# Tools for Wt1-based lineage tracing to analyse its role in embryonic development and normal organ homeostasis

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i. Summary / Abstract

The Wilms' tumour suppressor gene 1 (*Wt1*) is critically involved in a number of developmental processes in vertebrates, including cell differentiation, control of the epithelial/mesenchymal phenotype, proliferation and apoptosis. Wt1 proteins act as transcriptional and post-transcriptional regulators, in mRNA splicing and in protein-protein interactions. Furthermore, Wt1 is involved in adult tissue homeostasis, kidney function and cancer. For these reasons, Wt1 function has been extensively studied in a number of animal models to establish its spatiotemporal expression pattern and the developmental fate of the cells expressing this gene. In this chapter, we review the developmental anatomy of Wt1, collecting information about its dynamic expression in mesothelium, kidney, gonads, cardiovascular system, spleen, nervous system, lung and liver. We also describe the adult expression of Wt1 in kidney podocytes, gonads, mesothelia, visceral adipose tissue and a small fraction of bone marrow cells. We have reviewed the available animal models for Wt1-expressing cell lineage analysis, including direct Wt1 expression reporters and systems for permanent Wt1 lineage tracing, based on constitutive or inducible Cre recombinase expression under control of a Wt1 promoter. Finally we provide a number of laboratory protocols to be used with these animal models in order to assess reporter expression.

ii. Key words

Wt1, Wilms' tumour suppressor gene, cell lineage tracing.

## 1. Introduction

## Developmental anatomy of Wt1

The Wilms' tumour suppressor gene 1 (*Wt1*) is well known for its dynamic expression pattern during embryonic development, both in mouse and human. The gene gives rise to at least 24 protein isoforms in human, that are involved in regulation of expression of target genes acting in tissue development, growth, differentiation, and apoptosis. Target gene expression is frequently regulated through binding to co-regulators. Furthermore, Wt1 proteins also act as post-transcriptional regulators in mRNA splicing and in protein-protein interaction (1).

In this chapter, we first give a short overview of the distribution of Wt1 expression at gene and/or protein level during development in mice and other animal models, and describe the adult expression of Wt1. We then review the murine models available for the study of Wt1 function, and provide protocols that include the use of some of these models, with special emphasis on *Wt1*-expressing cell lineage tracing (in short: Wt1 lineage tracing).

### Mesothelium:

The mesothelium is a simple epithelium that lines the coelomic cavities and the organs that develop within these cavities. It forms from the lateral mesoderm at around E9 in the mouse embryo. By this time *Wt1* transcripts start to be detected in the parietal coelomic lining, and over the heart, intestine and urogenital ridge (2). Expression of Wt1 protein appears first over the urogenital ridge at E9.5, but between E10.5 and E11.5 Wt1 protein is present in the mesothelial layers of the parietal coelomic linings, over the heart, intestine, lungs and liver, and also in the septum transversum and developing diaphragm (3-7). Wt1 is

also found in many submesothelial mesenchymal cells in the parietal layers of the coelom at later stages of development (8).

Kidney:

Expression analysis of the murine *Wt1* gene in the developing kidney by *in situ* hybridisation and immunohistological analysis revealed that *Wt1* mRNA/Wt1 protein is expressed in the urogenital ridge from E9, the pro- and mesonephric tissues from E10, and the metanephric mesenchyme (MM) from E12 (9,10). The functional postnatal kidney develops from two tissues that have their origin in the intermediate mesoderm, the metanephric mesenchyme and the ureteric bud (UB). Reciprocal induction events between MM and UB lead to the formation of the functional kidney. This involves several rounds of induction and branching events, involving MM condensation into the mesenchymal cap around the UB, and a subsequent differentiation of condensed cap mesenchyme via mesenchymal-to-epithelial transition (MET) into epithelial cells forming the nephron. Expression of Wt1 in metanephric structures is highly dynamic with highest levels of expression in the condensing cap mesenchyme, comma- and s-shaped body of the developing nephrons, and finally remaining restricted to the podocytes of the glomeruli (11-13). This was confirmed in transgenic mice expressing LacZ under control of YAC fragments that harboured the human *WT1* gene locus including flanking regions of 470 or 280kb (14). Wt1 expression is not found in the UB.

Specifically, Wt1 is intimately involved in the regulation of the early steps of nephron formation and the maintenance of the glomeruli, through interactions with a range of nephrogenic and renal proteins. Wt1 regulates kidney development from early stages leading to complete renal agenesis in loss of Wt1 mutants through apoptosis of the

mesonephric tubules and the metanephric mesenchyme, resulting in failure to induce ureteric bud outgrowth (15). Apoptosis of renal progenitors in absence of Wt1 has recently been related with downregulation of fibroblast growth factor and induction of BMP/pSMAD signalling, and this apoptosis can be rescued by recombinant FGFs or inhibition of pSMAD signalling (16). Analysis of subsequent stages of kidney development, which is precluded due to metanephric apoptosis in the Wt1 null mutants, has been performed using kidney organoid culture in combination with siRNA approaches (17), and conditional inactivation of Wt1 *in vivo* (18). These studies showed that Wt1 controls MET to allow formation of renal vesicles and subsequent stages towards nephron formation from MM, through control of Wnt4 expression. Wnt4 has been identified as a crucial regulator of MET during nephron formation (19,20). Furthermore, a specific role for Wt1 in the control of Wnt4 expression had been described since Wt1 expression precedes that of Wnt4, Wt1 can control Wnt4 expression *in vitro*, and Wnt4 expression is lost in the embryonic kidney mesenchyme when Wt1 is inactivated (18, 21,22).

At later stages, Wt1 protein controls formation of the podocytes and their homeostasis through transcriptional regulation of Pax2, Nephrin and Podocalyxin (13, 23-26).

Wt1 also regulates expression of Nestin, an intermediate filament protein, in the glomeruli, although the significance of Nestin expression in the kidney is not well understood (27). Conditional deletion of Wt1 in embryonic kidneys using a Nestin-Cre model leads to a failure in MET and nephron formation (18).

Using ChIP-PCR to identify Wt1 target sites *in vivo*, a recent systemic study demonstrated that a range of factors important for kidney development are transcriptional targets of Wt1, including Bmp7 and Sall1 (28). Taken together, recent studies in the developing kidney have

shown that Wt1 is a key regulator of a range of molecular pathways that lead to the formation of functional nephrons from the metanephric mesenchyme.

Gonads:

Gonads develop from the urogenital ridge, initially as indifferent primordia, but later they specify into testis and ovaries. They start to arise at around E11 from the mesonephros, the embryonic kidney that forms only transiently, and the overlying coelomic mesothelium. The coelomic mesothelial cells contribute to gonad formation by migrating into the gonadal ridge, forming the primary sex cords and later giving rise to the Sertoli cells (male) or granulosa cells (female) (29, 30). In situ hybridisation and immunohistochemical studies have shown that Wt1 is expressed in the mesonephros and the overlying coelomic epithelium from around E10, but as the urogenital ridges thickens during gonad formation, Wt1 is strongly expressed in the mesenchymal component (12, 13).

Development into male or female gonads and genital organs is regulated by Sry expression, leading to testes formation and differentiation of the Wolffian ducts into seminal vesicles, epididymis and vas deferens in the male, or ovary formation and the emergence of the oviducts, Fallopian tubes, uterus and upper vagina from the Müllerian ducts in the female. However, most components of the testis arise from mesonephric cells migrating into the gonad, including peritubular and vascular endothelial cells, while the Leydig cells are formed in several waves from primary mesonephric and mesonephric-derived cells (31-33). One can speculate that since Wt1 is expressed in the gonadal anlagen from early on, most gonadal cells have their origin in cells originally expressing Wt1.

*Testis*: A complex hierarchical cascade of transcription and signalling factors controls the formation and maintenance of the male gonads. Wt1 is involved in this cascade at several levels since Wt1 expression is required for the survival of the early gonadal anlagen (34). A range of studies have shown that Wt1 is an important regulator of sex determination by controlling the expression of the *Sry* gene (34-39). Loss of function studies have demonstrated that Wt1 regulates the expression of steroidogenic factor 1 (*Sf1*) in the indifferent gonad (40). In addition, molecular and *in vitro* data indicate that Wt1 acts in concert with Sf1 to regulate the expression of the Müllerian inhibiting substance (*MIS*, also called anti-Müllerian hormone, AMH) (41), and it probably indirectly activates Dax1 during early gonadal development (42).

Using a mouse model for testis-specific conditional ablation of Wt1, Vicky Huff and collaborators showed that Wt1 is required for the formation and maintenance of the seminiferous tubules, Sertoli cells and germ cells in the testicular cords (43). Specifically, proteins expressed in Sertoli cells including Sox8, Sox9 and MIS were lost when Wt1 function was abolished. Importantly, loss of Wt1 in the testes leads to β-catenin accumulation which in turn results in testicular cord disruption (44). Further evidence for a role of Wt1 in testicular cord and Sertoli cell maintenance and germ cell survival stems from the finding that testicular cord integrity is associated with expression of *Col4a1* and *Col4a2* as these collagens are downregulated in the testes of mice with testis-specific loss of Wt1 (45). Using an siRNA approach and transgenic mice expressing dominant negative *Wt1*, similar results were reported, supporting an essential role for Wt1 in Sertoli cell and germ cell integrity and survival (46).

*Ovary*: During female gonad development, Wt1 is expressed in stromal cells, granulosa cells and the overlying coelomic mesothelium of the ovary (10). Specifically, granulosa cells of the primordial, primary and secondary follicles express Wt1 during ovary development, and expression is maintained throughout adult life (47), suggesting that Wt1 is involved in folliculogenesis.

*Germ cells*: Wt1 is expressed in germ cells when they start converting from primary germ cells to gonadal germ cells, beginning at embryonic day E11.5. Chimera experimentation has shown that loss of *Wt1* in ES cells leads to their exclusion from the germ cell lineage, suggesting that Wt1 is involved in germ cells proliferation, maturation or survival (48).

Heart and blood vessels:

Wt1 expression in the heart is predominantly, but not exclusively, associated with epicardial development. The earliest expression of Wt1 during cardiac morphogenesis is detected in mouse embryos at E9.5 in the proepicardium, which is the epicardial primordium; subsequently, Wt1 expression continues during the epicardial covering of the heart (2, 49). Wt1 expression is maintained in the epicardial-derived mesenchymal cells (EPDC) which delaminate from the epicardium and invade first the subepicardial space, and then the myocardium. This expression is progressively downregulated as EPDC differentiate and contribute to the vascular and connective tissue of the heart.

The role played by Wt1 in the developing epicardium seems to be critical, since conditional Wt1 loss of function in this tissue leads to impaired generation of EPDC, abnormal coronary morphogenesis and thinning of the myocardium, resulting in embryonic lethality (50). The

mechanism by which Wt1 acts in the epicardium is not completely understood, but results from recent studies suggest that the balance between Snail and E-cadherin activity (50) and the canonical β-catenin pathway (51) serve as main downstream effectors of Wt1 in regulating epicardial to mesenchymal transition. Additionally, recent data indicate that in the epicardium Wt1 regulates the transcriptional activation of Raldh2, which represents the main retinoic acid synthesising enzyme in mesodermal tissues (52). It had been previously established that cross-talk between epicardium and myocardium, facilitating development of both components, is dependent on retinoic acid signaling (53, 54).

Other genes activated by Wt1 in the epicardium include the neurotrophin receptor *TrkB* (55) and *α4 integrin*, required to maintain epicardial adhesion to the myocardium (56). Wt1 regulates expression of the erythropoietin receptor (57) in hematopoietic cells and its ligand erythropoietin in *in vitro* assays (58). Since the erythropoietin signaling system also acts in the epicardium and its failure causes myocardial thinning (59), Wt1 may also be involved through this pathway in epicardial-myocardial interaction, thus supporting development and differentiation of both tissues. Finally, an unsuspected role of Wt1 in the developing epicardium is the regulation of the expression of some chemokines. Specifically, Wt1 down-regulates Ccl5 and Cxcl10, two chemokines that inhibit EPDC migration and myocardial proliferation. This role is performed through increasing of the levels of Irf7 (60).

In summary, in the epicardium Wt1 activates a set of genes related with epicardial adhesion, epithelial-mesenchymal transition and migration. Thus, *Wt1* represents a key gene for epicardial development and function.

Wt1 expression has also been found in non-epicardial derived, cardiac cells. A few cells expressing Wt1 are already present in the endocardium and possibly in the myocardium of E9.5 embryos (61).

Lineage tracing studies of Wt1-expressing cells using Cre-LoxP technology have shed further light onto the role of Wt1 during cardiovascular development and function. Importantly, these studies have shown that the fate of Wt1-expressing cells in the heart is clearly related to coronary vascularization and the formation of cardiac connective tissue. Specifically, Wt1-lineage studies have confirmed an extensive contribution to coronary smooth muscle and cardiac fibroblasts (62). EPDC-derived Wt1-expressing cells have also been shown to contribute to the lateral atrioventricular cushions where they differentiate into fibroblastic cells of the valves (63). However, contribution of Wt1-expressing cells to coronary endothelium has been more controversial. Using different lineage tracing approaches, it was shown that the proportion of coronary endothelial cells originating from Wt1-expressing cells comprises less than 15% (3, 62). Recent data (Cano et al., submitted) demonstrate a large, but not complete, overlap between the Wt1 lineage and a *bona fide* epicardial-derived lineage characterized by the activation of a Gata4 enhancer in the septum transversum and proepicardium. These epicardial-derived cells contribute to a minor, but significant fraction of the coronary endothelium (about 20% of all the endothelial cells), at least during embryonic life and early postnatal stages. This agrees with recent findings reporting that the endocardium is a major contributor to the coronary endothelium (64, 65). Wagner and colleagues showed that Wt1 is expressed in the coronary endothelium of late gestation mouse embryos, while $Wt1^{-/-}$ embryos that survive to close to term, reveal a dramatic lack in coronary vasculature (55). In addition, the group could identify the neurotrophin receptor TrkB as a downstream target of Wt1 in the coronary endothelium

(55), and argued that loss of the coronary vasculature in Wt1 mutant embryos was directly linked to down-regulation of TrkB expression.

Furthermore, using *in vitro* experiments, Wt1 was shown to bind to the VEGF promoter and regulate its expression (66, 67). The intermediate filament and progenitor marker Nestin has also been shown to be downstream of Wt1 in the developing coronary vasculature (27), and to be co-expressed the vasa vasorum of human tissue samples (68.The finding that Wt1 regulates VE-cadherin expression *in vitro* and *in vivo* since VE-cadherin expression is reduced in the liver and hearts of Wt1 mutant embryos (69), corroborates the hypothesis that Wt1 is important for the regulation of blood vessel formation.

Lineage tracing studies have also added to controversy around the contribution of Wt1-expressing cells to the myocardium. Of note, this hypothetical contribution may originate from two sources, i) the EPDC and ii) migration of myocardial progenitors from the posterior secondary cardiac field, where Wt1 expression is prominent in mesenchymal cells of the transverse septum. Original evidence for Wt1-derived myocardial cells provided by Zhou et al. (62) was questioned by Rudat and Kispert (61) on the basis of the unsuitability of the Cre drivers used. Zhou and Pu (70) responded by providing new validating evidence for the existence of cardiomyocytes derived from Wt1-expressing cells, which they considered as epicardial-derived. On the other hand, the existence of a sinus venosus defect in Wt1-deficient mouse embryos (8) could be interpreted as the lack of a Wt1 lineage population contributing to the inflow tract myocardium. This possibility was ruled out by Norden et al. (8) who, by using two different models (LacZ reporter and Wt1-Cre) found that Wt1-expressing cells did not give rise to myocardial cells. These authors conclude that the involvement of the Wt1-lineage in sinus venosus development seems to be indirect.

Developmental haematopoiesis:

Molecular evidence for Wt1 as a regulator of developmental haematopoiesis is based on studies showing that Wt1 regulates expression of both Epo and its receptor EpoR in the fetal liver as the primary haematopoietic organ during mid-gestation (57,58). Furthermore, loss of Wt1 affects *in vitro* differentiation of fetal liver cells, suggesting that Wt1 regulates possibly in synergy with EpoR the differentiation potential of fetal haematopoietic stem cells (57). However, transplantation studies into lethally irradiated mice showed that fetal liver-derived $Wt1^{-/-}$ haematopoietic stem cells were as potent in restoring bone marrow and peripheral blood cells as wild type cells (71).

Spleen:

Wt1 is expressed in the spleen rudiment of the dorsal mesogastrium of mouse embryos by E10.5, continuing in the spleen capsule and epithelium by E14.5 (12). Herzer et al. (72) reported expression by E12.5 and described failure of spleen development in $Wt1^{-/-}$ embryos. Koehler et al. (73) found that the expression of *Wt1* in the spleen follows that of *Hox11* (a homeobox gene required for spleen development) with a delay of one day, while $Hox11^{-/-}$ embryos show reduced expression of Wt1 in the spleen rudiment, suggesting that Wt1 is acting downstream of Hox11 in spleen development.

Body muscle:

Expression of Wt1 in musculature of the body wall of E12-E13 mice embryos was described by Armstrong et al. (2), but this observation has not been confirmed by further reports. It is possible that the presence of mesenchymal cells migrating from the dorsolateral coelomic epithelium to the lateral body wall is related with this early description.

Nervous system and eye:

Besides its extensive expression in mesodermal cells, there are only a few specific domains of Wt1 expression in the neuroectoderm. In mouse embryos, Wt1 is expressed from E11 in a narrow linear domain located between the mantle and the ependymal layers. This expression domain becomes more pronounced by E12 and expands by E13 before turning more diffuse, extending to the ventral part of the marginal area of the medulla and finally disappearing at the end of gestation (2, 12, 14). This expression is anatomically related to the area where motoneurons differentiate. A second area of expression is found in the roof of the 4th ventricle, in a diverticulum of the ependymal layer, close to the rostral part of the medulla oblongata (2, 12).

Wt1 is also expressed in developing retina, as shown by RT-PCR in E12.5 mice embryos. In humans, retina expression of Wt1 has been detected in day 42 fetuses (2). Wt1 seems to be required for retinal development since Wt1-deficient mouse embryos show defects in retinal ganglion cells (74). This effect could be due to the activation of Pou4f2, a transcription factor essential for survival of retinal ganglion cells.

Lung:

Wt1 is expressed in mesothelial cells of the murine lungs from the early sprouting of the lung buds onwards (6, 12, 75). Differently to the heart, Wt1 is rapidly down-regulated in cells delaminating from the mesothelium and incorporating into the pulmonary mesenchyme. These mesothelial-derived cells contribute to most pulmonary mesodermal tissues, including vascular and bronchial smooth muscle, tracheal cartilage and a small fraction of the vascular endothelium (6, 75). In neonates, about 1.5% of all the dissociated pulmonary cells and about 11% of all the endothelial cells derive from theWt1-expressing cell lineage (6). Another difference with the epicardium is that the migration of the mesothelial-derived cells inside the pulmonary stroma is dependent of hedgehog signaling (5).

Liver:

Wt1 is expressed in the liver mesothelium from of the early stages of hepatic development (2, 14). Liver mesothelial cells continue to express Wt1 when they migrate from the surface and intermingle with the hepatoblasts and the hematopoietic cells to differentiate into sinusoidal endothelium and stellate cells (14). In contrast to the heart and the lung, in the liver Wt1 is not down-regulated with onset of differentiation of mesothelium-derived cells. In fact, Wt1 expression is still detectable in sinusoidal endothelial cells (14). This invasion of mesothelial derived cells is necessary for proper hepatic development (76, 77).

Adult expression of Wt1:

Wt1 expression has been reported in a few sites of adult mice, namely kidney podocytes, Sertoli cells of the testes, granulosa cells of the ovary, mesothelia, pancreatic stellate cells,

the stromal vascular component of several fat bodies including visceral adipose tissue progenitors, and a small fraction of bone marrow cells (3, 78-80).

The podocytes are the most prominent site of adult Wt1 expression, and in fact podocyte maintenance and function depends on Wt1 (80). In postnatal stages, Wt1 is involved in the regulation of the maintenance of the glomerular filtration function of the kidney, as shown through a range of studies. Mice with reduced Wt1 expression and subsequent downregulation of nephrin and podocalyxin expression showed increased glomerulosclerosis (26). The damage to the glomeruli is possibly caused by insufficient levels of podocalyxin and nephrin both of which are required for the functional morphology of the slit diaphragm and foot processes of the glomerular filtration membrane. Furthermore, recent study from the Ai lab has shown that Wt1 is important for maintaining cross-talk between podocytes and glomerular endothelial cells across glomerular filtration membrane. Specifically, Wt1 controls the expression of the 6-O-endosulfatases Sulf1 and Sulf2 which in turn regulate signalling of VEGFA from podocytes to glomerular endothelial cells across the glomerular filtration barrier (81).

Wt1 expression is not maintained in all adult mesothelial tissues: while it is present in the adult intestine (3) and the mesothelium lining the visceral fat (78, 79), there are conflicting findings about Wt1 expression in the lung mesothelium, with Dixit and colleagues reporting downregulation of Wt1 in postnatal and adult mice, while Que and colleagues have shown continued expression in P45 animals (5, 79). Karki et al. (82) also stated that expression of Wt1 remains in the adult pulmonary mesothelium, and its loss is correlated with mesenchymalization and fibrosis. Wt1 expression in the liver mesothelium seems to be

downregulated after E13.5 (77). It is possible that a low basal level of Wt1 expression in adult mesothelium is the basis for these discrepancies.

The expression of Wt1 in the visceral fat mesothelium and in the progenitors of the visceral white adipose tissue (WAT) establishes a key difference with other fat bodies such as subcutaneous WAT and brown adipose tissue that do not develop from Wt1-expressing cells (79). This difference could be significant given the different potential of visceral and subcutaneous WAT as risk factor for a number of diseases.

Expression of Wt1 is maintained into adulthood in the Sertoli and granulosa cells (2, 10). Wt1 regulates Sertoli cell polarity in the testes, and it is essential for germ cell survival, differentiation and spermatogenesis (83, 84). Additionally, the expression of Wt1 in Sertoli cell is essential to maintain steroidogenesis in Leydig cells (85, 86). In the ovary, Wt1 is also expressed in granulosa cells, controlling their polarity and differentiation (87). In a mouse model mimicking the Denys-Drash Syndrome (DSS), heterozygous mice have reduced ovulation rates, premature differentiation of granulosa cells, leading to disturbed development of follicles (87). This study supports the notion that Wt1 is not only required for normal spermatogenesis, but also for oogenesis.

Besides the ovary, Wt1 expression has also been reported in the embryonic and adult uterus, specifically the myometrium and human endometrium (10, 88, 89).

Wt1 expression was detected in the bone marrow of mice and humans for the first time by Fraizer et al. (90). A range of studies showed that Wt1 expression in hematopoietic cells is restricted to the phase of expansion of hematopoietic progenitor cells while expression was found to be reduced in mature haematopoietic cells and absent in the mature peripheral blood (91, and references therein). Furthermore, it was found that Wt1 expression was

downregulated in haematopoietic cell lines that underwent differentiation, while high expression of Wt1 was correlated with induced proliferation of cells in culture (92, 93). Wt1 seems to have conflicting roles in different stages of haematopoiesis since it can induce quiescence in early (CD34+ CD38-) progenitors, while it stimulates differentiative behaviour in more committed progenitor cells. Wt1 is present in erythroblastic progenitors, where it transactivates the EPO receptor (56). Wt1 is also involved in granulocyte differentiation (94). Single cell qPCR of cells during haematopoiesis revealed a biphasic expression pattern with high activity in quiescent primitive precursor cells and specific myeloid cell populations (95,96). Interestingly, using a genetically modified mouse line which expresses GFP under control of the endogenous Wt1 locus, Hosen and colleagues came to a slightly different finding, since in $Wt1^{GFP/+}$ mice, Wt1 expression was absent or very low in haematopoietic stem cells or fully differentiated granulocytes, respectively, while expression was higher in myeloid progenitor cells (97). Loss of Wt1 in haematopoietic stem cells was shown to affect their differentiation potential (98). Furthermore, in an independent study using ES cells lacking Wt1 protein that were differentiated towards the haematopoietic lineage, similar observations were made, as the colony forming/differentiation potential of the cells was greatly reduced (99). The authors could show that $Wt1^{-/-}$ ES cells undergo apoptosis that is dependent of Vegfa, and that Wt1 is responsible for splicing of Vegfa into functional isoforms. The function of Wt1 in blood cell differentiation could be mediated by p21cip1 induction, leading to growth arrest (95). This would explain the role played by Wt1 mutations in leukemogenesis (see below).

In some pathological conditions, the adult expression of Wt1 becomes more prominent. Wt1 is expressed in coronary arteries (endothelium and smooth muscle) after myocardial infarction (100). This is probably due to the hypoxia produced by the local ischemia, since

the upregulation of the *Wt1* gene, mediated by a hypoxia responsive element in the Wt1 promoter, is mimicked by exposing rats to hypoxic conditions (101). Thus, low oxygen tension could be a driver for Wt1-regulated angiogenesis (102).

Wt1 was named after its supposed role in the development of Wilms' tumor (103), although only a fraction of these tumours shows alterations in WT1 expression. In contrast, abnormal overexpression of WT1 has been reported in a number of tumour cells (104), and it is particularly prominent in acute myeloid leukemia (AML) (105-107). WT1 is overexpressed in malignant cells of 90% of patients with AML and appears mutated in approximately 10% of these patients (108). Importantly, these observations have raised expectations for Wt1 as a target in cancer immunotherapy (109, 110).

Wt1 expression in non-mammalian animal models:

The developmental expression of Wt1 in chicken embryos is in principle similar to that described for mammals (111). Wt1 has been used as a marker of proepicardial, epicardial and epicardial-derived cells by a number of groups studying chick development (112-116).

In zebrafish, two *Wt1* genes, *wt1a* and *wt1b*, have been reported, both showing +KTS and -KTS isoforms (117). In early embryos the expression of both genes is dynamic and restricted to intermediate mesoderm. Expression of wt1a in the zebrafish pronephros is regulated by retinoic acid through a highly conserved enhancer (118). In addition, wt1a has recently be reported to regulate expression of osr1, thus controlling the differentiation of zebrafish podocytes (119). The expression domains of wt1a and wt1b in adult fish tissues are more extensive than in mammals, including gonads, kidneys, heart, spleen and muscle. Both wt1a and wt1b, have also been reported in other fish, such as *Oncorhynchus*, *Oryzias*, *Takifugu*

and *Tetraodon* (reviewed in 120). In *Tetraodon*, the highly conserved motif KTS, distinguishing DNA-binding to DNA non-binding isoforms, is changed to KPS (120).

Information on Wt1 expression in amphibians is more limited. Wt1 is expressed in Sertoli cells, spermatogonia and mature sperm stages in the testes of the newt *Cynops pyrrhogaster* (121). In *Xenopus*, Wt1 expression is first restricted to the developing nephric system, and later is also detected in the developing heart (122, 123). Since Wt1 is not detected in developing pronephric tubules and ducts, its function seems to be related with the development of the glomeruli. In fact, when Wt1 was ectopically expressed in *Xenopus* embryos by mRNA injection, it inhibited pronephric tubule development (124).

Regarding invertebrates, a Wt1 ortholog has been found in the cephalochordate *Branchiostoma floridae* (125). Furthermore, the *Drosophila* gene *Klumpfuss* has been considered as a Wt1 ortholog, and is involved in neuronal (126, 127) and hemocyte differentiation (128). However, despite the similarity between the four zinc-finger domains with those of the vertebrate Wt1, the N-terminal region is clearly different making this orthology very doubtful.

Details of animal model systems and approaches to study systemic and inducible loss of function of Wt1 will be described in Chapter XYZ.

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