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Wnt-4 regulation by the Wilms' tumour suppressor gene, *WT1*

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The Wilms' tumour suppressor gene, *WT1*, encodes multiple nuclear protein isoforms, all containing four C-terminal zinc finger motifs. *WT1* proteins can both activate and repress putative target genes *in vitro*, although the *in vivo* relevance of these putative target genes is often unverified. *WT1* mutations can result in Wilms' tumour and the Denys-Drash Syndrome (DDS) of infantile nephropathy, XY pseudohermaphroditism and predisposition to Wilms' tumour. We have established stable transfectants of the mouse mesonephric cell line, M15, which express *WT1* harbouring a common DDS point mutation (R394W). A comparison of the expression profiles of M15 and transfectant C2A was performed using Nylon-based arrays. Very few genes showed differential expression. However *Wnt-4*, a member of the *Wnt* gene family of secreted glycoproteins, was downregulated in C2A and other similar clones. Doxycycline induction of *WT1-A* or *WT1-D* expression in HEK293 stable transfectants also elicited an elevation in *Wnt4* expression. *Wnt4* is critical for the mesenchyme-to-epithelial transition during kidney development, making it an attractive putative *WT1* target. We have mapped human *Wnt-4* gene to chromosome 1p35-36, a region of frequent LOH in WT, have characterized the genomic structure of the human *Wnt-4* gene and isolated 9 kb of immediate promoter. While several potential *WT1* binding sites exist within this promoter, reporter analysis does not strongly support the direct regulation of *Wnt4* by *WT1*. We propose that *Wnt-4* regulation by *WT1* occurs at a more distant promoter or enhancer site, or is indirect.
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Introduction

Wilms' tumour (WT) or nephroblastoma is an embryonal renal neoplasm affecting one out of 10 000 infants, thus accounting for about 6% of all childhood malignancies (Matsunaga, 1981). The tumour characteristically displays a triphasic histology consisting of undifferentiated blastemal stem cells, an epithelial component, and fibroblastic stromal elements (Miller *et al*., 1964). It is thought to arise from metanephric blastemal cells that failed to properly differentiate into the epithelial components of the kidney, possibly due to the inability of blastemal stem cells to respond to normal differentiation signals (Miller *et al*., 1964). WT can occur in association with other congenital anomalies, one of which is the WAGR syndrome of Wilms' tumour, Aniridia (lack or defect of the iris), Genitourinary anomalies, and mental Retardation. The detection of cytogenetically detectable microdeletions at chromosome position 11p13 in WAGR patients (Francke *et al*., 1979) facilitated the positional cloning of the WT suppressor gene, *WT1* (Call *et al*., 1990; Gessler *et al*., 1990). While numerous inactivating mutations have been found in this gene in WT, the percentage of WT cases with *WT1* mutations is only 10–15% (Little and Wells, 1997). WT show regions of LOH at 11p13, but also on many other chromosomes, including chromosomes 1p, 1q, 7p, 16q, and 17p (Slater and Mannens, 1992). It is possible that these loci represent other WT genes, which may lie up or downstream of *WT1*. Constitutional heterozygous *WT1* mutations are also found in the congenital anomaly of Denys-Drash syndrome (DDS) (XY pseudohermaphroditism, mesangial sclerosis, and predisposition to *WT1*) (Pelletier *et al*., 1991). The most common of these, C1180T, occurs within exon 9 of *WT1* resulting in the substitution of arginine by tryptophan (R394W) (Little and Wells, 1997).

The *WT1* gene encodes multiple nuclear proteins by virtue of three different transcription start sites, RNA editing of a single nucleotide and two alternatively spliced regions; exon 5 (17 amino acids) and an alternate splice donor site after exon 9 (Haber *et al*., 1991; Sharma *et al*., 1994; Bruening and Pelletier, 1996; Scharnhorst *et al*., 1999). The latter results in the addition of three amino acids (KTS) between exons 9 and 10. All isoforms contain the four C-terminal zinc fingers. Taking the

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