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Editorial

Mutations in the Wilms' Tumour Gene, *WT1*. What Do They Mean?

K. Pritchard-Jones

Institute of Cancer Research/Royal Marsden NHS Trust, Downs Road, Sutton, Surrey SM2 5PT, U.K.

INTRODUCTION

SINCE ITS simultaneous discovery by David Housman's and Gail Bruns' groups [1, 2] in 1990, the Wilms' tumour gene, *WT1*, has been the subject of much structural and functional investigation. The gene encodes a zinc finger protein, which can function as a transcription factor and which is subjected to alternative splicing to insert either exon 5 (splice I) or an extra three amino acids between the third and fourth zinc fingers (splice II) (Figure 1) [3]. Originally, a simple mechanism of loss of function mutations of this supposed tumour suppressor gene was expected to underlie the majority of Wilms' tumours, in line with the molecular findings in another childhood embryonal tumour, retinoblastoma. However, subsequent molecular screening of well over 500 Wilms' tumours worldwide has shown that only approximately 10–15% of tumours involve mutation of the coding sequence of *WT1* [4–9]. Investigation of children with the rare Denys–Drash syndrome (DDS, a triad of ambiguous genitalia, early onset nephropathy and high risk of Wilms' tumour) revealed a further mechanism of disrupting *WT1* function [10]. While most of these children carry a germline mis-sense mutation in the zinc finger region of the WT1 protein, a few cases have intronic mutations, which prevent the insertion of the extra three amino acids (KTS) at splice II. In this issue, Simms and colleagues [11] (pp. 2270–2276) describe a further mechanism, that of exon skipping, which may be numerically more important in disrupting *WT1* function in Wilms' tumours. They observe an almost 2-fold increase in the relative amount of *WT1* messenger RNA lacking exon 5 (splice I), which occurs independently of splicing at site II. No alterations of the exonic or adjacent intronic *WT1* sequence could be found, suggesting either that the primary mutation must lie within another protein which controls splice selection at splice I or that this is an epigenetic phenomenon. In a sample of 10 tumours, seven show this splicing defect, implying that *WT1* may be disregulated in the majority of Wilms' tumours, even though the gene itself is mutated only infrequently.

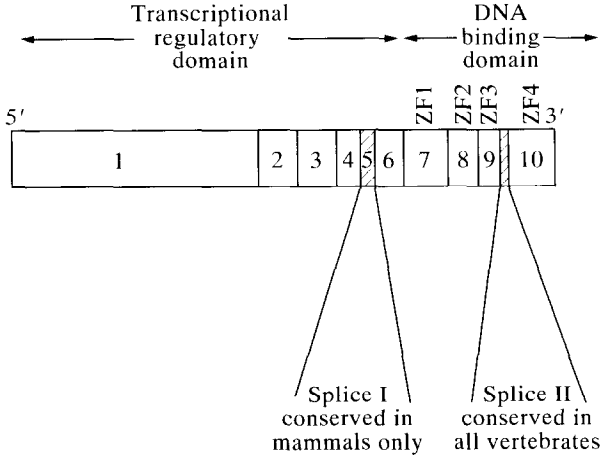

Figure 1. Structure of the *WT1* gene, showing exon structure and conservation of splice sites through vertebrate evolution. ZF, zinc finger. Data taken from Kent and associates [14].

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IS THIS A TRUE MUTATIONAL EVENT OR AN EPIGENETIC PHENOMENON?

There are several possible mechanisms which could explain the findings of Simms and colleagues. First, this could be a true mutational event occurring in a gene which specifically controls *WT1* splicing. Second, this could result from the clonal expansion of a cell type with a predetermined splicing pattern which simply reflects its stage of differentiation at the time of the separate tumorigenic event. Third, some genes show variation in splicing according to their rate of transcription [12]. Since the level of *WT1* expression in Wilms' tumours varies greatly, this mechanism could be operating here to explain the variation in exon 5 levels. There could be a degree of overlap between the latter two epigenetic mechanisms, although there is increasing evidence for tissue-specific splicing of *WT1*. The magnitude (less than 2-fold) of the change observed by Simms and colleagues makes an epigenetic mechanism more likely than mutation of a WT1-specific splice control protein, as the latter might be expected to exert a more profound effect.

HOW IS *WT1* SPLICED NORMALLY?

The *WT1* gene is expressed in a limited range of tissues, mainly in the developing genitourinary system, but also in mesothelium, spleen and some cells of haemopoietic origin

[13]. The amino acid sequence is highly conserved throughout vertebrate evolution, including the KTS insertion at splice II, whereas the insertion of exon 5 at splice I is confined to mammals [14]. Haber and associates found that the ratios of the splicing variants were fairly uniform in kidney and gonadal tissues, with a −/+ exon 5 ratio of 0.4 to 0.6 [3]. There is evidence for tissue-specific variations in exon 5 splicing in rat testis and mesothelial cell lines [15] and in haemopoietic cells (J. Renshaw & K.P-J, unpublished data), although others find no differences in mesothelial tumours or cell lines [16].

HOW CAN DISRUPTION OF SPLICING AFFECT *WT1* FUNCTION?

That correct splicing of the *WT1* gene is essential for normal function is illustrated by children with DDS, whose sole defect is their inability to produce the +KTS form of *WT1* at splice II. Disruption of the normal balance of *WT1* isoforms has a dominant effect on genitourinary development, although tumorigenesis may still require mutation of the remaining *WT1* allele. The presence or absence of splice II is known to have profound effects on the selection of DNA sequences to which *WT1* will bind and therefore presumably on which target genes it regulates [17, 18]. Splicing at this site also appears to determine the localisation of the WT1 protein within the nucleus, the −KTS form being found in areas of transcriptional activity whereas the +KTS form associates with splicing factors [19]. This raises the possibility that *WT1* may have more than one function, either as a transcription factor or controlling tissue-specific splice selection.

What is known about the functional effects of the presence of the 17 amino acids which comprise exon 5? *In vitro* transient transfection studies have shown that *WT1* can behave as either a transcriptional activator or repressor depending on the number and type of *WT1* binding sites in the target gene's promoter [20]. Whatever the effect on transcription, the presence of exon 5 enhances this relative to the −exon 5 form. A further role for exon 5 has been suggested by recent experiments by a Japanese group who show that *WT1*+exon 5 can inhibit the progression of cells through the cell cycle and that this inhibition can be overcome by increasing the amount of activated cyclin/CDK complexes in the cell [21]. Whether this is mediated by a direct effect on cell cycle proteins is unclear, as is its physiological relevance. However, these observations raise the possibility that the findings of Simms and associates, of a relative decrease in +exon 5 isoforms in Wilms' tumours, may underlie the increased proliferative capacity of these tumour cells. Further evidence to support this view comes from the findings of disrupted splicing of both exons 2 and 5 in Wilms' tumours and in cell lines [22]. Skipping of exon 2 maintains the reading frame of *WT1* to produce an N-terminal truncated protein with altered transactivation properties and which was detectable in 14/14 Wilms' tumour cell lines and 6/6 primary Wilms' tumours. Exon 5 was also completely absent in the cell line in which the exon 2 skipping was most marked. Neither exonic nor intronic *WT1* mutations could be found to account for this splicing abnormality. The authors postulate that disruption of a regulatory gene specific for *WT1* splice selection may underlie their observations. Simms and associates did not find any defect of exon 2 splicing in their primary tumour panel and Haber and associates do not report on exon 5 splicing in their primary tumour samples. However since the *WT1-del2* comprised less than 10% of *WT1* mRNA in primary tumour samples analysed by RNase protection, this apparent discrepancy may be due to the failure of Simms' analysis to detect low levels of expression of the aberrant transcript.

IS *WT1* ESSENTIAL FOR NORMAL DEVELOPMENT?

In the mouse, gene knockout experiments have shown that *WT1* is essential for formation of the kidney and gonad [23]. However, since the absence of the genitourinary system should not prevent survival to term, *WT1* must have other effects whose absence are embryo lethal at mid-gestation. The heterozygous *WT1* +/− mice do not have an increased rate of genitourinary malformation nor of tumours, unlike their human counterparts with the Wilms' tumour-aniridia syndrome, which is caused by a constitutional deletion of the *WT1* and aniridia genes on chromosome 11p13. Nature has performed her own experiment in producing a child who is constitutionally homozygous for a mis-sense mutation of the *WT1* gene [24]. The mutation causes the substitution of tyrosine for a critical cysteine in exon 8, which should disrupt formation of zinc finger 2. This type of mutation has been shown to prevent DNA binding by *WT1*, but the protein would still retain its N-terminal regulatory region which includes domains for protein–protein interaction. Splicing of exon 5 should be unaffected. Thus, this mutant *WT1* may be able to fulfill at least some of its normal functions through this region, allowing development of a viable human being, albeit with the Denys–Drash syndrome (she had nephropathy and Wilms' tumour). Of interest, in this child, homozygosity for a mutant *WT1* was not sufficient for malignant transformation, as this genotype was present in both the kidney and the tumour.

IS DISRUPTION OF *WT1* THE COMMONEST GENETIC DEFECT IN WILMS' TUMOUR?

There is evidence for at least four Wilms' tumour genes, located at chromosome 11p13 (*WT1*), 11p15 and 16q, plus a familial Wilms' tumour gene which maps outside these loci. To date, only one of these genes, *WT1*, has been isolated. It is unknown whether these genes may each lead independently to Wilms' tumour or whether they are components of a developmental pathway whose disruption results in the common phenotype of Wilms' tumour. Certainly, the finding of mutation of a single *WT1* allele in some Wilms' tumours which also show loss of heterozygosity for chromosome 11p15, suggests that these two genes may cooperate in some way during tumorigenesis. Attempts have been made to link histological subtypes of Wilms' tumour to underlying genotypes. There is a tendency for tumours with *WT1* mutations to be more likely to show heterologous differentiation of the mesenchymal components into muscle, fat, cartilage and bone, suggesting that they may originate at an earlier stage of nephrogenic commitment than the classic triphasic Wilms' tumour. However, the association is neither absolute nor, indeed clear cut, and both familial Wilms' tumour (i.e. non-chromosome 11) and tumours with complete loss of *WT1* can show identical triphasic histology. The effects of *WT1* splicing disruption on Wilms' tumour histology is also unclear. In the series of Simms and colleagues, the two tumours with predominantly epithelial differentiation did not show disruption of exon 5 splicing ratios but two tumours with triphasic histology, which includes epithelial components, did. Thus, whatever the function of exon 5 splicing in determining nephrogenic differentiation, there is obviously a great deal of plasticity in the system.

Whether skipping of exon 5 of *WT1* is truly found in the majority of Wilms' tumours requires further investigation. Brenner and associates analysed 36 Wilms' tumours with a semi-

quantitative RT-PCR method and reported variable levels of +/− exon 5 products [25]. Certainly, some of their tumours appeared to have a relative excess of the + exon 5 form, as did the five Wilms' tumours analysed by RNase protection by Haber and associates [3]. These findings are contrary to those of Simms and colleagues and indicate that further quantitative analysis of a large series of Wilms' tumours is merited.

In conclusion, alternative splicing of *WT1* is clearly an important mechanism in determining the normal function of the protein and it is possible that *WT1* regulates its own splicing. Mutations affecting splicing may have dominant effects on urogenital development and promote tumorigenesis. *WT1* undergoes tissue-specific splicing, affecting particularly exon 5. The mechanisms by which this is achieved for *WT1* are at present unknown, but comparisons with similar pathways controlling physiological exon skipping in both *Drosophila* [26] and in man [27] should suggest avenues for exploration. Even with the reservations about the relevance of mouse models of *WT1* genotype to humans, one way of analysing the role of exon 5 in *WT1* function would be to produce mice homozygous for the − exon 5 isoform of *WT1*. Given the above discussion, the expected phenotype is hard to predict, but may give some insights into whether the presence of exon 5 allows "suppression of tumours".

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