

Chapter 13

WT1 in Cardiac Development and Disease

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Abstract

The heart is essential for realizing the distribution of oxygen and nutrients throughout the body. Therefore, the heart is the first organ to develop and is already functional in its most primitive structure during embryogenesis. Recent studies indicate that the transcription factor Wilms' tumor-1 (WT1) is important for many aspects of cardiac development. WT1 expression is first observed in the proepicardium, a group of progenitor cells that give rise to a mesothelial sheet covering the heart, the epicardium. WT1 expression in epicardial cells is required for their epithelial-to-mesenchymal transformation forming epicardium-derived cells that will contribute to the formation of coronary vessels and interstitial fibroblasts. Endothelial cells within the heart also express WT1, whereas the endothelial cells in other

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parts of the embryo do not. The endothelial expression of WT1 during cardiac development is likely to be important for vascular formation. After cardiac injury, WT1 is temporally upregulated in the epicardium and in the endothelial cells in the infarcted area and border zone, which points to a potential important role for WT1 in cardiac repair and regeneration. In this chapter, we describe the many faces of WT1 within the heart.

Key words: Cardiac development; Cardiac regeneration; Endothelial cells; Epicardium; Wilms' tumor-1

Introduction

The pleiotropic molecule Wilms' tumor-1 (WT1) is a transcription factor that was first discovered in renal tumors (1, 2). It contains four zinc-finger motifs at the C-terminus which are important for the binding of DNA to activate gene expression. Besides its function to activate the transcription of genes, WT1 is also involved in posttranscriptional processes (3). The expression of WT1 is essential during development of multiple organs, including kidneys, gonads, spleen, and the heart (4, 5). In the developing heart, WT1 is strongly expressed in the outer layer, i.e. the epicardium, and in the cardiac endothelial cells. After myocardial infarction this WT1 expression reemerges in both lineages. In this chapter, we describe the dynamic expression of WT1 during development and after cardiac injury. We focus on the multiple roles of WT1 including epithelial-to-mesenchymal transformation and angiogenesis in cardiac development, repair and regeneration.

WT1 in cardiac development

The expression of WT1 in the epicardium during early cardiac development

Knockout of *Wt1* in mice revealed that this transcription factor plays an essential role in cardiac development. In the absence of WT1, the vasculature of the heart is not formed, which disturbs proper formation of the heart and therefore results in prenatal death (3, 4). Already in the developing embryo, the heart is essential for the supply of oxygen and nutrients. Therefore, it is the first organ to develop and function during embryogenesis. The heart has a mesodermal origin and is formed through gastrulation. The earliest recognizable structure is the primitive heart tube, which is formed at embryonic day (E) 8 in mouse, corresponding with day 21 postfertilization in human. This hollow structure consists of two cardiac cell populations, namely cardiomyocytes on the outside and endothelial cells on the inside, which are separated by cardiac jelly (5) (Figure 1). The primitive tube elongates and undergoes rightward looping between E8.5 and E10.0 in mouse (days 23 and 28 in human) (Figure 1). Subsequent remodeling of the heart involves formation and expansion of the chambers, and formation of valves and septa, resulting in a septated four-chambered heart (6, 7)

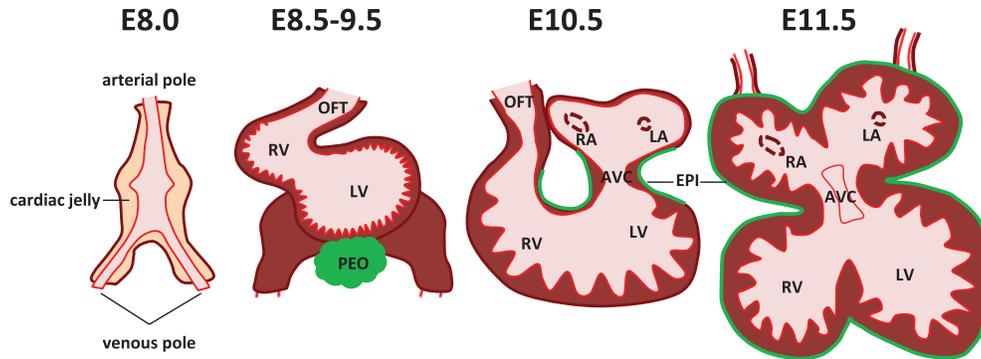


Figure 1. Early cardiac development and the expression of WT1. E8.0: The primitive tube consists of cardiomyocytes (brown) on the outside and endothelial cells (red) on the inside, which are separated by cardiac jelly. E8.5–E10.5: The primitive tube elongates and undergoes rightward looping. At the inflow tract of the developing heart, the proepicardium arises and the epicardium formation has initiated. E11.5: The four chambers of the heart can be recognized, and septa and valves are being formed. The expression of WT1 is indicated in green. AVC, atrioventricular cushion; EPI, epicardium; LA, left atrium; LV, left ventricle; OFT, outflow tract; PEO, proepicardium; RA, right atrium; RV, right ventricle.

(Figure 1). Even though the heart is already functional at an early stage of fetal development, the growth and maturation of the heart are not completed until after birth (8).

A third population of cardiac cells envelopes the heart during development, the epicardium. The cells forming the epicardium are derived from the proepicardium, a heterogeneous transient cluster of cells (9), located at the base of the inflow tract of the developing heart (Figure 1). The earliest expression of WT1 in the heart is found in cells of the proepicardium, at E9.5 (10–12) (Figure 1 and 2a). Proepicardial cells reach the bare heart tube by formation of a tissue bridge or by free-floating vesicles and spread over the myocardium covering the complete heart, forming the epicardium. Recently, we have shown that the covering of the myocardium with WT1-positive cells occurs in a dorsal to ventral pattern between E9.75 and E10.5. In addition, the epicardial covering of the right ventricle is delayed and less dense compared to the left ventricle. Complete covering of the myocardium with WT1-positive cells is established in the mouse by E12.5 (11) (Figure 1). During human cardiac development, complete covering of the myocardium is observed at week 5 (13). After enveloping the entire heart, the epicardium remains positive for WT1 both in mouse and in human during embryonic development (3, 10–18) (Figure 1 and Figure 2a).

After the formation of the epicardium, a subset of epicardial cells undergoes epithelial-to-mesenchymal transition (EMT), resulting in epicardium-derived cells (EPDCs) (19, 20)

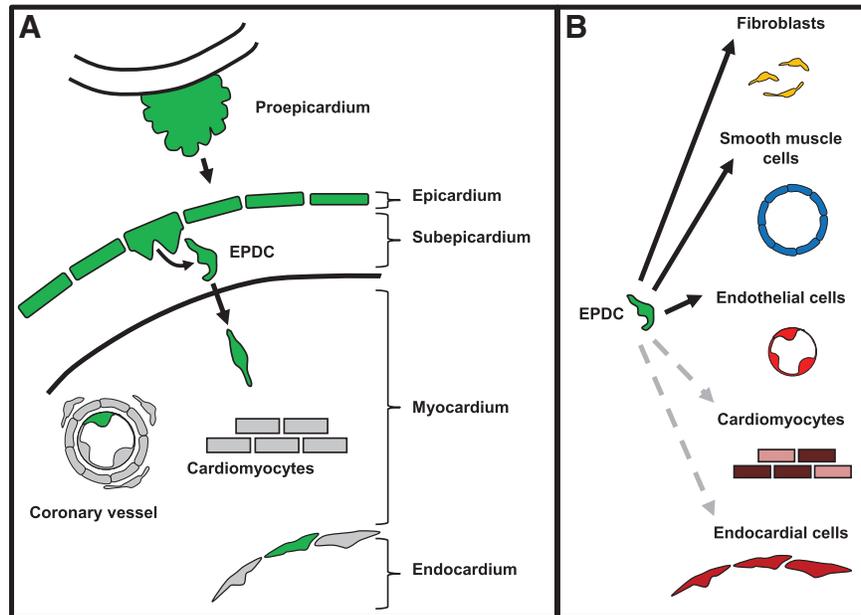


Figure 2. Expression of WT1 and the fate of epicardial cells. (a) The epicardium arises from the proepicardium. A subset of cells undergo EMT and migrate into the subepicardium and myocardium. WT1 (green) is expressed by cells in the proepicardium, epicardium, EPDC, endothelial cells, and endocardial cells. (b) EPDCs contribute to the formation of fibroblasts, smooth muscle cells and endothelial cells; the contribution to cardiomyocytes and endocardial cells is controversial. Cardiac fibroblasts can be subdivided into interstitial fibroblasts, perivascular fibroblast, and valvular interstitial cells.

(Figure 2a). Lineage-tracing studies have shown that EPDCs migrate into the myocardium and contribute to cardiac fibroblasts, endothelial and smooth muscle cells of the cardiac blood vessels, and cardiomyocytes (9, 12, 19, 21–23), although the contribution to the latter is still under debate (11, 14, 16, 24) (Figure 2b). The contribution of WT1-positive cells to endothelial cells however is minimal (12).

The role of WT1 in embryonic epithelial-to-mesenchymal transition

In addition to the essential role of WT1 in the formation of the epicardium (3, 4), several studies have shown that WT1 serves as a regulator of epicardial EMT. *Wt1*-knockout mice show a reduction of subepicardial mesenchyme (3). In addition, epicardial cells are unable to detach from the epicardium, and EPDCs do not migrate into the subepicardium (18, 25).

Epicardial EMT is regulated by WT1 via multiple genes and pathways. Knockdown of *Wt1* in epicardial cells reduced the expression of SNAIL and SLUG, whereas the

WT1 in the heart

downstream target E-cadherin was upregulated. SNAIL and SLUG are key regulators of the EMT process and have an inhibitory effect on the expression of the epithelial marker E-cadherin (26). WT1 directly promotes EMT by enhancing the expression of SNAIL and inhibiting the expression of E-cadherin (25). Furthermore, WT1 was shown to be a positive upstream regulator of the Wnt pathway, which influences diverse aspects of cardiogenesis and is important for epicardial EMT (27). Knockdown of *Wt1* resulted in a decrease of the downstream effectors of the Wnt pathway, *Ctnnb1* and *Lef1* (18). WT1 directly regulates RALDH2, an enzyme involved in retinoic acid (RA) synthesis, and is expressed in the epicardium (18, 28, 29). RA signaling is essential during embryonic development, and RA deficiency has been shown to result in cardiac abnormalities, similar to the phenotype of *Wt1*-knockout mice (20, 30). In chicken, induction of RA signaling in EPDCs results in upregulation of WT1 (31), indicating a positive feedback loop between WT1 and RA.

Although most *in vivo* studies suggest an inducing role for WT1 in epicardial EMT, *in vitro* repression of WT1 induced the transformation of both human and mice cobblestone-like EPDCs into spindle-shaped cells, indicating a context-dependent and possibly, concentration-dependent function of WT1 (32, 33).

Expression of WT1 in cardiac endothelial cells

The classical consensus is that EPDCs lose their expression of WT1 during their migration from subepicardium into the myocardial layer (12, 34). We have recently shown that the expression of WT1 is not restricted to the epicardium and subepicardium but is also present in the myocardial layer during development from E12.5 onward in mice and from week 5 after fertilization in humans (11, 13, 14) (Figure 2a and Figure 3). This expression of WT1 starts at the epicardial side and expands toward the luminal site of the heart (13, 14). The myocardial wall of the heart consists of an outer condensed part, the compact layer, and an inner loosely arranged part, the trabecular layer. Before birth, the expression of WT1 within the myocardium in mice is restricted to the compact layer and expands into the trabeculae in the neonatal heart (14). In human, the trabecular expression of WT1 is already observed before birth (13). The cellular composition of the heart comprises cardiomyocytes, cardiac fibroblasts, smooth muscle cells, and endothelial cells (35). Interestingly, in the myocardial layer of the heart, we found the expression of WT1 in endothelial cells of both small capillaries and the larger coronary vessels in mice and human (13, 14) (Figure 2a and Figure 3). In mice, the expression of WT1 in endothelial cells is still present at neonatal stages but gradually decreases before adulthood (14). In human, the expression of WT1 in endothelial cells of at least the arteries decreases before birth (13). Another difference between mice and human is

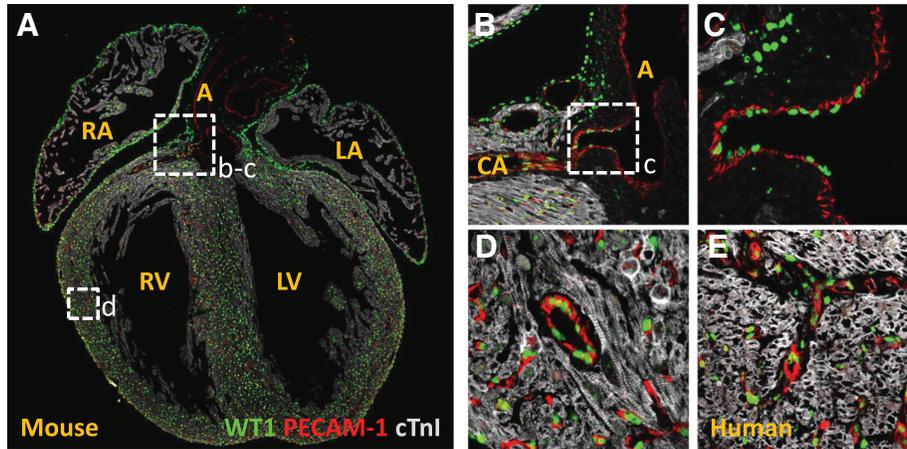


Figure 3. WT1 is expressed by cardiac endothelial cells during development. (a) Overview of the mouse heart at E17.5. WT1 is expressed in the epicardium and endothelial cells [coexpression of WT1 (green) and PECAM-1 (red)]. (b, c) Magnification of the connection between aorta and coronary vessel shows the specific cardiac expression of WT1 in endothelial cells. (d) WT1 is expressed in the vasculature of the heart during murine development. (e) WT1 is expressed in the vasculature of the heart during human development. A, aorta; CA, coronary artery; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

the widespread expression of WT1 in endocardial cells at early stages during human cardiogenesis (Figure 2a). The differences in WT1 expression between mouse and human can be explained by the difference in maturation time during pregnancy, as well as the differences in dimensions (36, 37).

WT1 in the infarcted heart

The epicardial expression of WT1 decreases after birth and remains at low levels during normal homeostasis (Figure 4a). The endothelial expression of WT1 in the adult heart is low and mostly observed in some capillaries and cardiac veins (14). In contrast to the quiescent appearance during adult physiological conditions, after myocardial infarction (MI) the epicardial and endothelial cells re-express WT1 (Figure 4d).

MI is the most common type of ischemic heart disease and the leading cause of death in the Western world (38, 39). During MI, coronary occlusion leads to a reduced supply of oxygen to the cardiac muscle, resulting in massive cell death of cardiomyocytes. The extracellular matrix (ECM) is degraded ensuring infiltration of inflammatory cells, which remove the cellular debris generated during acute cardiac injury (40, 41). The expression of WT1 in the epicardium is already upregulated 1 day after infarction and is induced throughout the entire

epicardial layer of the heart (42). The subepicardium thickens by resurgence of the EMT process and is most pronounced at the infarcted area (43) (Figure 4b). The re-expression of WT1 and the revival of epicardial EMT suggest that WT1 regains its fetal role after MI. In the subepicardium, WT1 is expressed in the α SMA-positive cells but not in the endothelial cells (44) (data not shown) (Figure 4d). Lineage tracing of epicardial cells indicated that the WT1-positive cells do not migrate into the infarcted area (44, 45). Remarkably, priming the mouse heart before MI with Thymosin beta-4 resulted in the migration of epicardial cells into the myocardium and functional differentiation into cardiomyocytes after MI (22).

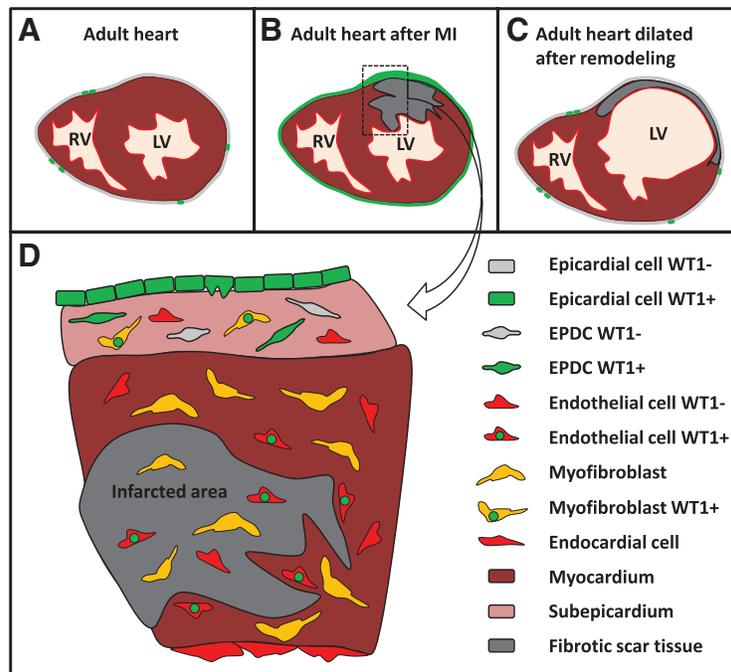


Figure 4. Expression of WT1 in the adult and injured heart. (a) The epicardial expression of WT1 is almost absent in the adult heart. (b) Myocardial infarction results in the loss of cardiomyocytes and the replacement by fibrotic scar tissue. After myocardial infarction, the epicardial expression of WT1 is reactivated in the entire heart. The thickening of the epicardium is most pronounced at the infarcted area. (c) Cardiac remodeling following myocardial infarction results in ventricular dilatation and impairment of cardiac pumping. (d) Myocardial infarction results in re-expression of WT1 in the epicardium, epicardial EMT, migration of EPDCs into the subepicardium, expression of WT1 in myofibroblasts of the subepicardium and in endothelial cells of the infarcted area and border zone. The expression of WT1 throughout adulthood and after injury is indicated in green. LV, left ventricle; RV, right ventricle.

In the infarcted area, an increase in granulation tissue is observed approximately 3 days after MI, which is characterized by the presence of interstitial fibroblasts, myofibroblasts, and forming blood vessels (12, 14, 43, 46, 47). As opposed to the expression in the subepicardium, upregulation of WT1 expression is present in endothelial cells and not in (myo) fibroblasts in the myocardial layer (14) (data not shown; Figure 4). Initially, upregulation of WT1 is observed in endothelial cells of the border zone and subsequently in the infarcted area, with a peak endothelial expression of WT1 at day 7 after MI (47). As time progresses, vessels in the infarcted area become more mature, fibrotic scar forms, and the expression of WT1 disappears (14). Interestingly, in the border zone, the expression of WT1 in endothelial cells is still detectable 4 weeks after MI, indicating that this region still undergoes active remodeling. The expression of WT1 in the epicardium gradual decreases after the first week to return to quiescent levels at 3 months after infarction (42) (Figure 4c). The remodeling and maturation of the fibrotic scar result in ventricular dilatation and impairment of cardiac functioning (48, 49) (Figure 4c).

The molecular mechanism causing WT1 reactivation in epicardial and endothelial cells is unclear. Inflammation might be a potential trigger for the activation of WT1 expression after MI. Proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, are upregulated within the first few hours after injury (50). These cytokines are able to activate the transcription of NF- κ B (51), which is highly present after MI (52-54). NF- κ B upregulates WT1 expression (55), thereby potentially activating the epicardium after MI (Figure 5). The peak of NF- κ B induction is found at day 3 after MI (56), the same day the first signs of angiogenesis, including the endothelial WT1 expression, are visible in the border zone (47). Interestingly, WT1 has an anti-inflammatory role because it inhibits the expression of inflammatory cytokines by stimulation of IL-10 (57) (Figure 5).

The upregulation of epicardial WT1 after injury might also be caused by soluble factors released by the myocardium within the pericardial fluid (PF). Injection of PF from MI patients into the pericardial cavity of mice induces the expression of WT1 in epicardial cells in the absence of infarction (58, 59). In addition, PF of patients affects gene expression in epicardial cells that are involved in EMT; among others, the expression of SNAIL and TWIST is stimulated (59). After MI, PF contains an increased number of exosomes, which are small extracellular microvesicles. These vesicles contain bioactive molecules and are important for intracellular communication and activation (60). Recent proteomic analysis by Foglio et al. (59) showed that clusterin is highly enriched in exosomes of PF of patients after MI. Clusterin is involved in EMT in prostate cancer (61), and administration of clusterin in the pericardial cavity induced EMT in epicardial cells (59).

The upregulation of endothelial WT1 after injury might be caused by hypoxia (Figure 5). Hypoxia is a well-known condition that induces vascular formation during development

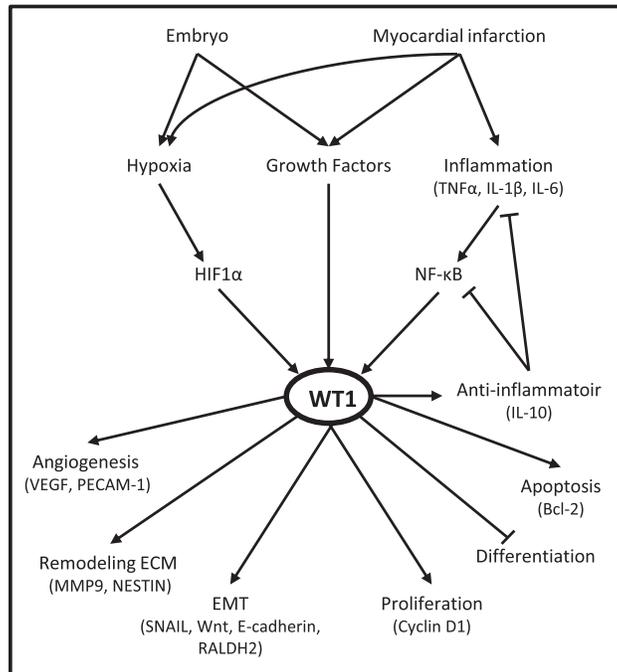


Figure 5. Working model of the regulation of WT1 and its target genes. The expression of WT1 is activated during embryonic development and in the adult heart after injury. Upregulation of WT1 is caused by hypoxia, growth factors, and inflammation. WT1 in turn is able to activate multiple genes that are important for the regulation of different processes, including EMT, angiogenesis, remodeling of the ECM.

and after MI via the hypoxia-inducible factor-1-alpha (HIF1 α) (62). The expression of WT1 can be directly upregulated by hypoxia through the HIF1 α -responsive elements in the WT1 promoter (63) (Figure 5). *In vitro* exposure of human endothelial cells to hypoxia increased the expression of WT1 (14, 64). Important for the response to hypoxia is that one of the downstream targets of WT1 is VEGF (65), one of the most potent angiogenic factors, both in embryonic vascular formation and in the growth of blood vessels after injury (66) (Figure 5).

The role of WT1 in endothelial cells

The expression of WT1 in endothelial cells is only found in the heart and not in other organs of the developing embryo (14) (Figure 3a-3c). The cardiac-specific expression of WT1 is supported by a recent study that identified the unique gene expression profiles of endothelial cells, isolated from different organs (67). In both human and mouse, the expression of WT1

in cardiac endothelial cells is significantly higher compared to noncardiac endothelial cells, confirming cardiac-specific expression of WT1 in endothelial cells (67). In fact, overexpression of WT1 was sufficient to differentiate endothelial cells into a more cardiac specialized population. The importance of WT1 for the development of blood vessels is highlighted by the re-expression in the cardiac vasculature after MI in mouse and after exposure of rats to hypoxia (14, 64). A study by Coosemans et al. (68) claimed the expression of WT1 in cardiac endothelial cells of patients that died after MI. Although the expression of WT1 in cardiac endothelial cells is unique during normal conditions, the expression is also present in endothelial cells in other organs in a pathological condition. WT1 is found in endothelial cells of the skin in patients with chronic dermatitis (69), and WT1 has been observed in endothelial cells in a wide variety of tumors (68–72).

It is unclear why under physiological conditions WT1 expression is found only in cardiac endothelial cells. In contrast to other organs, the heart has the unique feature that it is exposed to cyclic strain (73). It is known that mechanical forces during early development play an important role in cardiac morphology (74). In addition, cyclic strain is able to regulate the process of EMT (75). It is therefore tempting to speculate that stimulation of EMT by cyclic strain is regulated by an upregulation of the expression of WT1. Alternatively, WT1 might be induced by TGF β , which is known to be upregulated by cyclic strain. TGF β is able to upregulate WT1 expression via Par-4 (76–78). On the contrary, WT1 works as a negative feedback loop on TGF β , by repressing its expression (79, 80).

At the very early stages of development, the fetal heart is predominantly dependent on glucose metabolism and shortly after birth the heart energy metabolism switches to fatty acid oxidation (81). Facilitating the uptake of fatty acids is a unique feature of cardiac endothelial cells (67, 82). WT1 expression is known to be essential for the cardiac endothelial fingerprint; therefore, WT1 might be important for the regulation of cardiac endothelial cell metabolism.

Patients with Denys–Drash syndrome (DDS), carrying partial-loss-of-function mutations in the *WT1* gene, develop glomerulosclerosis. In addition, the capillaries of the glomeruli show abnormal development. A cause of these malformations is found in a strong decrease in the expression of platelet endothelial cell adhesion molecule-1 (PECAM-1) in endothelial cell of the glomeruli (83). PECAM-1 is part of intercellular junctions and is present in mature vascular structures; additionally, its expression is upregulated during formation and remodeling of vascular networks (84, 85). WT1 is a positive regulator of PECAM-1 (71); this may explain the poor organization of capillaries in patients with DDS. Knockdown of *WT1* in human endothelial cells confirms the importance of WT1 in the formation of vascular networks, as these cells are unable to form proper networks (14, 64). The angiogenic role of WT1 is supported by a reduced sprouting capacity in an aortic ring angiogenesis

assay from mice lacking WT1 expression in endothelial cells. In addition, the same study revealed that the vessel density in matrigel plugs after subcutaneously injection, in mice lacking WT1 expression in endothelial cells, is significantly reduced compared to wild-type animals (71). Furthermore, deletion of WT1 in endothelial cells resulted in major reduction in cardiac vessel formation during mouse cardiac development supporting the presence of WT1 and the essential role of WT in cardiac endothelial cells (86).

Endothelial cells are anchored to a basement membrane that ensures structural and organizational stability. During vascular formation and remodeling, reorganization of the ECM is essential (87, 88). Remodeling of the ECM is the net result between synthesis and decomposition of the ECM (89). Degradation of the ECM is facilitated by an increase and activation of latent matrix metalloproteinases (MMPs). WT1 is able to directly upregulate the expression of MMP9 (70), thereby facilitating the degradation of ECM. The basement membrane is mostly made up of collagen IV, which is degraded by MMP9 (90). Interestingly, the study of Johnson et al. (91) showed that in the absence of MMP9 revascularization of infarcted tissue is strongly impaired, confirming that remodeling is essential for angiogenesis. The role of WT1 in remodeling is further strengthened by proteomic analysis in patients with DDS. Glomerular podocytes with WT1 mutations have a disturbed production of proteins forming the cytoskeleton (92). Furthermore, the expression of intermediate filament Nestin is regulated by WT1 (93). The expression of Nestin is increased in regenerating tissue and is believed to participate in cellular remodeling and angiogenesis (94–96). Coexpression of WT1 and Nestin was found in the epicardium and endothelial cells of the embryonic heart in mice (93) and in vascular endothelium of patients who died after MI (97, 98).

Changes in the cytoskeleton are also required for cells to adapt to a less differentiated phenotype, allowing them to proliferate and migrate. Nestin is present in proliferating progenitor cells and positively regulates proliferation and migration (96). Within the epicardium, a positive correlation was found between WT1 and proliferation (99). In addition, WT1 plays a role in regulating the cell cycle. *In vitro* studies knocking down WT1 in human endothelial cells show reduced proliferation and migration (14, 72). Proliferation of endothelial cells is directly regulated by WT1 via Cyclin D1 (14, 100), one of the many regulators of the cell cycle and present in the G1 phase (101). Interestingly, the expression of WT1 is upregulated in embryonic stem cells during embryonic body differentiation, a proliferative period for mesenchymal cells. Upon cellular differentiation, the expression of WT1 was reduced (25). The positive role of WT1 on migration might be the result of direct repression of the promoter of Cxcl10, an inhibitory chemokine preventing angiogenesis (17).

Finally, WT1 is known to play a role in apoptosis. This was already noticed in 1993 in *Wt1*-knockout mice; embryonic tissue of the kidney showed more cell death compared to wild-type littermates (4). Over the last years, it has become clear that WT1 can directly

regulate genes involved in apoptosis; however, it depends on the cellular context if WT1 has a pro- or anti-apoptotic effect (102). Future research is required to investigate the role of WT1 in apoptosis of endothelial cells; potentially WT1 protects the forming and maturing blood vessels against cell death.

Together these observations suggest a role for WT1 in the remodeling, proliferation, and migration of cardiac endothelial cells and the formation of a proper vascular network (Figure 5). Stress factors such as hypoxia and inflammation are likely to play a role in the activation of WT1 both during cardiac development and in the response after injury (Figure 5). Future research, focusing on the molecular mechanisms, can hopefully reveal all pathways by which the angiogenic function of WT1 can be explained.

Clinical perspective

Restoring the cardiac blood flow is the most important treatment of ischemic cardiomyopathy at this moment. To improve cardiac output, the cardiac wall consisting of cardiomyocytes, fibroblasts, endothelial and smooth muscle cells, has to be rebuilt. Transplantation of cardiac stem cells after infarction improved the function (103–105); however, difficulties in acquisition of human tissue and *in vitro* expansion of cells limit the clinical applicability. An interesting approach would be to take advantage of the properties of WT1-expressing cells. The differentiation potential of WT1-expressing epicardial cells during development into the vasculature, fibroblasts and cardiomyocytes has positioned the epicardium as a promising target (20, 106, 107). WT1-expressing stem cell-like cells are residing in the epicardium (58, 108). In addition, Chong and colleagues (109) showed that cardiac colony-forming units originate from WT1-positive cells within the epicardium. Finally, activation of WT1 expression within the epicardium after injury revives the fetal differentiation potential in the epicardial cells. Facilitating the differentiation of these WT1-expressing cells towards cardiomyocytes, fibroblasts, endothelial and smooth muscles cells could provide a great tool to improve cardiac regeneration after injury. In addition, the indicated role of WT1 in the endothelial cells during development and injury has positioned the WT1-expressing endothelial cells as a potential target for improving angiogenesis in the diseased area.

A recent study indicates a role for the epicardium in autonomic modulation during early development. Within the initial stages of epicardial formation, WT1 in epicardial cells is coexpressed with the neuronal markers TUBB3 and NCAM (15). Interestingly, the expression of WT1 was also found in the ventral region of the neural tube, as well as the roof of the 4th ventricle of the brain, supporting the neuronal phenotype of the epicardium. Dysfunctioning of the cardiac autonomic nervous system plays a role in the pathogenesis of arrhythmias (110) and hypertension (111) and is involved in disease progression in heart failure (112). Understanding the mechanistic role of WT1 in the formation of the cANS

might help to unravel processes that govern normal cANS development and opens possibilities for treatment after cardiac injury.

WT1 is associated with major generation processes during cardiac development like formation of epicardium, cardiac vasculature, valves, cANS, and also myocardial wall maturation, but also with major regeneration processes during cardiac repair like scar formation and angiogenesis. More knowledge on the upregulation of WT1 in cardiac cells and their subsequent response can contribute to the development and improvement of therapeutic strategies for cardiac repair, and thereby restoring a functional contractile cardiac wall.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

Acknowledgment

Not relevant.

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