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POLYMORPHIC AND TISSUE-SPECIFIC IMPRINTING OF THE HUMAN WILMS TUMOR GENE, *WT1*

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Summary We previously demonstrated maternal monoallelic expression of the Wilms tumor suppressor gene, *WT1*, in about half of pre-term placental villus and fetal brain tissues examined. There were two alternative explanations for this pattern of the *WT1* expression, *i.e.*, an imprinting polymorphism *vs.* a developmental stage-dependent switching from monoallelic to biallelic expression of the gene. To investigate these possibilities, we examined *WT1* expression in a larger number of villus samples (46 samples) with gestational ages ranging from 4 to 21 weeks, using reverse transcriptase-based polymerase chain reaction (RT-PCR) to amplify the sequences for polymorphic sites in the 3'-untranslated region (UTR) of *WT1*. Maternal monoallelic expression was observed in 7 (39%) of 18 samples informative for the polymorphism, while the expression of the remaining 11 samples was biallelic. In addition, there was no correlation between expression patterns and gestational ages of the samples. The results indicate that the pattern of expression (monoallelic *vs.* biallelic) is polymorphic. The expression patterns were also studied in five different organs from a 21-week-old fetus, showing monoallelic expression only in the placenta and biallelic expression in other organs (heart, lung, liver and intestine). The finding supports the tissue specificity of the *WT1* monoallelic expression.

Key Words Wilms tumor gene, *WT1*, paternal imprinting, polymorphism, tissue-specific monoallelic expression

INTRODUCTION

Genomic imprinting is a phenomenon in which maternal and paternal alleles are epigenetically modified before conception and the modified and unmodified parental alleles are differentially expressed in offspring. Nine imprinted genes have

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been identified in man: the insulin-like growth factor II gene (*IGF2*) (Ohlsson *et al.*, 1993; Giannoukakis *et al.*, 1993) and its receptor gene (*IGF2R*) (Xu *et al.*, 1993), H19 gene (*H19*) (Zang and Tycko, 1992), the $p57^{KIP2}$ gene (Hatada *et al.*, 1996), the Wilms tumor suppressor gene (*WT1*) (Jinno *et al.*, 1994), the small nuclear ribonucleoprotein-associated polypeptide N gene (*SNRPN*) (Özçelik *et al.*, 1993), and transcripts (*IPW*, *PAR1* and *PAR5*) from the Prader-Willi syndrome critical region (Wevrick *et al.*, 1994; Sutcliffe *et al.*, 1994). Among them, *IGF2*, *SNRPN*, *IPW*, *PAR1* and *PAR5* are paternally expressed, and the remaining 3 are maternally expressed genes. Abnormal expression of these genes may have phenotypic effects, *e.g.*, an excess of the active paternal *IGF2* allele may lead to an overgrowth syndrome, Wiedemann-Beckwith syndrome (Weksberg *et al.*, 1993) or result in Wilms tumor (Rainier *et al.*, 1993; Ogawa *et al.*, 1993a, b) and lung cancer (Suzuki *et al.*, 1994).

We previously demonstrated maternal monoallelic-expression of human *WT1* in human placental and fetal brain tissues (Jinno *et al.*, 1994). In contrast to other human imprinted genes, monoallelic expression of *WT1* was not universally observed in the villus or brain samples examined. In 4 of 9 villus samples, the gene was expressed biparentally. Thus, our data suggested that *WT1* imprinting is tissue-specific and polymorphic in the human population. Alternatively, the *WT1* imprinting might be developmental stage-specific and erased in later stages of development, resulting in biparental expression. In order to test these two possibilities, we analyzed *WT1* expression in a large number of samples with different gestational ages and in different organs.

MATERIALS AND METHODS

Preparation of genomic DNA, mRNA and its cDNA. A total of 46 placental villus samples aged from 4 to 21 gestational weeks were collected from induced abortuses. Four organs (heart, lung, liver and intestine) were obtained from a 21-week-old fetus. An informed consent was obtained from each pregnant woman, and only the samples from women who had given consent were subjected to this study. All the samples were carefully washed to separate them from maternal blood and decidual tissues, immediately frozen in liquid nitrogen, and then ground to powder. A part of the powdered tissue was treated with proteinase K/SDS and genomic DNA was prepared with phenol/chloroform treatment and ethanol precipitation. RNA was extracted from the powdered tissue using an RNA extraction kit (Nippon Gene, Tokyo) or according to the guanidine thiocyanate-phenol/chloroform method. Single-stranded cDNAs were synthesized from 5–10 mg of total RNA using the oligo-dT primer (GIBCO BRL, USA) as described previously (Jinno *et al.*, 1994).

Reverse transcriptase-based (RT) PCR and detection of allele-specific expression of WT1. Allele-specific transcription of *WT1* was studied on PCR

products that were amplified using two sets of primers (Jinno *et al.*, 1994), *i.e.*, one set (WTCA3 and WTCB3) flanking the polymorphic CA repeat sequences (Gessler *et al.*, 1992) and the other set (WTHfa and WTHfb) for a *Hin*fI restriction fragment length polymorphism (RFLP) (Hoban and Kesley, 1990), both located in the 3′-untranslated region (UTR) of *WT1*. The sequences of the two primer sets (5′ to 3′) are as follows: WTCA3, ATCCATTGTTTAAAGATGGTCG; WTCB3, GTAAATAATAAATTCCCTCCCTT; WTHfa, AATCAGAGAGCAAGGCATCG; WTHfb, GTGCAAGGAGGTATGTACATC. The cDNA (2–10 ng) and genomic DNA (250 ng) were subjected to PCR with denaturation at 94°C for 1.5 min, annealing at 55°C for 1.5 min and extension at 72°C for 1.75 min, in a 50 μl standard reaction mixture. After 20, 25, 30 and 35 cycles of PCR, the amount of amplified DNA was estimated by electrophoresis on a 4% polyacrylamide gel. A second round of PCR was carried out for 14 cycles in a 10 μl reaction mixture containing WTCA3 and WTCB3 primers, 1 μl each of [α-^{32}P]dCTP and the first PCR products, with the same PCR conditions. The radiolabeled product (2 μl) was electrophoresed in 6% polyacrylamide/8 M urea sequencing gel. The *Hin*fI RFLP was detected by *Hin*fI-digestion of PCR products, followed by electrophoresis in 6% acrylamide gel and staining with ethidium bromide.

RESULTS

Maternal monoallelic expression in chorionic villi

Of the 46 RT-PCR products from respective placental villus samples, 18 were informative for the differential expression between the maternal and paternal alleles (Table 1). Among the 18 informative samples, 7 (39%) showed monoallelic *WT1* expression, the expressed allele being mainly or exclusively maternal, while the remaining 11 samples showed biallelic expression (Figs. 1 and 2).

Monoallelic expression of WT1 is not correlated with gestational weeks

Ages of the villus samples with monoallelic or biallelic expressions tended to be diversely distributed at a period of 4–21 gestational weeks (Table 1). In this period of development, there was no correlation of monoallelic expression with gestational age, *e.g.*, the villus from a 4-week-old embryo, the youngest sample in our series, showed biallelic expression of *WT1*, while that from a 21-week-old fetus, the oldest sample, exhibited monoallelic expression.

Table 1. Number of chorionic villus samples with monoallelic or biallelic expression.

Expression	Gestational ages (weeks)							
	4	5	6	7	8	9	14	21
Monoallelic	0	2	1	1	1	0	1	1
Biallelic	1	2	0	5	1	1	1	0

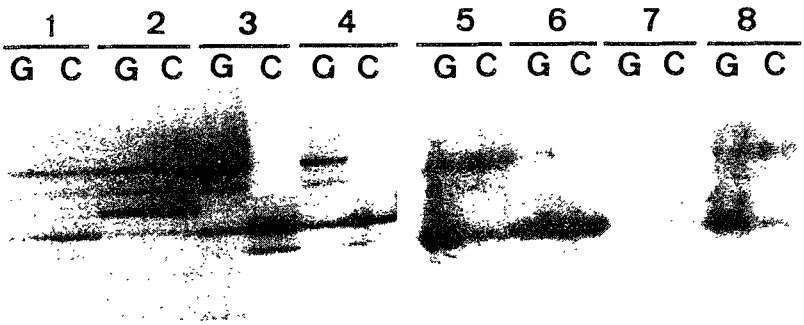

Fig. 1. Polymorphic patterns of a CA repeat in the *WT1* 3′-UTR, showing monoallelic (lanes 3, 4 and 6) or biallelic expression (lanes 1, 2, 5, 7 and 8). Lanes 1-8 are PCR products of genomic DNA (G) and cDNA (C) from 4, 5, 5, 7, 7, 8, 8 and 11-week-old placental villi, respectively.

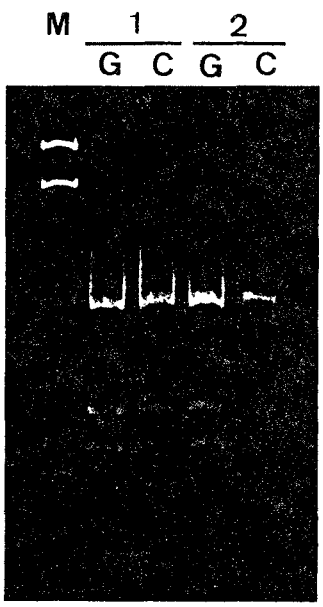

Fig. 2. *Hin*fI RFLP at the 3′-UTR of *WT1* in genomic DNA (G) and cDNA (C) from the villus tissue of a 14-week-old fetus, showing biallelic (lane 1), and monoallelic expression (lane 2). M, a size marker, *Alu*I-cleaved pUC19.

Tissue-specific monoallelic expression of WT1

Analysis of *WT1* expression in different organs was possible only in a 21-week-old fetus. All four organs (heart, lung, liver and intestine) from the fetus showed biallelic *WT1* expression, although the expression of the villus sample of the same fetus was monoallelic (Fig. 3). The brain tissue of the fetus was not available.

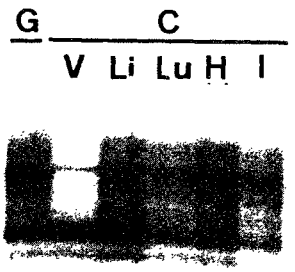

Fig. 3. Tissue-specific allele expression patterns of *WT1*. PCR-amplified 3′-UTR CA repeat sequences of genomic DNA (G) and those of cDNA (C) from the villus (V), liver (Li), lung (Lu), heart (H) and intestine (I) of a 21-week-old fetus.

DISCUSSION

The results of our previous study showed that *WT1* expression in pre-term placentae was either monoallelic or biallelic (Jinno *et al.*, 1994). However, we were unable to exclude the possibility that the expression patterns observed would change with increasing fetal age. In other words, *WT1* would first be expressed from the maternal allele in an early developmental stage and subsequently from both alleles by "loss of imprinting," as observed in the human *H19* gene (Jinno *et al.*, 1995), through a putative gene silencer-mediated mechanism similar to that in the mouse *Igf2* (Surani, 1993). However, the present study provided unequivocal evidence for an imprinting polymorphism of *WT1*, since the different expression patterns of the gene did not correlate to developmental stages. The human *IGF2R* is another imprinted gene possibly showing an imprinting polymorphism (Xu *et al.*, 1993). Although imprinting of *IGF2R*, whose mouse homologue (*Igf2r*) was reported to be maternally expressed, had first been denied (Kalscheuer *et al.*, 1993), its maternal monoallelic expression was demonstrated in 2 of 14 villus tissues examined (Xu *et al.*, 1993).

WT1 contributes to the differentiation of mesenchymal cells or renal vesicles in an early stage of human development. The gene product contains a DNA binding domain consisting of 4 zinc finger motifs (Call *et al.*, 1990) and binds a recognition element common to the early growth response (EGR) family of zinc finger transcriptional activators (Rauscher, 1993). In contrast to other EGR transcription factors, *WT1* behaves as a transcription repressor in transient transfection assays (Madden *et al.*, 1991). *IGF2* has EGR/*WT1* binding sites in its promotors. By binding to the sites, *WT1* functions as a potent repressor of *IGF2 in vivo* (Drumond *et al.*, 1992). *IGF2R* also acts as a regulator for *IGF2*. The *IGF2* gene product, IGF-II, deteriorates by binding to its receptor, the *IFG2R* product (Xu *et al.*, 1993). From this point of view, it is of great interest why polymorphic

imprinting occurs in these two regulating genes. It is plausible that the imprinting polymorphism in *WT1* and *IGF2R* may relate to the *IGF2* regulation and a common regulating system may exist involving the three genes. It remains to be seen whether the expression patterns observed in *WT1* and *IGF2R* are related to each other. Alternatively, these two genes might have lost their imprinting through the evolutionary process in mammals. It seems that the imprinting is still maintained in a part of the human population.

Among the 5 organs examined in this study, only the placental villus showed monoallelic expression of *WT1*. The result, together with the previous finding of monoallelic expression in the villus and fetal brain tissues (Jinno *et al.*, 1994), confirmed the tissue-specific expression of the gene, although the number of fetuses and organs examined was small in the present study. It remains to be seen whether the tissue specificity plays a role in the development of these organs. We did not observe any phenotypic differences among a series of the placenta and other tissues, irrespective of *WT1* expression patterns. This may imply that a growth factor, IGF-II, is regulated normally in these organs, regardless of biallelic or monoallelic expression or even a hemizygous condition of *WT1*. Tissue-specific imprinting has been reported in the mouse *Igf2* (DeChiara *et al.*, 1991) and the human *IGF2* (Kalscheuer *et al.*, 1993). A concept of a regional imprinting factor has recently been proposed through the analysis of the *IGF2* promotor (Vu and Hoffman, 1994). *IGF2* has at least 4 different promotors, P1, P2, P3 and P4. *IGF2* transcripts from P1 are always derived from both parental alleles, whereas those from other promotors are from one parental allele (Vu and Hoffman, 1994). Thus, it is likely that there exists a similar system which regulates different expression patterns of *WT1* and this system may lead to tissue-specific expression of the gene.

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