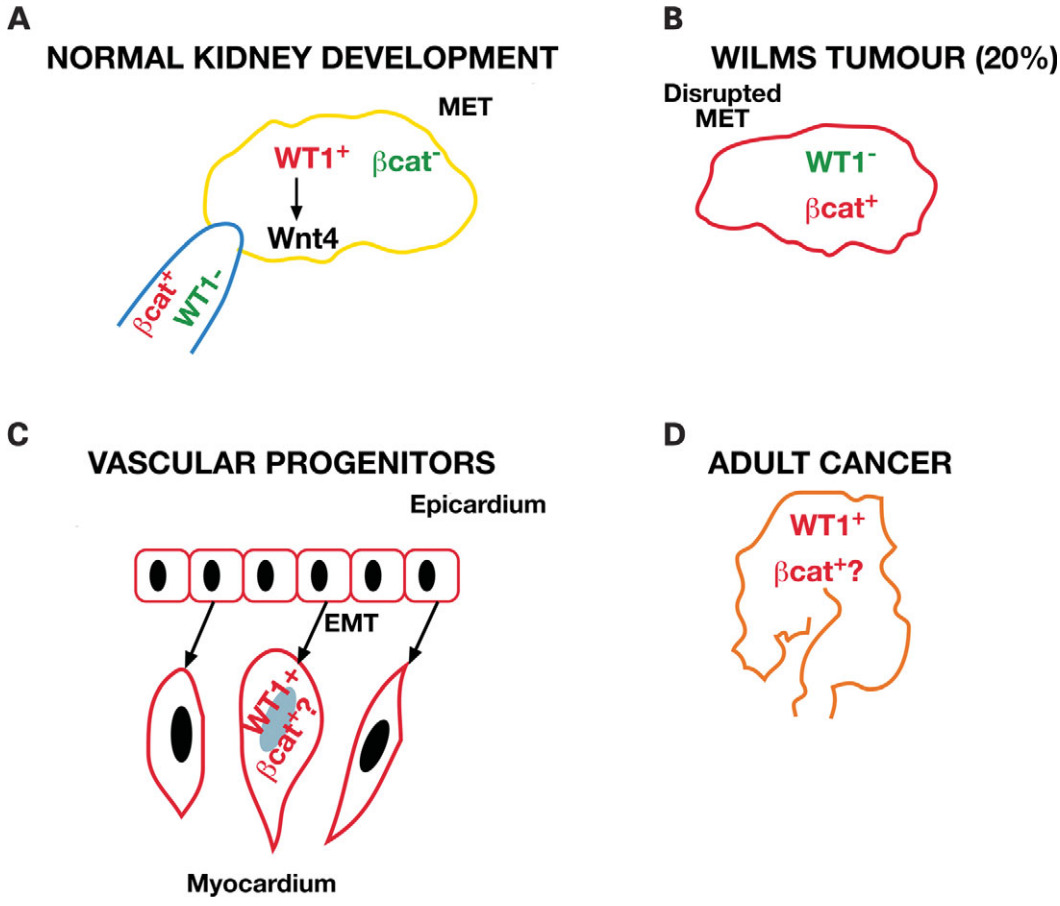

Figure 2. WT1/β-catenin interactions in development and mesenchymal–epithelial balance. (**A**) In the induced mesenchyme, WT1 is driving the Wnt-4-mediated MET, leading to nephron induction in the absence of β-catenin activity. (**B**) WT1-mutant Wilms' tumours, resulting from the disturbance of this MET, select for activating mutations in β-catenin. (**C**) In contrast, in the developing heart, WT1 drives the EMT in the epicardium; β-catenin activity in these cells remains to be confirmed. (**D**) Similarly, WT1 might be involved in the EMT during the formation of many adult cancers outside the urogenital system. Many of these tumours have activated β-catenin, but it is not known whether the WT1 positive and β-catenin positive subsets of tumours overlap.

context-dependent bi-directional control of mesenchymal–epithelial fate might partially explain how WT1 can function as a tumour suppressor gene in some tissues and as a potential oncogene in others. The adult cancers where *WT1* is expressed are generally derived from epithelial cells. These tumours will undergo an EMT during their development, and this is often linked to a worse prognosis. If WT1 is involved in maintaining the mesenchymal–epithelial balance in cells, activating its expression might help establishing or maintaining this mesenchymal status in these tumour cells. In contrast, Wilms' tumours are derived from mesenchymal cells and WT1 normally forces the cells towards an epithelial state. In this case, losing WT1 might keep the cells in the desired mesenchymal state.

The role of WT1 loss in the development of Wilms' tumours was further complicated by the observation that *WT1*-mutant tumours select for oncogenic activation of β-catenin (36). Somehow, the two genetic aberrations must be linked. The obvious candidate for this would be Wnt4 if it would signal via the canonical, β-catenin-mediated, pathway. However, the BAT-gal reporter mouse for β-catenin activity shows no activity in the mesenchyme where Wnt4 is active (37), strongly suggesting that Wnt4 in the kidney cannot signal via β-catenin. Yet, there clearly is β-catenin activity in the ureteric bud, so the reciprocal interactions between bud and mesenchyme in the kidney development might still provide a more indirect link between *WT1* and β-catenin mutations in Wilms' tumours.

Interestingly, expression of activated β-catenin can force cells into premature EMT (38). And although a role for β-catenin in the epicardial EMT where Wt1 is active has not been studied, other EMT processes in the developing heart are clearly linked to canonical Wnt signalling (39). Again, there is a contrast between cells in the urogenital system and outside it. All data suggest that there is a strong link between WT1, β-catenin and regulation of the mesenchymal–epithelial balance, which might explain the role of WT1 in both Wilms' tumours and adult cancers (Fig. 2). It might be relevant that subsets of many of the adult cancers where *WT1* is found activated are known to have oncogenic mutations in β-catenin. It would be interesting to see whether there is overlap in the tumours with β-catenin mutations and *WT1* activations in these tumours, comparable with the situation in Wilms' tumours.

CONCLUSION

It is clear that many inconsistencies with respect to WT1 still exist. However, there is the possibility that these inconsistencies might in fact begin to reveal the true functions of WT1 in normal development and disease. Many phenotypes in cancer and *Wt1*-deficient mice can be explained by a role for the protein in regulating the mesenchymal–epithelial balance. In fact, even the glomerular sclerosis found in different *Wt1* mouse models has been suggested to be linked to changes in differentiation state and mesenchymal–epithelial balance (40). The potential involvement of β-catenin signalling in all these processes is tantalizing at least.

Many of the apparent discrepancies in the WT1 literature are not likely to be resolved using the introduction of WT1 constructs into WT1-negative cell lines. The only kidney-derived embryonic cell line that expresses high endogenous levels of Wt1, M15 (18), is difficult to manipulate experimentally; therefore, its use is limited as well. Resolving most issues regarding the role of WT1 in normal development and different pathogenic conditions will have to wait for the development of a conditional *Wt1* mouse model. Very recently a conditional mouse model leading to an in-frame deletion of exon 8-9 (Zn-fingers 2-3) was published (41). In addition, we have now generated a conditional model that completely recapitulates the normal knockout mouse by placing exon 1 between IoxP sites, leading to an exact phenocopy of the published null phenotype when crossed with a germline Cre deleter mouse (Peter Hohenstein, Laura Lettice, Nicholas D. Hastie, unpublished data). Although its use in the development of a mouse model for Wilms' tumours might be complicated for the moment by the unavailability of appropriate Cre-expressing mice, at least, it should allow assessing phenotypes due to *Wt1*-deficiency beyond its earliest functions and its role in heart development. It should also provide new systems in which Wt1-positive cells derived from these mice can be studied *ex vivo* in induced *Wt1*-deficient conditions. We expect that this will greatly enhance our understanding of WT1 and clarify many of its current paradoxes.

Conflict of Interest statement. None declared.

REFERENCES

1. Haber, D.A., Buckler, A.J., Glaser, T., Call, K.M., Pelletier, J., Sohn, R.L., Douglass, E.C. and Housman, D.E. (1990) An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor. *Cell*, **61**, 1257–1269.
2. Dallosso, A.R., Hancock, A.L., Brown, K.W., Williams, A.C., Jackson, S. and Malik, K. (2004) Genomic imprinting at the WT1 gene involves a novel coding transcript (AWT1) that shows deregulation in Wilms' tumours. *Hum. Mol. Genet.*, **13**, 405–415.
3. Dechsukhum, C., Ware, J.L., Ferreira-Gonzalez, A., Wilkinson, D.S. and Garrett, C.T. (2000) Detection of a novel truncated WT1 transcript in human neoplasia. *Mol. Diagn.*, **5**, 117–128.
4. Hammes, A., Guo, J.K., Lutsch, G., Leheste, J.R., Landrock, D., Ziegler, U., Gubler, M.C. and Schedl, A. (2001) Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. *Cell*, **106**, 319–329.
5. Hastie, N.D. (2001) Life, sex, and WT1 isoforms—three amino acids can make all the difference. *Cell*, **106**, 391–394.
6. Wagner, N., Wagner, K.D., Hammes, A., Kirschner, K.M., Vidal, V.P., Schedl, A. and Scholz, H. (2005) A splice variant of the Wilms' tumour suppressor Wt1 is required for normal development of the olfactory system. *Development*, **132**, 1327–1336.
7. Natoli, T.A., McDonald, A., Alberta, J.A., Taglienti, M.E., Housman, D.E. and Kreidberg, J.A. (2002) A mammal-specific exon of WT1 is not required for development or fertility. *Mol. Cell. Biol.*, **22**, 4433–4438.
8. Miles, C.G., Slight, J., Spraggon, L., O'Sullivan, M., Patek, C. and Hastie, N.D. (2003) Mice lacking the 68-amino-acid, mammal-specific N-terminal extension of WT1 develop normally and are fertile. *Mol. Cell. Biol.*, **23**, 2608–2613.
9. Roberts, S.G. (2005) Transcriptional regulation by WT1 in development. *Curr. Opin. Genet. Dev.*, **15**, 542–547.
10. Lee, S.B., Huang, K., Palmer, R., Truong, V.B., Herzlinger, D., Kolquist, K.A., Wong, J., Paulding, C., Yoon, S.K., Gerald, W. *et al*. (1999) The Wilms tumor suppressor WT1 encodes a transcriptional activator of amphiregulin. *Cell*, **98**, 663–673.
11. Gross, I., Morrison, D.J., Hyink, D.P., Georgas, K., English, M.A., Mericskay, M., Hosono, S., Sassoon, D., Wilson, P.D., Little, M. *et al*. (2003) The receptor tyrosine kinase regulator Sprouty1 is a target of the tumor suppressor WT1 and important for kidney development. *J. Biol. Chem.*, **278**, 41420–41430.
12. Wagner, N., Wagner, K.D., Theres, H., Englert, C., Schedl, A. and Scholz, H. (2005) Coronary vessel development requires activation of the TrkB neurotrophin receptor by the Wilms' tumor transcription factor Wt1. *Genes Dev.*, **19**, 2631–2642.
13. Wagner, N., Wagner, K.D., Xing, Y., Scholz, H. and Schedl, A. (2004) The major podocyte protein nephrin is transcriptionally activated by the Wilms' tumor suppressor WT1. *J. Am. Soc. Nephrol.*, **15**, 3044–3051.
14. Wagner, N., Wagner, K.D., Scholz, H., Kirschner, K.M. and Schedl, A. (2006) The intermediate filament protein nestin is expressed in the developing kidney and heart and might be regulated by the Wilms' tumor suppressor Wt1. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **291**, R779–R787.
15. Wagner, K.D., Wagner, N., Schley, G., Theres, H. and Scholz, H. (2003) The Wilms' tumor suppressor Wt1 encodes a transcriptional activator of the class IV POU-domain factor Pou4f2 (Brn-3b). *Gene*, **305**, 217–223.
16. Carpenter, B., Hill, K.J., Charalambous, M., Wagner, K.J., Lahiri, D., James, D.I., Andersen, J.S., Schumacher, V., Royer-Pokora, B., Mann, M. *et al*. (2004) BASP1 is a transcriptional cosuppressor for the Wilms' tumor suppressor protein WT1. *Mol. Cell. Biol.*, **24**, 537–549.
17. Srichai, M.B., Konieczkowski, M., Padiyar, A., Konieczkowski, D.J., Mukherjee, A., Hayden, P.S., Kamat, S., El-Meanawy, M.A., Khan, S., Mundel, P. *et al*. (2004) A WT1 co-regulator controls podocyte phenotype by shuttling between adhesion structures and nucleus. *J. Biol. Chem.*, **279**, 14398–14408.
18. Larsson, S.H., Charlieu, J.P., Miyagawa, K., Engelkamp, D., Rassoulzadegan, M., Ross, A., Cuzin, F., van Heyningen, V. and Hastie, N.D. (1995) Subnuclear localization of WT1 in splicing or transcription factor domains is regulated by alternative splicing. *Cell*, **81**, 391–401.
19. Davies, R.C., Calvio, C., Bratt, E., Larsson, S.H., Lamond, A.I. and Hastie, N.D. (1998) WT1 interacts with the splicing factor U2AF65 in an isoform-dependent manner and can be incorporated into spliceosomes. *Genes Dev.*, **12**, 3217–3225.
20. Ladomery, M., Sommerville, J., Woolner, S., Slight, J. and Hastie, N. (2003) Expression in *Xenopus* oocytes shows that WT1 binds transcripts *in vivo*, with a central role for zinc finger one. *J. Cell Sci.*, **116**, 1539–1549.
21. Niksic, M., Slight, J., Sanford, J.R., Caceres, J.F. and Hastie, N.D. (2004) The Wilms' tumour protein (WT1) shuttles between nucleus and cytoplasm and is present in functional polysomes. *Hum. Mol. Genet.*, **13**, 463–471.
22. Bor, Y.C., Swartz, J., Morrison, A., Rekosh, D., Ladomery, M. and Hammarskjold, M.L. (2006) The Wilms' tumor 1 (WT1) gene (+KTS isoform) functions with a CTE to enhance translation from an unspliced RNA with a retained intron. *Genes Dev.*, **20**, 1597–1608.
23. Kreidberg, J.A., Sariola, H., Loring, J.M., Maeda, M., Pelletier, J., Housman, D. and Jaenisch, R. (1993) WT-1 is required for early kidney development. *Cell*, **74**, 679–691.
24. Davies, J.A., Ladomery, M., Hohenstein, P., Michael, L., Shafe, A., Spraggon, L. and Hastie, N. (2004) Development of an siRNA-based method for repressing specific genes in renal organ culture and its use to show that the Wt1 tumour suppressor is required for nephron differentiation. *Hum. Mol. Genet.*, **13**, 235–246.

25. Hastie, N.D. (1994) The genetics of Wilms' tumor–a case of disrupted development. *Annu. Rev. Genet.*, **28**, 523–558.
26. Sim, U.-H., Smith, A., Szilagi, E., Rae, F., Ioannou, P., Lindsay, M.H. and Little, M.H. (2002) *Wnt-4* regulation by the Wilms' tumour suppressor gene WT1. *Oncogene*, **21**, 2948–2960.
27. Rao, M.K., Pham, J., Imam, J.S., MacLean, J.A., Murali, D., Furuta, Y., Sinha-Hikim, A.P. and Wilkinson, M.F. (2006) Tissue-specific RNAi reveals that WT1 expression in nurse cells controls germ cell survival and spermatogenesis. *Genes Dev.*, **20**, 147–152.
28. Moore, A.W., McInnes, L., Kreidberg, J., Hastie, N.D. and Schedl, A. (1999) YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development*, **126**, 1845–1857.
29. Moore, A.W., Schedl, A., McInnes, L., Doyle, M., Hecksher-Sorensen, J. and Hastie, N.D. (1998) YAC transgenic analysis reveals Wilms' tumour 1 gene activity in the proliferating coelomic epithelium, developing diaphragm and limb. *Mech. Dev.*, **79**, 169–184.
30. Koesters, R., Linnebacher, M., Coy, J.F., Germann, A., Schwitalle, Y., Findeisen, P. and von Knebel Doeberitz, M. (2004) WT1 is a tumor-associated antigen in colon cancer that can be recognized by *in vitro* stimulated cytotoxic T cells. *Int. J. Cancer*, **109**, 385–392.
31. Loeb, D.M., Evron, E., Patel, C.B., Sharma, P.M., Niranjan, B., Buluwela, L., Weitzman, S.A., Korz, D. and Sukumar, S. (2001) Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. *Cancer Res.*, **61**, 921–925.
32. Amini Nik, S., Hohenstein, P., Jadidizadeh, A., Van Dam, K., Bastidas, A., Berry, R.L., Patek, C.E., Van der Schueren, B., Cassiman, J.J. and Tejpar, S. (2005) Upregulation of Wilms' tumor gene 1 (WT1) in desmoid tumors. *Int. J. Cancer*, **114**, 202–208.
33. Oji, Y., Suzuki, T., Nakano, Y., Maruno, M., Nakatsuka, S., Jomgeow, T., Abeno, S., Tatsumi, N., Yokota, A., Aoyagi, S. *et al*. (2004) Overexpression of the Wilms' tumor gene W T1 in primary astrocytic tumors. *Cancer Sci.*, **95**, 822–827.
34. Tuna, M., Chavez-Reyes, A. and Tari, A.M. (2005) HER2/neu increases the expression of Wilms' tumor 1 (WT1) protein to stimulate S-phase proliferation and inhibit apoptosis in breast cancer cells. *Oncogene*, **24**, 1648–1652.
35. Oka, Y., Tsuboi, A., Taguchi, T., Osaki, T., Kyo, T., Nakajima, H., Elisseeva, O.A., Oji, Y., Kawakami, M., Ikegame, K. *et al*. (2004) Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc. Natl Acad. Sci. USA*, **101**, 13885–13890.
36. Maiti, S., Alam, R., Amos, C.I. and Huff, V. (2000) Frequent association of beta-catenin and WT1 mutations in Wilms tumors. *Cancer Res.*, **60**, 6288–6292.
37. Maretto, S., Cordenonsi, M., Dupont, S., Braghetta, P., Broccoli, V., Hassan, A.B., Volpin, D., Bressan, G.M. and Piccolo, S. (2003) Mapping Wnt/beta-catenin signaling during mouse development and in colorectal tumors. *Proc. Natl Acad. Sci. USA*, **100**, 3299–3304.
38. Kemler, R., Hierholzer, A., Kanzler, B., Kuppig, S., Hansen, K., Taketo, M.M., de Vries, W.N., Knowles, B.B. and Solter, D. (2004) Stabilization of beta-catenin in the mouse zygote leads to premature epithelial-mesenchymal transition in the epiblast. *Development*, **131**, 5817–5824.
39. Liebner, S., Cattelino, A., Gallini, R., Rudini, N., Iurlaro, M., Piccolo, S. and Dejana, E. (2004) Beta-catenin is required for endothelial-mesenchymal transformation during heart cushion development in the mouse. *J. Cell Biol.*, **166**, 359–367.
40. Patek, C.E., Fleming, S., Miles, C.G., Bellamy, C.O., Ladomery, M., Spraggon, L., Mullins, J., Hastie, N.D. and Hooper, M.L. (2003) Murine Denys-Drash syndrome: evidence of podocyte de-differentiation and systemic mediation of glomerulosclerosis. *Hum. Mol. Genet.*, **12**, 2379–2394.
41. Gao, F., Maiti, S., Alam, N., Zhang, Z., Deng, J.M., Behringer, R.R., Lecureuil, C., Guillou, F. and Huff, V. (2006) The Wilms tumor gene, Wt1, is required for Sox9 expression and maintenance of tubular architecture in the developing testis. *Proc Natl Acad Sci USA*, **103**, 11987–11992.