Genetic and epigenetic alterations on the short arm of chromosome 11 are involved in a majority of sporadic Wilms’ tumours

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Wilms’ tumour is one of the most common solid tumours of childhood. 11p13 (WT1 locus) and 11p15.5 (WT2 locus) are known to have genetic or epigenetic aberrations in these tumours. In Wilms’ tumours, mutation of the Wilms tumour 1 (WT1) gene at the WT1 locus has been reported, and the WT2 locus, comprising the two independent imprinted domains IGF2/H19 and KIP2/LIT1, can undergo maternal deletion or alterations associated with imprinting. Although these alterations have been identified in many studies, it is still not clear how frequently combined genetic and epigenetic alterations of these loci are involved in Wilms’ tumours or how these alterations occur. To answer both questions, we performed genetic and epigenetic analyses of these loci, together with an additional gene, CTNNB1, in 35 sporadic Wilms’ tumours. Loss of heterozygosity of 11p15.5 and loss of imprinting of IGF2 were the most frequent genetic (29%) and epigenetic (40%) alterations in Wilms’ tumours, respectively. In total, 83% of the tumours had at least one alteration at 11p15.5 and/or 11p13. One-third of the tumours had alterations at multiple loci. Our results suggest that chromosome 11p is not only genetically but also epigenetically critical for the majority of Wilms’ tumours.

Keywords: Wilms’ tumour; genetics; epigenetics; loss of heterozygosity; loss of imprinting; DNA methylation

Wilms’ tumour, also known as nephroblastoma, is one of the most common solid tumours of childhood, accounting for approximately 6% of all childhood malignancies. Chromosomal region 11p13 was first identified as a Wilms’ tumour locus, WT1, because the region was found to be deleted in Wilms’ tumours (Kaneko et al., 1981; Huff, 1998; Dome and Coppes, 2002, OMIM 194070). The Wilms tumour 1 (WT1) gene, isolated from the WT1 locus, was the first causative gene for Wilms’ tumour (Call et al., 1990; Gessler et al., 1990). However, WT1 aberrations, such as deletions and point mutations, are observed in only approximately 10–20% of Wilms’ tumours (Huff, 1998; Nakadate et al., 2001). The small number of WT1 mutations in Wilms’ tumours suggests that WT1 can be inactivated by alterations that would not be detected by mutational analysis (Huff, 1998). On the other hand, although WT1 mutation is not frequent, WT1 mutation and CTNNB1 (β-catenin) mutation at 3p21 are significantly correlated with Wilms’ tumours (Maiti et al., 2000).

Loss of heterozygosity (LOH) of 11p15.5, which is known as the WT2 locus (OMIM 194071), is observed in Wilms’ tumours. LOH occurs on the maternal chromosome, suggesting the involvement of genomic imprinting in Wilms’ tumorigenesis. This imprinted region is well characterised, and is divided into two imprinted domains, IGF2/H19 and KIP2/LIT1 (Feinberg, 2000). It has been reported that IGF2 and H19 within the IGF2/H19 domain are expressed abnormally in Wilms’ tumours. IGF2 encodes an embryonal growth factor and is transcribed exclusively from the paternal allele (Reik and Murrell, 2000), and H19 is a noncoding RNA with reciprocal transcription from the maternal allele. In Wilms’ tumours, abnormally high levels of IGF2 mRNA and loss of imprinting (LOI) of IGF2, allowing both paternal and maternal alleles to be transcribed, have been observed (Reeve et al., 1985; Ogawa et al., 1993; Rainier et al., 1993). LOI of IGF2 is always accompanied by H19 biallelic hypermethylation, leading to inactivation of H19 (in the normal situation, the region upstream of H19 is methylated only on the paternal allele) (Moulton et al., 1994; Steenman et al., 1994). Demethylation of DMR-LIT1, an imprinting control region (ICR) of the KIP2/LIT1 domain, occurs in half of all patients with Beckwith–Wiedemann syndrome (BWS) (OMIM 130650), which predisposes patients to embryonal tumours, and in a variety of adult tumours. The p57KIP2 (KIP2)/CDKN1C gene within the KIP2/LIT1 domain, which is expressed predominantly from the maternal allele, encodes a cyclin-dependent kinase inhibitor and is a putative tumour suppressor. In several adult tumours, KIP2 expression is epigenetically reduced (Shin et al., 2000; Kikuchi et al., 2002; Li et al., 2002; Soejima et al., 2004). However, KIP2 expression has been found to be reduced in Wilms’ tumours in some studies, but not in others (Chung et al.,
1996; Hatada et al, 1996; Thompson et al, 1996; O’Keefe et al, 1997; Taniguchi et al, 1997; Soejima et al, 1998).

Although several genes involved in Wilms’ tumour have been identified, as described above, the alteration frequencies of these genes (loci) and how many loci are altered in the tumour are still unknown. To investigate this, we comprehensively investigated genetic and epigenetic alterations of three loci – WT1 (11p13), WT2 (11p15.5), and CTNNB1 (3p21) — in 35 sporadic Wilms’ tumours (Figure 1). Our data indicate that genetic and/or epigenetic alterations of genes at these loci, especially WT1 and WT2, is involved in the majority of Wilms’ tumours, and that alterations of multiple loci occur in one-third of tumours. These findings suggest that genetic and epigenetic alterations on the short arm of chromosome 11 play an important role in Wilms’ tumorigenesis.

MATERIALS AND METHODS

DNA and RNA

In all, 35 tissue samples from sporadic Wilms’ tumours and five tissue samples from mid-gestational fetal kidneys were obtained from Saitama Cancer Center Hospital (Saitama, Japan) and the fetal tissue bank at the University of Washington, (WA, USA), respectively. Genomic DNA and total RNA were extracted with a QiAamp DNA mini kit (Qiagen, Hilden, Germany) and Isogen (Nippon Gene, Tokyo, Japan), respectively.

Mutation and LOH analyses

Genetic analyses of WT1 were carried out as previously described (Nakadate et al, 1999, 2001). Briefly, WT1 loci were screened for mutations by single-strand conformation polymorphism (SSCP) analysis of all exons and splice-donor/acceptor sites. When an aberrant band was identified by SSCP, the band was excised and sequenced. Loss of heterozygosity was also analysed using polymorphic DNA markers as follows to compare tumour tissue with adjacent normal tissue or peripheral blood: D11S16, D11S325, PAX6, D11S324, WT1, and CAT for 11p13; and D11S12, D11S922, D11S932, IGF2, INS, and TH for 11p15.5. Mutations in exon 3 of the CTNNB1 gene were investigated by PCR-directed sequencing as previously described (Satoh et al, 2003).

Quantitative real-time reverse transcription (RT) – polymerase chain reaction (PCR)

Total RNA (500 ng) was treated with RNase-free DNase I (Roche, Basel, Switzerland) and reverse-transcribed with ReverTra Ace reverse transcriptase (Toyobo, Japan) and random primers (TaKaRa, Japan). Quantitative real-time RT–PCR was performed with the LightCycler® system (Roche) according to the manufacturer’s protocol. The expression of WT1 was normalised with that of β-actin, as previously described (Satoh et al, 2003). The average WT1 expression of four mid-gestational fetal kidneys was employed as a standard. All experiments were performed in triplicate.

Allele-specific expression of IGF2

Genotyping of IGF2 was performed by PCR-restriction fragment length polymorphism (RFLP) using a polymorphic HaeIII (Apal or Avall) site in exon 9, as previously described (Soejima and Yun, 1998). To eliminate genomic DNA contamination, the RNA-specific product was excised from the purified product (1120 bp) was amplified by using an exon connection primer pair (5'-TCCTGGAGACGTCTGTGCTA-3' and 5'-GGTCTGTCGACAAATTACATTTCA-3'). To further eliminate contaminating DNA, the RNA-specific product was excised from 1% agarose gel after electrophoresis and purified. Then, the purified product was subjected to nested PCR and RFLP analysis with HaeIII (Yun et al, 1999).

Methylation analyses

Combined bisulphite restriction analyses (COBRA) using the hot-stop method were employed to determine the extent of methylation at the differentially methylated region (DMR) of the H19 promoter (H19-pro-DMR), DMR-LIT1, and WT1 promoter. Although an ICR of the H19 promoter (H19-pro-DMR), DMR-LIT1, and WT1 promoter.

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follows: 5′-GGAGGGTTTTGGTTGTTG-3′, 5′-ACTCTCTCT TCAAACACCCCTCTCT-3′, and Hini for H19-pro-DMR; and 5′- CGTATCAGTTTGGTTGAGTT-3′, 5′-ACTACGCTAATCTCC CAAAATC-3′, and Hini for the WT1 promoter. For several samples, methylation of H19-pro-DMR was confirmed by using hot-stop COBRA for a region immediately downstream of CTCF binding site 6 (CTCF6) in H19-DMR. The primer pairs and the restriction endonuclease used were 5′-GAGTGGGTTTTGGTTGAGTT-3′ TATAATGTTCCACCTAAATCTAA-3′, and MluI. DMR-LIT1 was analysed as previously described (Soejima et al., 2004). The hot-stop COBRA products were separated by 7.5% polyacrylamide gel electrophoresis (PAGE) and quantified with BAS2000 (Fujiﬁlm, Japan). All experiments were performed three times independently.

RESULTS

Genetic and epigenetic alteration of the IGF2/H19 imprinted domain at 11p15.5

Of 35 tumours, 10 (29%) showed LOH of 11p15 and 25 showed retention of heterozygosity (ROH) at this locus (Tables 1 and 2).

Table 1 Genetic or epigenetic alterations in Wilms’ tumours

| Sample no. | 11p15.5 LOH | IGF2 LOI | DMR-LIT1 | 11p13 LOH | WT1 mutation | WT1 expression | CTNNB1 mutation | Alteration type |
|-----------|-------------|---------|---------|-----------|--------------|----------------|----------------|---------------|
| 1         | Normal      | ROI (p) | Normal  | –         | –            | 128.1          | –              | None          |
| 2         | Normal      | ROI (p) | Normal  | ND        | –            | 114.3          | –              | None          |
| 3         | Normal      | ROI (p) | Normal  | –         | –            | 128.6          | –              | None          |
| 4         | Normal      | ROI (p) | Normal  | ND        | –            | 385.7          | –              | None          |
| 5         | Normal      | ROI (m) | Normal  | –         | –            | 57.1           | –              | None          |
| 6         | Normal      | ROI (m) | Normal  | ND        | –            | 385.7          | –              | None          |
| 7         | Normal      | ROI (m) | Normal  | –         | –            | 0.0            | –              | E             |
| 8         | Normal      | ROI (m) | Normal  | ND        | –            | 0.0            | –              | E             |
| 9         | Normal      | ROI (m) | Normal  | HD        | HD           | 28.6           | –              | G             |
| 10        | Normal      | ROI (m) | Normal  | HD        | HD           | 28.6           | –              | G             |
| 11        | Normal      | ROI (p) | Hypo    | HD        | HD           | 0.0            | –              | G             |
| 12        | Normal      | ROI (p) | Normal  | –         | –            | 14.3           | –              | G             |
| 13        | Normal      | ROI (p) | Normal  | –         | –            | 85.7           | –              | G             |
| 14        | Normal      | ROI (p) | Normal  | –         | –            | 28.6           | –              | G             |
| 15        | Normal      | ROI (m) | Normal  | –         | –            | 57.1           | –              | G             |
| 16        | Normal      | ROI (m) | Normal  | –         | –            | 114.3          | –              | G             |
| 17        | Normal      | ROI (m) | Normal  | –         | –            | 442.9          | –              | G             |
| 18        | Normal      | ROI (m) | Normal  | ND        | –            | 28.6           | –              | G             |
| 19        | Normal      | ROI (m) | Normal  | ND        | –            | 85.7           | –              | G             |
| 20        | Normal      | ROI (m) | Normal  | ND        | –            | 1557.1         | –              | G             |
| 21        | Normal      | ROI (m) | Normal  | –         | –            | 157.1          | –              | G             |
| 22        | Normal      | ROI (m) | Normal  | –         | –            | 0.8            | –              | E, E          |
| 23        | Normal      | ROI (m) | Hypo    | ND        | –            | 142.9          | –              | E             |
| 24        | Normal      | ROI (m) | Hypo    | NI        | –            | 171.4          | –              | E             |
| 25        | Normal      | ROI (m) | Hypo    | ND        | –            | 0.6            | –              | E, E          |
| 26        | Hyper       | Hyper   | Normal  | +         | +            | 1.2            | –              | G, G, E       |
| 27        | Hyper       | Hyper   | Hypo    | +         | +            | 1.4            | –              | G, G, E       |
| 28        | Hyper       | Hyper   | Hypo    | +         | +            | 28.6           | –              | G, G          |
| 29        | Hyper       | Hyper   | Hypo    | +         | +            | 85.8           | –              | G, G          |
| 30        | Hyper       | Hyper   | Hypo    | +         | +            | 228.6          | –              | G, G          |
| 31        | Hyper       | Hyper   | Hypo    | +         | +            | 857.1          | –              | G             |
| 32        | Hyper       | Hyper   | Hypo    | ND        | –            | 5.7            | –              | G, E          |
| 33        | Hyper       | Hyper   | Hypo    | NI        | –            | 385.7          | –              | G             |
| 34        | Hyper       | Hyper   | Hypo    | NI        | –            | 14.3           | –              | G             |
| 35        | Hyper       | Hyper   | Hypo    | ND        | –            | 100.0          | –              | G             |

Genetic and epigenetic alterations are indicated by blue and red bold, respectively. IGF2 LOI was examined by RT–PCR–RFLP with HaeIII polymorphism (p) or methylation analysis of H19-pro-DMR (m). Hypermethylation of H19-pro-DMR in 11p15.5 LOH cases was not indicated by red color because it was due to LOH. WT1 expression in #11 is not indicated by red color because the reduction of this sample was secondary alteration caused by a genetic alteration, homozygous deletion. LOI = loss of imprinting; hyper = hypermethylation of H19-pro DMR; hypo = hypomethylation of DMR-LIT1; ND = not done; NI = not informative; HD = homozygous deletion. WT1 expression less than 10% of fetal kidneys is considered epigenetic alteration. Genetic alteration and epigenetic alteration are indicated by G and E, respectively. Number of G or E indicates number of altered loci. These were considered ROH because methylation of H19-pro-DMR and DMR-LIT1 were maintained. This sample showed promoter hypermethylation. These were considered LOH because of H19-pro-DMR hypermethylation and DMR-LIT1 hypomethylation.
as hypermethylation. The total number of tumours showing \( \text{H19-pro-DMR} \) hypermethylation was 21, comprising 11 with ROH and 10 with LOH (Table 1, Figure 2A). Because LOH occurs with the maternal chromosome, only the methylated paternal chromosome remains in LOH tumour cells, resulting in hypermethylation. Thus, biallelic hypermethylation leading to \( \text{IGF2} \) LOI occurred in 11 tumours with ROH. Indeed, all three tumours (#12, #13, #14) that were heterozygous for the polymorphism and showed biallelic expression also showed hypermethylation (data not shown). Furthermore, representative samples with hypermethylation at \( \text{H19-pro-DMR} \) also underwent hypermethylation at \( \text{H19-DMR} \), CTCF6 (data not shown). A total of 14 out of 35 tumours (40%) had LOI (Tables 1 and 2); and LOI occurred in 56% of ROH tumours (14 out of 25).

### Epigenetic alteration of the KIP2/LIT1 imprinted domain at 11p15.5

We investigated methylation of \( \text{DMR-LIT1} \) in the KIP2/LIT1 imprinted domain (Tables 1 and 2, Figure 2B) relative to the average percentage methylation in fetal kidneys, which was 44.3 ± 7.5% (data not shown). We defined methylation of less than the average of the fetal kidneys – 2 s.d. as hypomethylation. Although 12 tumours, eight with LOH and four with ROH, showed hypomethylation, the four with ROH had biallelic hypomethylation because maternal \( \text{DMR-LIT1} \) is normally methylated. In spite of LOH, two tumours (#26 and #32) did not have a methylation level that was less than the average for the fetal kidneys – 2 s.d., but still had a low level of methylation (29.7 and 39.8%). These findings might be due to contamination with nontumour cells.

We also investigated expression and promoter methylation of KIP2, because this imprinted gene is a putative tumour suppressor gene, but no somatic mutation has been found in tumours to date. KIP2 expression varied from zero to approximately 800% of that of the control fetal kidneys, and the promoter region was not methylated in any sample (data not shown). In addition, there was no correlation between \( \text{KIP2} \) expression and \( \text{DMR-LIT1} \) methylation.

A total of 25 (71%) tumours showed alteration of \( \text{IGF2/H19} \) or \( \text{KIP2/LIT1} \) or both of the domains, of which 10 showed LOH, 11 showed \( \text{IGF2} \) LOI only, one showed \( \text{DMR-LIT1} \) hypomethylation only, and three showed both \( \text{IGF2} \) LOI and \( \text{DMR-LIT1} \) hypomethylation (Table 1).

### Genetic and epigenetic alteration of WT1 at 11p13

A total of 20 tumours were informative for polymorphisms on 11p13: 12 of these had preserved heterozygosity and five (25%)
showed 11p13 LOH, and these had concurrent 11p15.5 LOH, indicating a large LOH region (more than 30 Mb) in the short arm of chromosome 11 (Tables 1 and 2). WT1 gene mutation was also examined as a genetic alteration. Only three tumours had homozygous deletion of WT1, as previously described (Nakadate et al, 1999; Watanabe et al, 2006).

As epigenetic alterations, the expression and promoter methylation of WT1 were examined. We determined the quantity of WT1 expression normalised with β-actin expression. We defined expression of less than 10% of that of the control fetal kidneys as a significant reduction, and found seven tumours with such a reduction (Table 1). Two tumours (#9 and #10) expressed a certain level of WT1 in spite of a homozygous deletion, which might be due to contamination with nontumour cells. Excluding tumours with homozygous deletions, six tumours (17%) had a reduction in WT1 expression (Tables 1 and 2). Methylation analysis, however, revealed that only one tumour (#26) had promoter methylation, as previously described (Table 1 and Figure 2C) (Satoh et al, 2003). Promoter methylation was not found in any other tumours with reduction in WT1 expression.

In summary, genetic alterations of WT1 such as LOH or WT1 homozygous deletion were found in a total of eight tumours, and epigenetic alterations (i.e. reduction of WT1 expression) were found in six (Table 2).

**CTNNB1 mutation**

We found four missense mutations of the CTNNB1 gene in three tumours: Pro44Ala (CCT to GCT) and Ser45Pro (TCT to CCT) in #11, Thr41Ala (ACC to GCC) in #21, and Ser45Tyr (TCT to TAT) in #35 (Tables 1 and 2, Figure 2D). The tumours with CTNNB1 mutation had concurrent WT1 homozygous deletion and DMR-LIT1 hypomethylation, IGF2 LOI, and 11p15.5 LOH, respectively.

**DISCUSSION**

In this study, we investigated genetic and epigenetic alterations of three loci that are thought to be involved in Wilms’ tumour: the WT2 locus (11p15.5) including the IGF2/H19 and the KIP2/LIT1 imprinted domains, the WT1 locus (11p13) including the WT1 gene, and 3p21 locus including the CTNNB1 gene. Loss of heterozygosity of 11p15.5 was the most frequent genetic alteration (29%), and IGF2 LOI was the most frequent epigenetic alteration (40%) (Table 2). In ROH tumours only, IGF2 LOI frequency occurred in approximately 56% of cases (14/25). The data were consistent with the results of previous reports (Ogawa et al, 1993; Rainier et al, 1993; Steenman et al, 1994; Moulton et al, 1994, Yuan et al, 2005). It is intriguing that three tumours (#23–25) showed alterations of both IGF2/H19 and KIP2/LIT1 imprinted domains, because each domain is independently regulated, and BWS with both alterations is very rare (DeBaun et al, 2002). Furthermore, #25 had a reduction of WT1 expression. The data suggest that 11p is epigenetically unstable in Wilms’ tumours. With regard to the number of altered loci, 18 tumours (51%) showed alteration at only one locus and 11 (31%) showed alterations at multiple loci (Table 3). Six (18%) tumours did not show any alteration. Thus, 83% (29 out of 35) of Wilms’ tumours had alterations at one or more of the three loci. Furthermore, no tumour had mutation of CTNNB1 alone. These results indicate that the alterations observed in Wilms’ tumours are concentrated on the short arm of chromosome 11, that is 11p15.5-p13, and that the region is not only genetically but also epigenetically critical for Wilms’ tumorigenesis.

As shown in Figure 3, there were 10 and 15 tumours, respectively, with only genetic or only epigenetic alterations. Four tumours had both genetic and epigenetic alterations. The average age of patients at diagnosis for tumours with only genetic and only

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**Table 3** Number of altered loci in Wilms’ tumour

| Genes and loci | One locus | Two loci | Three loci | None |
|---------------|-----------|----------|------------|------|
| WT2 locus (11p15.5) | +         | +        | +          | +    |
| WT1 locus (11p13) | +         | +        | +          | +    |
| CTNNB1 (3p21) | +         | +        | +          | +    |

|       | 14 | 4  | 0  | 7  | 2  | 0  | 2  | 6  |

*Indicates genetic or epigenetic alteration at each locus. WT2 locus: 11p15.5 LOH or IGF2 LOI or DMR-LIT1 hypomethylation. WT1 locus: 11p13 LOH or WT1 mutation or WT1 reduction. CTNNB1: mutation.*
epigenetic alterations was 34.8 ± 33.3 and 46.5 ± 24.1 months, respectively, but there was no significant difference between them.

Because maternal LOH of 11p15.5 is uniformly accompanied by paternal duplication, it results in two paternal copies of the IGF2 gene and an increase of IGF2 expression. In addition, IGF2 LOI is observed in non-neoplastic kidney parenchyma and frequently in early-stage tumours, indicating the importance of IGF2 in Wilms’ tumorigenesis (Moulton et al., 1994; Okamoto et al., 1997; Yuan et al., 2005). However, in a recent study, IGF2 LOI was not observed in any of 21 Wilms’ tumours from Japanese patients (Fukuzawa et al., 2004). In that study, the HpaII site near the CTCF6 in H19-DMR, which is approximately 2 kb upstream from the H19 transcription initiation site, was used to analyse IGF2 LOI using real-time PCR. In the present study, we employed RT–PCR – RFLP and hot-stop COBRA for analysis of the methylation of H19-pro-DMR. Further, the results of H19-pro-DMR were confirmed by H19-DMC CTCF6 with hot-stop COBRA. Our results clearly show that IGF2 LOI occurs in Japanese patients with Wilms’ tumour. At present, we are not able to explain the discrepancy, but having a small sample size might have influenced the results.

Although KIP2 expression is epigenetically reduced in several adult tumours (Shin et al., 2000; Kikuchi et al., 2002; Li et al., 2002; Soejima et al., 2004), expression levels in Wilms’ tumour as measured in previous studies have varied (Chung et al., 1996; Hatada et al., 1996; Thompson et al., 1996; O’Keele et al., 1997; Taniguchi et al., 1997; Soejima et al., 1998). In the present study, KIP2 expression also varied, suggesting that at least in Wilms’ tumour, KIP2 may not be involved.

WT1 gene expression was reduced in six (17%) tumours. It is noteworthy that the frequency of WT1 reduction in expression is similar to that of WT1 mutation. WT1 expression reduction is correlated with predominant stromal histology (Pritchard-Jones et al., 1990; Miwa et al., 1992). Our tumours comprised one stromal, two triphasic, and three blastemal types. Although the precise histologic composition of tumours in the present study was unknown, whether or not there is a correlation between the WT1 expression reduction and histology is not clear because the number of tumours was small. Only one tumour (#26) had promoter hypermethylation, as described previously (Satoh et al., 2003). Since this tumour also had concurrent 11p13 LOH, ‘two-hit’ inactivation (LOH and methylation) led to a reduction of WT1 expression. Homozygous methylation was not found in any other tumours with WT1 expression reduction, thus promoter methylation might not be fundamentally involved in WT1 transcriptional repression. WT1 transcriptional regulation is remarkably complex, and our knowledge of it is still quite limited (Englert, 1998). Thus, other unknown mechanisms may be involved in the reduction of WT1 expression.

A highly significant correlation has been found between WT1 mutation and CTNNB1 mutation in Wilms’ tumours (Maiti et al., 2000). β-Catenin, a product of the CTNNB1 gene, is involved in the regulation of cell adhesion and in signal transduction through the WNT pathway. Abrogation of the WNT pathway by CTNNB1 mutations, resulting in reduced serine/threonine phosphorylation, has been recognised as playing an important role in the development of many tumours. All CTNNB1 mutations we found occurred at or near phosphorylation sites. Only one tumour had concurrent homozygous deletion of the WT1 gene. Whether or not there is a correlation between the gene mutations is not clear because the number of tumours with mutations was too small.

In conclusion, genetic and epigenetic alterations of chromosome 11p play an important role in the majority of Wilms’ tumours. There is a possibility that not only the genes investigated in this study but also unidentified genes existing in the region with unknown function also play an important role in Wilms’ tumorigenesis. In addition, six tumours did not have any alterations at the three loci studied, suggesting the involvement of genes at other loci. Chromosomes 1p, 4q, 7p, 11q, 14q, 16q, and 17p are also frequently lost in Wilms’ tumours, and the RASSF1A tumour suppressor is frequently silenced by promoter hypermethylation (Ehrlich et al., 2002; Harada et al., 2002; Wagner et al., 2002; Yuan et al., 2005). Identification of a novel gene or genes at these loci and those silenced by epigenetic mechanisms will be helpful to further understand Wilms’ tumorigenesis.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research on Priority Area ‘Applied Genomics’ (No. 17019054) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a Grant-in-Aid for Scientific Research (C) (No. 16590263 and No. 18590313) from the Japan Society for the Promotion of Science; Grants-in-Aid for the Third Term Comprehensive Ten-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare, Japan; the Public Trust Surgery Research Fund; an AstraZeneca Research Grant; and The Mother and Child Health Foundation.

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British Journal of Cancer (2006) 95(4), 541 – 547 © 2006 Cancer Research UK
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