

## WT1 expression in the human fetus during development

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### Abstract

Wilms' Tumor 1 (WT1) is a transcription factor involved in the development of the urogenital system. The purpose of this study was to analyze the immunoreactivity for WT1 protein in different tissues and organs in human fetuses in early phases of gestation. To this end, samples from multiple organs were obtained from 4 human fetuses, ranging from 7 up to 12 weeks of gestation. Each sample was formalin-fixed, paraffin embedded and immunostained for WT1. Our data show that WT1 is involved in development of multiple human organs in a more vast series of cells types than previously reported. Immunostaining for WT1 was characterized by a predominant cytoplasmic reactivity in the vast majority of cell types. Mesenchymal progenitors in the fetal lung, ductal plate progenitors in fetal liver, cap mesenchymal cells in the developing kidney, fetal zone cells in adrenal glands, atrial and ventricular cardiomyocytes in the fetal heart, radial glial cells in the fetal cerebral cortex and skeletal muscle cell precursors showed the highest levels of WT1 immunoreactivity. Future studies will be needed to detect differences in the expression of WT1 in various organs at different gestational ages, in order to better evaluate the role of WT1 in cell proliferation and differentiation during intrauterine human development.

### Introduction

The Wilms' Tumor 1 (WT1) protein is a zinc finger transcription factor encoded by the human gene WT1 which has a length of ~50 kb and consists of 10 exons.<sup>1</sup> WT1 shares a high degree of structural homology with the early growth response (EGR) transcription factor family<sup>2,3</sup> and it is involved in the onset of Wilms' tumor, the most common primary renal tumor in childhood.<sup>4</sup> The combination of alter-

native splicing of mRNA<sup>5</sup> and RNA editing<sup>6</sup> can generate more than 20 different gene products. The corresponding proteins, which are designated as WT1 (-KTS) and WT1 (+KTS) respectively, differ in the selective domain for DNA binding. Isoforms WT1 (-KTS) are potent transcriptional activators and preferentially bind to DNA, whereas the isoforms WT1 (+KTS) have a role in RNA binding.<sup>7</sup> The WT1 protein regulates the transcription of numerous genes and functions both as activator and as transcriptional co-activator or as repressor of gene expression.<sup>8</sup> Thanks to the ability of the WT1 to bind RNA, it can also be detected in the cytoplasm of many cells. A significant percentage of cytoplasmic WT1 is observed in association with the ribonucleoprotein particles (RNPs), strengthening the hypothesis of its involvement in RNA metabolism.<sup>9</sup> In addition, WT1 is associated with polysomes active in the translation, opening the possibility of its involvement in the regulation of translation.<sup>10</sup>

Due to its role as a transcription factor, WT1 recognizes genes promoters containing a DNA sequence rich in GC, including c-myc,<sup>11</sup> EGR1,<sup>12</sup> WT1<sup>13</sup> and PAX2.<sup>14</sup> Recently, it has been shown that even the genes promoters for nephrin,<sup>15</sup> nestin,<sup>16</sup> trkB,<sup>17</sup> SF1,<sup>18</sup> amphiregulin,<sup>17</sup> Bcl2,<sup>11</sup> vitamin D receptor,<sup>20,21</sup> podoxalysin<sup>22</sup> may represent physiological targets of WT1. WT1 is involved in development of several tissues, including early developing kidney,<sup>23,24</sup> in which it plays a role in both the induction of angioblasts and the regulation of angiogenesis. WT1 has been one of the first tumor suppressor genes identified for playing a role in kidney development<sup>25</sup> and it is considered a master control gene that regulates the expression of a large number of genes that play a critical role in early phases of nephrogenesis. Embryogenesis of the kidneys is induced by a reciprocal interaction of the metanephric mesenchyme and the invading ureteric bud.<sup>26</sup> WT1 is upregulated since the mesenchyme begins to condensate around the ureteric bud tips. In the absence of WT1, the mesenchyme becomes apoptotic and invasion of the ureteric bud does not occur, suggesting that WT1 acts as a survival factor for embryonic kidney progenitor cells. During the later stages of renal development, WT1 may inhibit mesenchymal cell proliferation, thereby allowing the formation of S-shaped bodies, which will elongate and eventually connect to the branching collecting duct tree, giving rise to the mature nephrons.<sup>27</sup>

WT1 protein was initially shown as a marker of stem cells in the mouse embryo.<sup>28</sup> Recently, a study from our group showed that WT1 is strongly expressed in the stem/progenitor cells of the human fetal kidney.<sup>29</sup> Evidences showing the expression of WT1 protein during normal kidney development at different stages of

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Key words: Wilms' tumor 1, WT1, human fetus, immunohistochemistry, human development, fetal stem cells.

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nephrogenesis suggest a possible role for WT1 in mesenchymal-epithelial transition (MET), as well as in the development and maturation of podocytes.<sup>29</sup> Recent studies have shown that WNT signaling pathway is a major target of WT1.<sup>30</sup> WT1 probably negatively regulates WNT/ $\beta$ -catenin signaling during kidney development.<sup>31</sup> Moreover, WT1 plays a complex role in the regulation of cell proliferation and apoptosis.<sup>32</sup> Recently, studies based on silencing WT1 by RNA interference have demonstrated the critical role played by WT1 in these processes that regulate the fetal development as well as tumor development.<sup>33</sup> A large number of studies on the expression of WT1 protein in different tissues and cell types during embryogenesis were performed in mice models. In these experiments, the expression of WT1 was restricted to specific cell types, most of which undergo MET during organogenesis.<sup>34</sup> Recently, it has been suggested a potential role of WT1 protein in the development of the nervous tissues in mouse, including retina and retinal ganglia<sup>35</sup> and of the olfactory system.<sup>36</sup>

Moreover, a strong WT1 cytoplasmic immunostaining in developing skeletal and cardiac muscle cells, in endothelial cells, in the sympathetic system and in the gastroenteric nervous system has been reported in human fetuses.<sup>37-38</sup> The purpose of this study was to analyze the WT1 immunoreactivity in different tissues and organs in human fetuses during early phases of development, and to assess its possible role in human ontogeny.

## Materials and Methods

The expression of WT1 was evaluated in several tissues from four human fetuses that we received from the Obstetric Division of the University of Cagliari, as voluntary termination of pregnancy (VTOP). All the fetuses included in this study had no congenital malformation. All procedures performed were approved by the Ethics Human Studies Committee of University Medical Centre of Cagliari (according to the instructions of the Declaration of Helsinki).

The fetuses, aged from 7<sup>th</sup> to 12<sup>th</sup> week of gestation (Table 1), have been completely sampled and histologically and immunohistochemically studied. Samples were fixed in 10% buffered formalin, routinely processed, and paraffin-embedded. Two serial 3 µm-thick sections were obtained from each paraffin block; after dewaxing and rehydrating, one of these was stained with hematoxylin-eosin, the other pre-treated for immunohistochemical analysis, with 10 min heat-induced epitope retrieval in buffer pH 9.00 (EnVision™ FLEX Target Retrieval Solution High pH, Code: K8004; Dako Denmark A/S, Glostrup, Denmark). Slides were then incubated for 20 min at room temperature with anti Wilms' Tumor (WT1) (Code: M3561; Dako North America, Carpinteria, CA, USA) mouse monoclonal antibody clone 6F-H2 at 1:100 dilution. Staining procedures were performed by Envision™ FLEX+ (Code: K8002; Dako Denmark A/S) Detection System and AutostainerLink 48 instrument following dealer's instructions. The data were obtained by evaluation of positivity (+) and negativity (-) for WT1 immunoreactivity in the various tissues and organs (Table 1).

## Results

Placental WT1 cytoplasmic immunoreactivity was restricted to two main cell types: muscular cells in the arterial wall and star-shaped cells in close relationship with the cytotrophoblast.

No significant reactivity for WT1 was detect-

**Table 1. Clinical and immunohistochemical data.**

	Case 1	Case 2	Case 3	Case 4
Gender	mv	M	F	M
Gestational age (weeks)	7	9	11	12
Body weight (g)	mv	3	12	14
<b>Placenta</b>				
Cito-trophoblast	-	mv	-	-
Sincitio-trophoblast	-	mv	-	-
Endothelial cells	+	mv	+	+
Interstitial cells	+	mv	+	+
Corion of villi	+	mv	+	+
<b>Gut</b>				
<i>Lamina propria</i>	mv	+	+	+
Enterocytes	-	-	-	-
Submucosa	-	+	+	+
Myoenteric plexus	+	+	+	+
<i>Tonaca muscularis propria</i>	+	-	-	-
<b>Kidney</b>				
Glomerular Podocyte	+	+	+	+
Proximal and distal tubules	-	-	-	-
Cap mesenchyme cells	mv	+	+	+
Metanephric mesenchyme	mv	+	+	+
Collecting tubules	mv	-	-	-
Capsular parietal epithelium	mv	+	+	+
<b>Adrenal glands</b>				
Fetal zone	mv	+	+	+
Definitive zone	mv	-	-	-
Capsule	mv	+	+	+
<b>Lung</b>				
Mesenchymal cells	mv	+	+	+
Ephitelial cells	mv	-	-	-
Pleura	mv	+	+	+
<b>Heart</b>				
Atrial cardiomyocytes	+	+	+	+
Ventricular cardiomyocytes	+	+	+	+
Endocardium	-	-	-	-
Epicardium	+	+	-	-
<b>Liver</b>				
Hepatocyte	-	-	-	-
Kupffer cells	mv	-	-	-
Hematopoietic cells	-	-	-	-
Ductal cells	-	-	+	+
Sinusoidal cells	+	+	+	+
<b>Central nervous system</b>				
Cerebral cortex	mv	+	+	+
Spinal cord	mv	+	+	+
Neural progenitor cells	+	+	+	+
<b>Skeletal muscle</b>				
Skeletal muscle	+	+	+	+
<b>Smooth muscle</b>				
Smooth muscle	-	-	-	-
<b>Cartilage</b>				
Cartilage	mv	-	-	-
<b>Skin</b>				
Epidermis	mv	-	-	-
Derma	mv	+	+	+
<b>Gonads</b>				
Testis	mv	+	mv	-
Ovary	mv	-	-	-

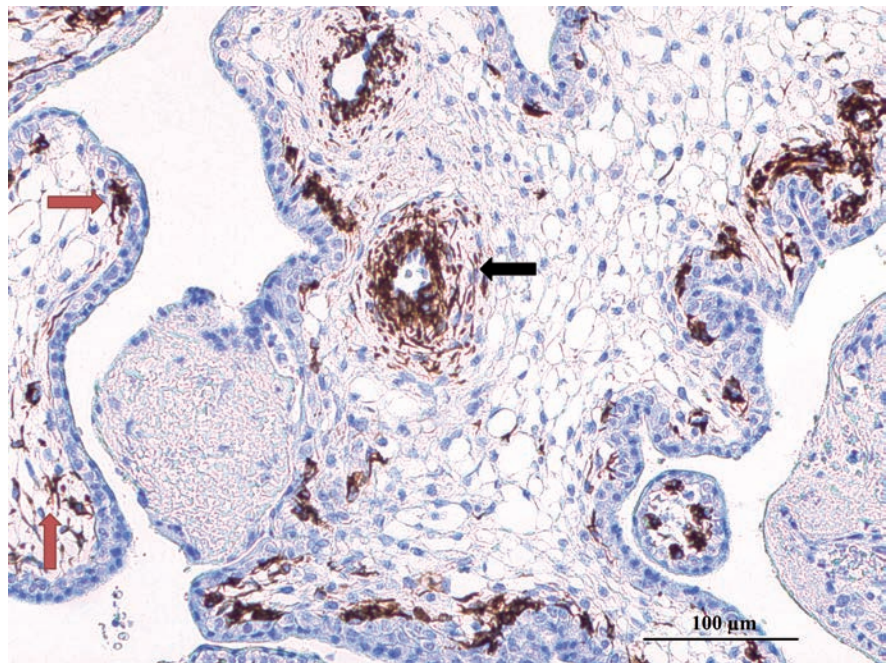
mv, missing value; +, positivity; -, negativity.



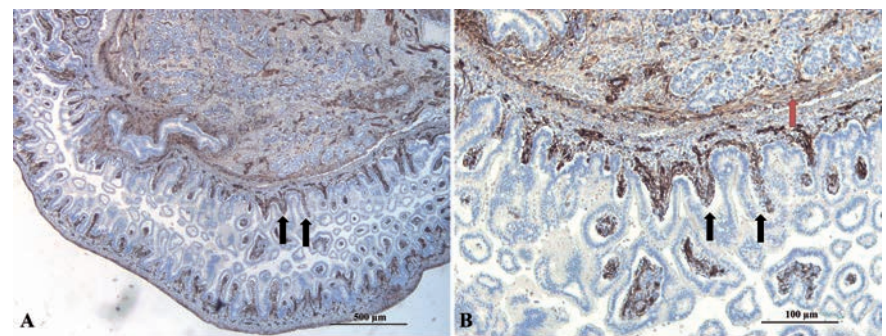
ed in both cyto- and syncytiotrophoblast (Figure 1). In the fetal gut, immunostaining for WT1 was detected in the cytoplasm of lamina propria of the mucosa, being particularly strong along the fibrovascular axis of villi, in the absence of any significant reactivity in the enterocytes. A cytoplasmic expression of WT1 were also observed in the submucosa and in the myoenteric plexus. No immunoreactivity for WT1 was found in the smooth muscle cells of the tonaca muscularis propria (Figure 2 A,B). In the developing fetal kidney, reactivity for WT1 was observed in different cell types and in different renal compartments. At panoramic view, immunostaining for WT1 was mainly located in the developing glomeruli, in the nephrogenic zone located under the renal capsule and in interstitial cells (Figure 3A). At higher magnification, proximal, distal and collecting tubules were completely negative, as well as the immature mesenchymal cells merged in a loose connective tissue located in the hilar regions. Staining for WT1 was detected in the cytoplasm of the interstitial cells surrounding tubular structures (Figure 3B). Inside glomeruli, WT1 was expressed in the cytoplasm of podocyte precursors and along the developing basal membranes. Reactivity for WT1 was also observed inside the nuclei of podocyte precursors, contrasting with the absence of immunostaining in the mesangial. No significant reactivity was detected in the parietal epithelial cells (Figure 3C). The study of the renal capsule evidenced a peculiar pattern regarding WT1 immunoreactivity. We found a marked variability among different capsular cells, WT1 being expressed only by scattered cells. Immunostaining was found in the cytoplasm of the cells of the nephrogenic zone, both in non-induced metanephric cells and in the Cap mesenchymal cells surrounding the tips of the branching ureteric bud. In the S-shaped bodies, WT1 preferentially immunostained the cytoplasm of podocyte progenitors. Reactivity for WT1 was also observed in spindle cells located in the renal interstitium (Figure 3D). In the fetal adrenal glands, cytoplasmic immunoreactivity for WT1 was mainly located in the cytoplasm of cells of the fetal zone, contrasting with the absence of reactivity in the definitive zone located under the adrenal capsule. A pattern similar to that observed in the renal capsule was also found in the cytoplasm of adrenal capsule cells, with some strongly reactive capsular cells intermingled with non-reactive cells. A strong cytoplasmic immunostaining was also found in the wall of the adrenal vessels (Figure 4). In the developing lung, cytoplasmic immunoreactivity for WT1 was restricted to mesenchymal cells and in the pleura, while the epithelial cells lining the branching tubules did not show any immunostaining. In particular, WT1 was

expressed in the developing vessels, in close proximity to the tubular structures. In the pulmonary arteries, a strong cytoplasmic reactivity for WT1 was found around the arterial lumen (Figure 5). In the fetal heart, immunoreactivity for WT1 was characterized by striking differences among different cardiac compartments (Figure 6). The highest levels of cytoplasmic immunostaining for WT1 were observed in the muscle cells of the atria. A weaker immunoreactivity was detected in the cytoplasm of cardiomyocytes of both ventricles. No significant staining was observed in the wall of the large arteries emerging from the

heart (Figure 6). In the developing human liver, WT1 was not expressed in the hepatocytes, in Kupffer cells nor in hematopoietic cells. A mild reactivity for WT1 was observed in the cytoplasm of mesenchymal cells of the developing portal tracts. WT1 was also expressed in the cytoplasm of cells of developing biliary structures, including remnants of the ductal plate (Figure 7). In the fetal brain, WT1 was expressed in the cerebral cortex (Figure 8A). Reactivity for WT1 were detected in the cytoplasm of Radial Glia cells involved in guiding the radial migration of developing neuronal precursor from the ventricular zone



**Figure 1.** WT1 immunostaining in placenta. WT1 immunoreactivity was restricted to cytoplasm of muscular cells in the arterial wall (black arrow) and star-shaped cells in close relationship with the cytotrophoblast (red arrows).



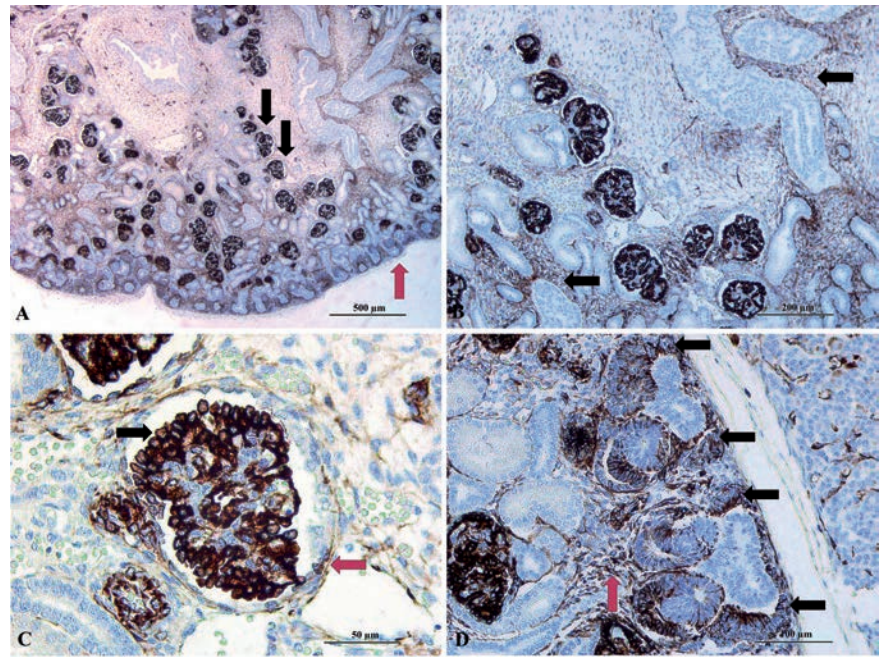
**Figure 2.** WT1 immunostaining in fetal gut. WT1 was detected A) in the cytoplasm of lamina propria of the mucosa and in the fibrovascular axis of villi (black arrows); B) in the submucosa (red arrow).



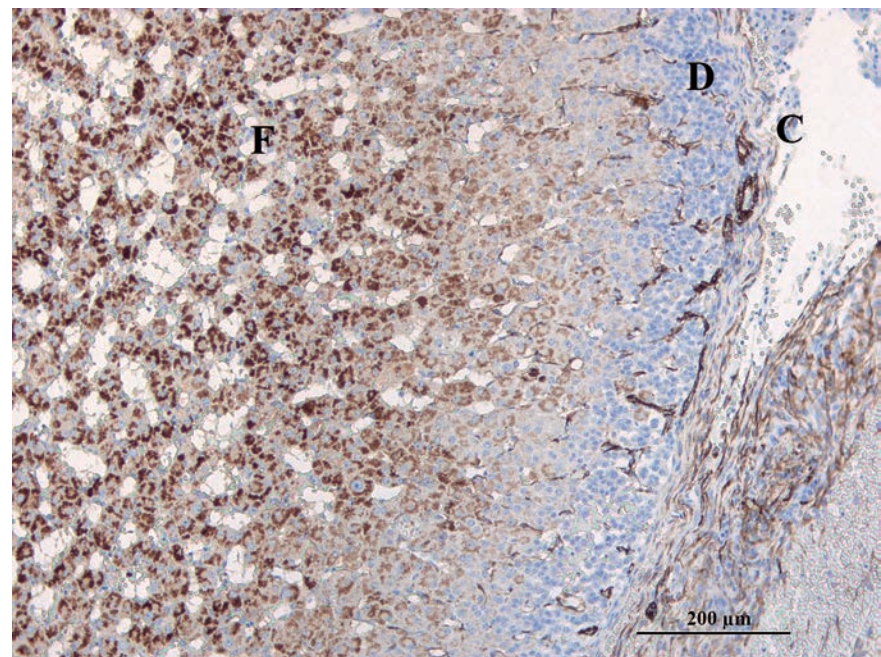
toward the marginal zone. Immunostaining for WT1 was also detected in afferent axonal fibers extending from the thalamus toward cerebral cortex, establishing synaptic contacts between neuronal precursor of the subplate zone. WT1 has been observed to be expressed in the cytoplasm of developing Radial Glia cells in the Spinal cord (Figure 8B). Cytoplasmic immunoreactivity for WT1 were observed in developing skeletal muscles (Figure 9A). The developing skin was characterized by a contrast between the absence of immunostaining in the epidermis and the cytoplasmic positivity observed in the progenitor cells of the developing derma (Figure 9B). A cytoplasmic immunostaining for WT1 was also detected in endothelial cells of blood vessels. No reactivity was found in superficial (epidermis, GI tract, endometrium, renal tubules, urothelium) and glandular (salivary glands, thyroid, pancreas, ovary) epithelia; biliary cells were the unique epithelial cells expressing WT1 in this study. No immunoreactivity for WT1 was detected in thymus, smooth muscle tissue, crystalline lens, cartilage and blood cells.

## Discussion

In recent years, many studies carried out on experimental animal models have shown that WT1 protein plays a key role in embryonic development. WT1 has been involved in the development of the urogenital system, particularly during early kidney development.<sup>25</sup> Other studies on mice models have shown that WT1 is involved in the development of spleen<sup>39</sup> and adrenal glands.<sup>40</sup> Studies on knock-out mice showed that WT1 is required for heart development<sup>27</sup> and for the development of the central nervous system, retina<sup>35</sup> and the olfactory system.<sup>36</sup> Few studies performed on human embryonic and fetal tissues showed that WT1 is involved in the development of human kidney<sup>23</sup> and female and male gonads.<sup>41</sup> WT1 expression was detected in nuclei of some fetal tissues including metanephros and mesonephros, spleen, gonads, peritoneal mesothelium,<sup>42</sup> skeletal muscle, smooth muscle of urinary bladder, ureter and arteries.<sup>43</sup> Previous immunohistochemical studies utilizing antibodies against C-terminal of WT1 protein showed a predominant nuclear localization of this transcription factor in several developing organs.<sup>34,41-43</sup> As compared to these previous studies, our work was based on the use of anti-WT1 monoclonal antibody specific for the N-terminal portion of this protein (clone 6F-H2). This new available antibody is able to detect both cytoplasmic and nuclear reactivity.<sup>35-38</sup> These localizations reflect the important role of WT1 as a nuclear transcription factor and its



**Figure 3.** WT1 immunostaining in fetal kidney. WT1 immunoreactivity was detected A) in the developing glomeruli (black arrows) and in the nephrogenic zone located under the renal capsule (red arrow); B) in the cytoplasm of interstitial cells surrounding tubular structures (black arrow); C) in the cytoplasm and nuclei of podocyte precursors (black arrows) and along the developing basal membranes inside glomeruli (red arrow); and D) in the cytoplasm of Cap mesenchymal cells (black arrows) and in spindle cells located in the renal interstitium (red arrow).



**Figure 4.** WT1 immunostaining in fetal adrenal gland. WT1 immunoreactivity was located in the cytoplasm of cells of the fetal zone and in the adrenal capsule, with some strongly reactive capsular cells intermingled with non-reactive cells (F, fetal zone; D, definitive zone; C, adrenal capsule).

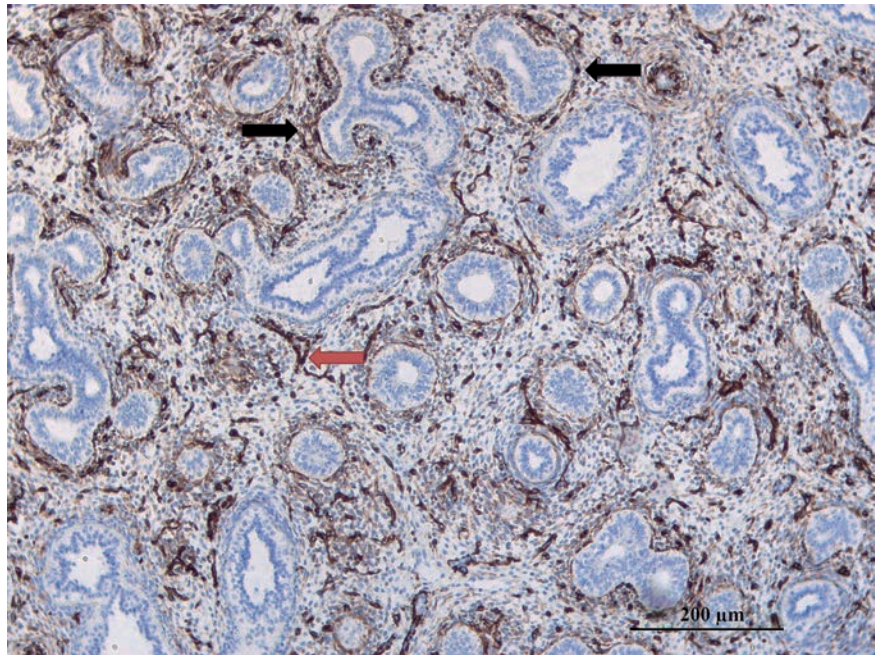


involvement in RNA metabolism and in the regulation of translation. Our study adds new data regarding a major role of WT1 in developing human organs, tissues and cell types. By an immunohistochemical approach, we demonstrated that in the developing embryo and fetus, WT1 is expressed in various human organs, in which WT1 immunoreactivity was not reported by previous studies. In the fetal lung, WT1 was expressed in the pleura and in mesenchymal progenitors surrounding branching epithelial structures (Figure 5). Moreover, WT1 immunostaining was detected in small vessels inside the pulmonary scarcely differentiated mesenchyme. Recent studies conducted on transgenic mouse models have shown an involvement of WT1 in the development of this organ, suggesting a major role for WT1 in pleura and lung development.<sup>44,45</sup> At the best of our knowledge, our data represent the first report of WT1 involvement in human lung morphogenesis, and confirm the relevance of its expression even in human lung development. The high immunoreactivity detected in developing small vessels surrounding the epithelial structures may indicate a role for WT1 in the formation of the peri-alveolar capillaries.

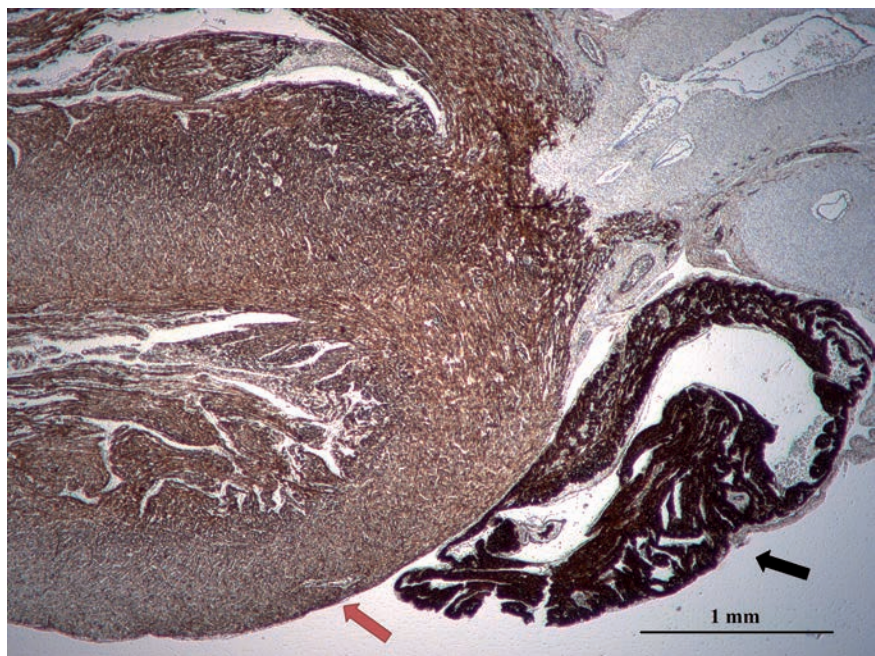
WT1 immunoreactivity in the liver was restricted to portal tracts, while hepatocytes and hematopoietic cells appeared non-immunoreactive for WT1 protein. Inside portal tracts, only specific cell types expressed WT1, including stromal cells, muscular cells of the portal vein and arterial wall, bile ductular cells and developing ductal cells of the ductal plate (Figure 7). Inside the liver acinus, a focal immunoreactivity for WT1 was detected in the cytoplasm of isolated sinusoidal cells. These findings lay stress on a possible relevant role for WT1 in the development of the biliary tree. The strong expression of WT1 protein in the cells of the ductal plate as well as in the cells of the developing biliary structures observed at the periphery of immature portal tracts suggests a role for WT1 at least in the early phases of the development of the human biliary tree. On the basis of these preliminary data, further studies are needed in order to better analyze WT1 expression and its role in the more advanced stages of maturation of the intrahepatic and extrahepatic biliary tree, as well as during the progression of vanishing bile duct diseases in childhood. Regarding WT1 expression in the developing kidney, our data confirm previous studies on a major role played by WT1 during kidney development.<sup>25,39</sup> In all fetal kidneys immunostained, WT1 immunoreactivity is extended from the nephrogenic zone to the capsule, the residual metanephric mesenchyme and to the vast majority of glomeruli (Figure 3). Regarding the renal cell types expressing WT1, the protein was mainly detect-

ed in the cytoplasm of immature stem/precursor cells, including a subset of intracapsular cells and the majority of metanephric mesenchymal cells detectable at the renal hilum. Inside the nephrogenic zone, WT1 was preferentially expressed by cap mesenchymal cells

aggregating around the tips of the branching ureteric bud. These data confirm previous reports from our group on a preferential WT1 expression in stem/progenitors of the developing human kidney and, in particular, in stem cells undergoing mesenchymal-epithelial tran-



**Figure 5.** WT1 immunostaining in fetal lung. WT1 was expressed in cytoplasm of mesenchymal progenitors surrounding branching epithelial structures (black arrows) and in small vessels inside the pulmonary scarcely differentiated mesenchyme (red arrow).



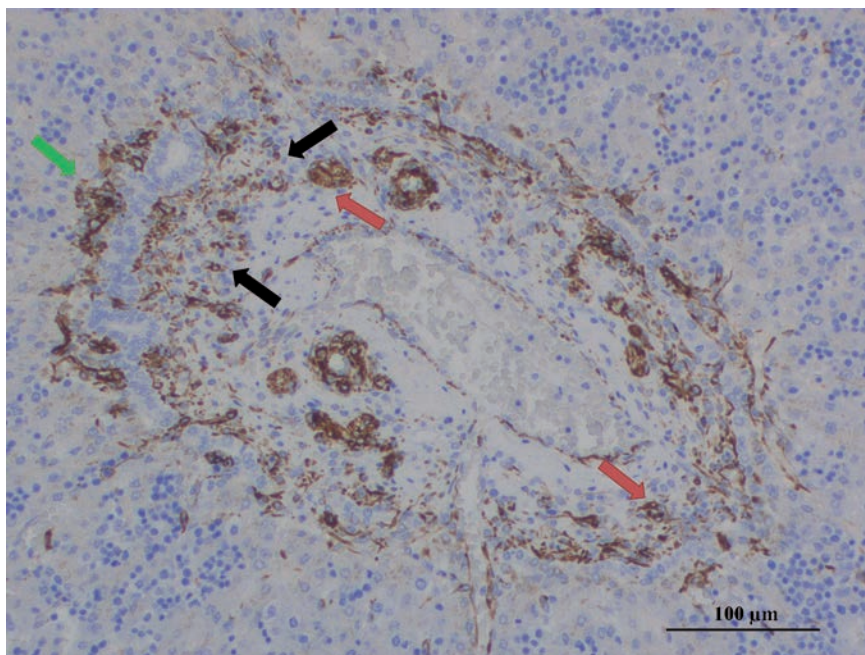
**Figure 6.** WT1 immunostaining in fetal heart. Atrial cardiomyocytes (black arrow) showed higher cytoplasmic levels of WT1 immunostaining as compared to those observed in both ventricles (red arrow).



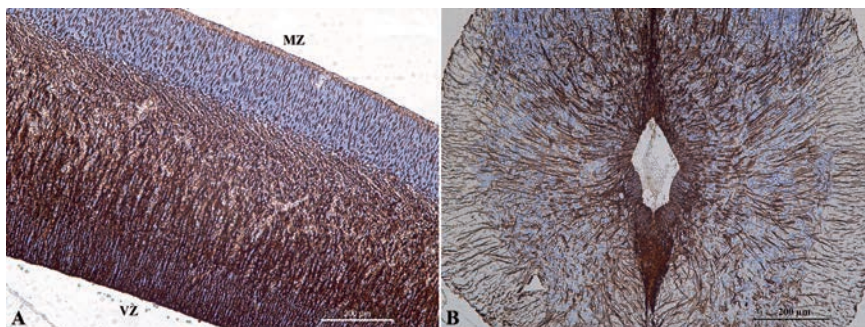
sition such as the Cap mesenchymal cells.<sup>29</sup> WT1 was also detectable in a specific differentiated glomerular cell: the podocyte. This finding suggests a role for WT1 not restricted to the early phases of kidney development, but also in the differentiation process of podocytes, as previously reported.<sup>29</sup> Further studies are mandatory in order to better analyze the role of WT1 in podocyte development in the different phases of kidney development, as well as during the insurgence of podocytopathies in childhood and in adult subjects.

A new finding emerging from our study is represented by the detection of a reactivity for WT1 in the human fetal adrenal glands (Figure 4). Previous studies had reported the involvement of WT1 in the development of adrenal glands in mice, without defining the cell type involved in WT1 expression. Here we describe, at the best of our knowledge for the first time, a preferential WT1 expression in a single cell type of the developing human adrenal glands, the cells of the fetal zone, in the absence of any significant reactivity in the cells of the definitive zone (Figure 4). The meaning of this expression in fetal zone cells is not clear. The association of WT1 expression in a subset of cells inside the adrenal capsule, putatively representing adrenal stem/progenitor cells, leads to hypothesize that WT1 might be mainly involved in the early phases of adrenal development, its role decreasing in more differentiated adrenal cells, including cells of the definitive zone.

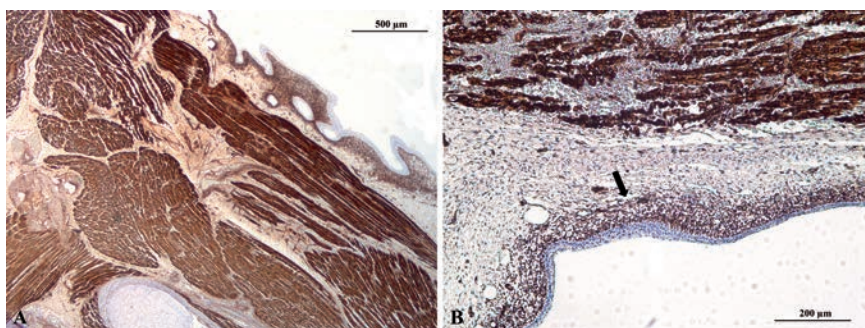
Interesting data emerge, in this study, from the analysis of WT1 expression in the developing fetal heart. The involvement of WT1 in heart development had been previously reported in mice.<sup>27</sup> In humans, a single study showed a strong WT1 cytoplasmic immunostaining in developing cardiac muscle cells.<sup>37</sup> In this study, we confirm the high levels of WT1 protein expression in cardiac cells in the fetal hearts studied. Regarding the cell type involved in WT1 expression, cardiomyocytes appeared as the site of the WT1 immunostaining. Moreover, differences were found regarding the level of WT1 expression in the different compartments of the developing heart. Atrial cardiomyocytes showed higher levels of immunostaining as compared to those observed in both ventricles (Figure 6). The immunostaining of atria and ventricles contrasted with the absence of reactivity in the large vessels emerging from the heart. Moreover, we detected WT1 immunostaining in epicardial cells, whereas no reactivity for the protein was found in endocardial cells. These data taken together indicate a complex pattern of WT1 expression in the developing human heart, with decreasing levels of WT1 expression from atrial cardiomyocytes to ventricle cardiomyocytes to the epicardium, end-



**Figure 7.** WT1 immunostaining in fetal liver. WT1 cytoplasmic immunoreactivity was observed in the of mesenchymal cells of the developing portal tracts (black arrows) and developing biliary structures (red arrows), including remnants of the ductal plate (green arrow).



**Figure 8.** WT1 immunostaining in fetal central nervous system. WT1 was detected A) in the cytoplasm of Radial glia and in the axons localized in the cerebral cortex (VZ, ventricular zone; MZ, marginal zone) and B) in the cytoplasm of Radial Glia of the spinal cord.



**Figure 9.** WT1 immunostaining in fetal arts. WT1 cytoplasmic immunoreactivity were observed A) in developing skeletal muscles and B) in the progenitor cells of the developing derma (black arrow).

ing with the absence of the expression of endocardium and large vessel cells. This spectrum of WT1 expression in the developing human heart deserves further studies in order to better specify the role of WT1 in the different cardiac cell types during the different phases of heart development.

A recent study on WT1 expression in the human developing gut showed WT1 expression to be restricted to the sympathetic neuroblasts of the fetal gastro-enteric nervous system, progressively decreasing with differentiation of these cells along both ganglionic and chromaffin cell lineage.<sup>38</sup> In our study, WT1 expression was found in the myoenteric plexus of the fetal gut confirming the finding of Parenti et al. Moreover, we detected high levels of WT1 in the lamina propria of the fetal gut and particularly in the fibrovascular axis of the intestinal villi (Figure 2). WT1 expression in muscularis propria was observed in one case, the 7-week-old fetus, suggesting a role for WT1 restricted to the very early phases of gut muscular cells development. The involvement of WT1 in central nervous system development had been previously reported in the human fetus.<sup>38</sup> Our study confirm a possible relevant role for WT1 in the development of the human central nervous system. The reactivity of WT1 was detected in radial glia cells in the cerebral cortex and in the spinal cord (Figure 8). The finding of reactivity for WT1 in radial Glia cells, the primary progenitors capable of generating neurons, astrocytes and oligodendrocytes<sup>46</sup> suggest the involvement of WT1 in the early phases of development of multiple cell lineages in CNS. Since neuronal precursor use radial Glia as scaffold in order to reach their final destination,<sup>47</sup> our data indicate that WT1 plays a role not only in differentiation but even in cell migration. In this study, WT1 immunostaining were also detected in developing skeletal muscle cells (Figure 9A), confirming previous reports.<sup>37</sup> This finding suggest a major role for WT1 in the development of human skeletal muscle, and deserves further studies on WT1 expression in these cells during the more advanced stages of the intrauterine development.

In conclusion, our study clearly indicates that WT1 is involved in development of a large number of human organs, tissues and cell types, suggesting a critical role in the early phases of morphogenesis of the majority of human organs. Further studies are needed in order to better analyze the immunohistochemical and the mRNA expression pattern of WT1 in the different phases of human development, in order to better understand the role of WT1 in the differentiation of human cell types in the perinatal period and the post-natal life.

## References

- Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. *Cell* 1990;60:509-520.
- Rauscher FJ 3rd, Morris JF, Tournay OE, Cook DM, Curran T. Binding of the Wilms' tumor locus zinc finger protein to the EGR-1 consensus sequence. *Science* 1990;250:1259-62.
- Hamilton TB, Barilla KC, Romaniuk PJ. High affinity binding sites for the Wilms' tumour suppressor protein WT1. *Nucleic Acids Res* 1995;23:277-84.
- Hastie ND. The genetics of Wilms' tumor - a case of disrupted development. *Annu Rev Genet* 1994;28:523-558.
- Haber DA, Sohn RL, Buckler AJ, Pelletier J, Call KM, Housman DE. Alternative splicing and genomic structure of the Wilms tumor gene WT1. *Proc Natl Acad Sci USA* 1991;88:9618-22.
- Sharma PM, Bowman M, Madden SL, Rauscher FJ 3rd, Sukumar S. RNA editing in the Wilms' tumor susceptibility gene, WT1. *Genes Dev* 1994;8:720-31.
- Hammes A, Guo JK, Lutsch G, Leheste JR, Landrock D, Ziegler U, et al. Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. *Cell* 2001;106:319-29.
- Lee SB, Haber DA. Wilms tumor and the WT1 gene. *Exp Cell Res* 2001;264:74-99.
- Hohenstein P, Hastie ND. The many facets of the Wilms' tumour gene, WT1. *Hum Mol Genet* 2006;15 Spec No 2:R196-201.
- Niksic M, Slight J, Sanford JR, Caceres JF, Hastie ND. The Wilms' tumour protein (WT1) shuttles between nucleus and cytoplasm and is present in functional polysomes. *Hum Mol Genet* 2004;13:463-71.
- Hewitt SM, Hamada S, McDonnell TJ, Rauscher FJ 3rd, Saunders GF. Regulation of the proto-oncogenes bcl-2 and c-myc by the Wilms' tumor suppressor gene WT1. *Cancer Res* 1995;55:5386-9.
- Madden SL, Cook DM, Morris JF, Gashler A, Sukhatme VP, Rauscher FJ 3rd. Transcriptional repression mediated by the WT1 Wilms tumor gene product. *Science* 1991;253:1550-3.
- Rupprecht HD, Drummond IA, Madden SL, Rauscher FJ 3rd, Sukhatme VP. The Wilms' tumor suppressor gene WT1 is negatively auto-regulated. *J Biol Chem* 1994;269:6198-206.
- Ryan G, Steele-Perkins V, Morris JF, Rauscher FJ 3rd, Dressler GR. Repression of Pax-2 by WT1 during normal kidney development. *Development* 1995;121:867-75.
- Wagner N, Wagner KD, Xing Y, Scholz H, Schedl A. The major podocyte protein nephrin is transcriptionally activated by the Wilms' tumor suppressor WT1. *J Am Soc Nephrol* 2004;15:3044-51.
- Wagner N, Wagner KD, Scholz H, Kirschner KM, Schedl A. Intermediate filament protein nestin is expressed in developing kidney and heart and might be regulated by the Wilms' tumor suppressor Wt1. *Am J Physiol Regul Integr Comp Physiol* 2006;291:R779-87.
- Wagner N, Wagner KD, Theres H, Englert C, Schedl A, Scholz H. Coronary vessel development requires activation of the TrkB neurotrophin receptor by the Wilms' tumor transcription factor Wt1. *Genes Dev* 2005;19:2631-42.
- Wilhelm D, Englert C. The Wilms tumor suppressor WT1 regulates early gonad development by activation of Sfl. *Genes Dev* 2002;16:1839-51.
- Lee SB, Huang K, Palmer R, Truong VB, Herzlinger D, Kolquist KA, et al. The Wilms tumor suppressor WT1 encodes a transcriptional activator of amphiregulin. *Cell* 1999;98:663-73.
- Maurer U, Jehan F, Englert C, Hubinger G, Weidmann E, DeLuca HF et al. The Wilms' tumor gene product (WT1) modulates the response to 1,25-dihydroxyvitamin D3 by induction of the vitamin D receptor. *J Biol Chem* 2001;276:3727-32.
- Wagner KD, Wagner N, Sukhatme VP, Scholz H: Activation of vitamin D receptor by the Wilms' tumor gene product mediates apoptosis of renal cells. *J Am Soc Nephrol* 2001;12:1188-96.
- Palmer RE, Kotsianti A, Cadman B, Boyd T, Gerald W, Haber DA. WT1 regulates the expression of the major glomerular podocyte membrane protein Podocalyxin. *Curr Biol* 2001;11:1805-9.
- Pritchard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, et al. The candidate Wilms' tumour gene is involved in genitourinary development. *Nature* 1990;346:194-7.
- Gao X, Chen X, Taglienti M, Rumballe B, Little MH, Kreidberg JA. Angioblast-mesenchyme induction of early kidney development is mediated by Wt1 and Vegfa. *Development* 2005;132:5437-49.
- Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, et al. WT-1 is required for early kidney development. *Cell* 1993;74:679-91.
- Faa G, Gerosa C, Fanni D, Monga G,



- Zaffanello M, Van Eyken P, et al. Morphogenesis and molecular mechanisms involved in human kidney development. *J Cell Physiol* 2012;227:1257-68.
27. Scholz H, Kirschner KM. A role for the Wilms' tumor protein WT1 in organ development. *Physiology (Bethesda)* 2005;20:54-9.
28. Kreidberg JA. WT1 and kidney progenitor cells. *Organogenesis* 2010;6:61-70.
29. Fanni D, Fanos V, Monga G, Gerosa C, Locci A, Nemolato S, et al. Expression of WT1 during normal human kidney development. *J Matern Fetal Neonatal Med* 2011;24(Suppl.2):44-7.
30. Kim MK, McGarry TJ, O Broin P, Flatow JM, Golden AA, Licht JD. An integrated genome screen identifies the Wnt signaling pathway as a major target of WT1. *Proc Natl Acad Sci USA* 2009;106:11154-9.
31. Kim MS, Yoon SK, Bollig F, Kitagaki J, Hur W, Whye NJ, et al. A novel Wilms tumor 1 (WT1) target gene negatively regulates the WNT signaling pathway. *J Biol Chem* 2010;285:14585-93.
32. Hartkamp J, Roberts SG. The role of the Wilms' tumour-suppressor protein WT1 in apoptosis. *Biochem Soc Trans* 2008;36(Pt 4):629-31.
33. Parenti R, Cardile V, Graziano AC, Parenti C, Venuti A, Bertuccio MP et al. Wilms' tumor gene 1 (WT1) silencing inhibits proliferation of malignant peripheral nerve sheath tumor sNF96.2 cell line. *PLoS One* 2014;9:e114333.
34. Armstrong JF, Pritchard-Jones K, Bickmore WA et al. The expression of the Wilms' tumour gene, WT1, in the developing mammalian embryo. *Mech Dev* 1993;40:85-97.
35. Wagner KD, Wagner N, Vidal VP, Schley G, Wilhelm D, Schedl A, et al. The Wilms' tumor gene Wt1 is required for normal development of the retina. *EMBO J* 2002;21:1398-405.
36. Wagner N, Wagner KD, Hammes A, Kirschner KM, Vidal VP, Schedl A, et al. A splice variant of the Wilms' tumour suppressor WT1 is required for normal development of the olfactory system. *Development* 2005;132:1327-36.
37. Parenti R, Perris R, Vecchio GM, Salvatorelli L, Torrisi A, Gravina L, et al. Immunohistochemical expression of Wilms' tumor protein (WT1) in developing human epithelial and mesenchymal tissues. *Acta Histochem* 2013;115:70-5.
38. Parenti R, Puzzo L, Vecchio GM, Gravina L, Salvatorelli L, Musumeci G, et al. Immunolocalization of Wilms' Tumor protein (WT1) in developing human peripheral sympathetic and gastroenteric nervous system. *Acta Histochem* 2014;116:48-54.
39. Herzer U, Crocoll A, Barton D, Howells N, Englert C. The Wilms tumor suppressor gene wt1 is required for development of the spleen. *Curr Biol* 1999;9:837-40.
40. Moore AW, McInnes L, Kreidberg J, Hastie ND, Schedl A. YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis. *Development* 1999;126:1845-57.
41. Mundlos S, Pelletier J, Darveau A, Bachmann M, Winterpacht A, Zabel B. Nuclear localization of the protein encoded by the Wilms' tumor gene WT1 in embryonic and adult tissues. *Development* 1993;119:1329-41.
42. Charles AK, Moore IE, Berry PJ. Immunohistochemical detection of the Wilms' tumour gene WT1 in desmoplastic small round cell tumour. *Histopathology* 1997;30:312-4.
43. Ramani P, Cowell JK. The expression pattern of Wilms' tumour gene (WT1) product in normal tissues and paediatric renal tumours. *J Pathol* 1996;179:162-8.
44. Cano E, Carmona R, Muñoz-Chápuli R. Wt1-expressing progenitors contribute to multiple tissues in the developing lung. *Am J Physiol Lung Cell Mol Physiol* 2013 15;305:L322-32.
45. Dixit R, Ai X, Fine A. Derivation of lung mesenchymal lineages from the fetal mesothelium requires hedgehog signaling for mesothelial cell entry. *Development* 2013;140:4398-406.
46. Campbell K, Götz M. Radial glia: multi-purpose cells for vertebrate brain development. *Trends Neurosci* 2002;25:235-8.
47. Malatesta P, Appolloni I, Calzolari F. Radial glia and neural stem cells. *Cell Tissue Res* 2008;331:165-78.