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Imprinting of the gene encoding a human cyclin-dependent kinase inhibitor, $p57^{KIP2}$, on chromosome 11p15

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ABSTRACT **Parental origin-specific alterations of chromosome 11p15 in human cancer suggest the involvement of one or more maternally expressed imprinted genes involved in embryonal tumor suppression and the cancer-predisposing Beckwith–Wiedemann syndrome (BWS). The gene encoding cyclin-dependent kinase inhibitor $p57^{KIP2}$, whose overexpression causes G_1 phase arrest, was recently cloned and mapped to this band. We find that the $p57^{KIP2}$ gene is imprinted, with preferential expression of the maternal allele. However, the imprint is not absolute, as the paternal allele is also expressed at low levels in most tissues, and at levels comparable to the maternal allele in fetal brain and some embryonal tumors. The biochemical function, chromosomal location, and imprinting of the $p57^{KIP2}$ gene match the properties predicted for a tumor suppressor gene at 11p15.5. However, as the $p57^{KIP2}$ gene is 500 kb centromeric to the gene encoding insulin-like growth factor 2, it is likely to be part of a large domain containing other imprinted genes. Thus, loss of heterozygosity or loss of imprinting might simultaneously affect several genes at this locus that together contribute to tumor and/or growth-suppressing functions that are disrupted in BWS and embryonal tumors.**

Genomic imprinting is a modification of a specific parental chromosome in the gamete or zygote, leading to differential expression of the two alleles of a gene in somatic cells (1). While the strict definition of imprinting does not require monoallelic expression, this is seen for most imprinted genes. A relatively small number of imprinted genes have been identified, although studies of chromosomal uniparental disomy in mice suggest that many more have yet to be identified (2).

Human chromosome 11 was predicted to contain an imprinted, maternally expressed tumor suppressor gene (3), because loss of heterozygosity (LOH) of chromosome 11p15 in Wilms tumor and other embryonal tumors preferentially involves the maternal chromosome (4). Furthermore, balanced chromosomal rearrangements of 11p15 always involve the maternal chromosome in Beckwith–Wiedemann syndrome (BWS) (5), which causes prenatal overgrowth and embryonal tumors such as Wilms tumor (5).

Two imprinted genes have been identified on 11p15. Insulin-like growth factor II gene (*IGF2*), a growth-promoting gene on 11p15, was found to be expressed from the paternal allele (6, 7). Embryonal tumors show loss of imprinting (LOI) and biallelic expression of *IGF2* (6, 7). Closely linked (<100 kb) to *IGF2* is the nontranslated, maternally expressed *H19* gene (6). Mouse embryo knockout experiments show that *H19* imprinting regulates *IGF2* expression, but these animals, while large, do not develop tumors (8). *H19* also lies outside the region of 11p15 that suppresses tumorigenicity in genetic complementation experiments (9). Thus, additional imprinted genes on 11p15 are likely to play a role in tumorigenesis. In addition to the imprinted genes on chromosome 11p15, a number of imprinted genes have been identified over a several megabase region of chromosome 15, including small nuclear ribonucleoprotein N (snRPN), which may be involved in the retardation and obesity-associated Prader–Willi syndrome (10).

$p57^{KIP2}$ is a cyclin-dependent kinase inhibitor that causes G_1 arrest (11, 12) and is homologous in its inhibitory domain to $p21^{CIP1/WAF1}$, a mediator of p53-directed cell cycle arrest (13–15). Since the gene encoding $p57^{KIP2}$ maps to 11p15 (11), we have examined the imprinted state of this gene in normal development and determined if the pattern of imprinting is consistent with a role of $p57^{KIP2}$ gene as a potential tumor-suppressor gene.

MATERIALS AND METHODS

Identification of a Transcribed Polymorphism in $p57^{KIP2}$. PCR amplifications were performed in a final volume of 25 μl of buffer containing 60 mM Tris·HCl (pH 9.0), 15 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 60 nM ^{32}P-labeled hR21 (5′-TTCGCGCCCTGCTCGGCGCTCTCTTGAGGC-3′), 400 nM hF19 (5′-TGCCCGCGTTCTACCGCGAGACGGTG-CAGG-3′), 12.5% dimethyl sulfoxide, 0.2 mM each dNTP, 150 ng of genomic DNA, and 1.25 units of exo⁻ *Pfu* DNA polymerase (Stratagene). Amplification conditions consisted of an initial denaturation of 5 min at 98°C, followed by 35 cycles of 1 min at 98°C, 1 min at 65°C, and 2 min at 75°C. A final extension of 10 min was carried out at 75°C. After PCR, the amplified fragment was digested with *Pvu* II and then applied to a 4% acrylamide sequencing gel. Allele sizes were determined by comparing migration relative to an M13 sequencing ladder. For sequence analysis, the PCR fragment amplified by using 400 nM each of hF19 and hR21 under the same conditions was cloned into the *Sma* I site in pBluescript (Stratagene). Multiple isolates were sequenced to eliminate PCR artifacts.

DNA and RNA Preparation from Tissue Samples. Wilms tumors and corresponding normal kidneys were obtained from snap-frozen surgical specimens, and fetal tissue samples were obtained from the University of Washington Fetal Tissue Bank.

DNA was extracted from tissue samples by proteinase K/SDS treatment. Pea-sized tissue fragments were pulverized in liquid nitrogen and resuspended in 5 ml of TE9 (500 mM

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Abbreviations: LOH, loss of heterozygosity; LOI, loss of imprinting; BWS, Beckwith–Wiedemann syndrome; RT, reverse transcription; *IGF2*, gene for insulin-like growth factor II.
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Tris/20 mM EDTA/10 mM NaCl, pH 9.0). One hundred microliters of proteinase K at 10 mg/ml and 250 μl of 20% SDS were added per sample, followed by incubation overnight at 55°C. Samples were extracted twice with phenol/chloroform and once with chloroform. DNA was precipitated by the addition of 0.3 volumes of 10 M NH_4OAc and 2.5 volumes of ethanol. DNA pellets were washed with 70% ethanol, air-dried, and resuspended in 100–500 μl of TE (3 mM Tris/0.2 mM EDTA, pH 7.5). For RNA extraction, tissue samples were diced with a sterile scalpel, and RNA was isolated by using the MicroFastTrack mRNA isolation kit (Invitrogen) and resuspended in 50 μl of the buffer provided.

Characterization of p57^{KIP2} PAPA Polymorphism in Tissue Samples. DNA isolated from human tissue samples identified individuals heterozygous for a transcribed polymorphism which involves a hexanucleotide repeat within exon 1 of the p57^{KIP2} gene encoding a proline-alanine repeat called the PAPA repeat. Primers hF19 and hRT1 (5′-CGCTGATCTCT-TGCGCTTGGCGAAGAAATC) were used for PCR amplification of the hexanucleotides encoding the PAPA repeat domain of p57^{KIP2}. PCR reactions were performed in a final volume of 50 μl of buffer A (Stratagene) containing 100 ng of each primer, 0.25 mM each dNTP, 10% dimethyl sulfoxide (DMSO), and 1–2 units of *Pfu* exo$^+$ DNA polymerase (Stratagene). PCR reactions were carried out as follows with the Perkin–Elmer/Cetus 9600 PCR thermal cycler: 98°C for 5 min; 35 cycles of 98°C for 1 min, 60°C for 1 min, and 72°C for 2 min (35 cycles); and an extension at 72°C for 10 min. PCR products were digested with *Pvu* II and electrophoresed on a 6% nondenaturing polyacrylamide gel to resolve the polymorphic size difference in the PAPA repeat region.

Reverse Transcription (RT)–PCR Reactions for Analyzing Allele-Specific Expression. RT-PCRs were done with mRNA purified from informative (heterozygous) tissues as described above. mRNA (1–5 μl) was initially treated with DNase to remove contaminating DNA in a final volume of 30 μl of *Taq* PCR buffer (Boehringer Mannheim) containing 10 units of DNase and 0.5 units of RNase Inhibitor (5′-3′). DNase treatment was carried out at 37°C for 60 min, followed by inactivation of the enzyme at 95°C for 7 min.

DNase-treated mRNA was cooled to 65°C, followed by addition of the primer hRT1 (final concentration, 5 ng/μl) and incubation for 5 min. Reactions were then cooled to 53°C, followed by addition of stock RT solution (1 μl of ×10 *Taq* PCR buffer/8 μl of dNTPs at 1.25 mM each) and 1 μl of avian myeloblastosis virus (AMV) reverse transcriptase (Seikagaku America, Rockville, MD; 30 units/μl). RT reactions were carried out at 53°C for 60 min, followed by heat inactivation at 95°C for 7 min. RT reactions were purified by using Qiagen (Chatsworth, CA) Qiaquick PCR purification columns per kit instructions. RT products were eluted in 40 μl of 10 mM Tris (pH 8.3). Twenty microliters of the purified reaction mixture was then used in the PAPA repeat PCR reaction with hF19 and hRT1 as the primers.

RT-PCRs were initially analyzed by electrophoresing 5–10 μl of the 50-μl PCR reaction mixture on a 1% agarose gel to ensure that no contaminating genomic DNA had been amplified during the PCR. When no contamination was present, 4–17 μl of the PCR reaction was digested with *Pvu* II and analyzed on a 6% nondenaturing polyacrylamide gel. Corresponding PCRs of genomic DNA from the same tissue were run simultaneously to assay allele-specific expression. Gels were transferred to Genescreen (NEN/DuPont) by electroblotting with a Trans-Blot Cell apparatus (Bio-Rad). Blots were probed with an [α-^{32}P]dATP-labeled probe spanning the PAPA repeat region. Digital quantification of expression levels was done with a PhosphorImager (Molecular Dynamics).

RESULTS

Identification of a Transcribed Polymorphism in p57^{KIP2}. Sequence analysis of human p57^{KIP2} gene revealed a hexanucleotide repeat within exon 1 encoding a proline–alanine repeat termed "PAPA" repeat (11). PCR amplification and sequencing of this domain from 60 individuals revealed two common and two rare alleles of a length polymorphism in this repeat (Fig. 1). Analysis of reference kindreds showed Mendelian segregation of these alleles (Fig. 1). Sequence analysis revealed that the polymorphism represented deletion of two to eight codons in the PAPA domain (Fig. 1).

Imprinting of the p57^{KIP2} Gene in Developing Tissues. To determine whether one or both alleles were expressed in heterozygous individuals, RT-PCR was performed with primers that spanned the PAPA repeat and first intron (Fig. 2). Conditions were developed to isolate the PCR-amplified cDNA product to examine allele-specific expression. Use of primers spanning the first intron allowed for distinction be-

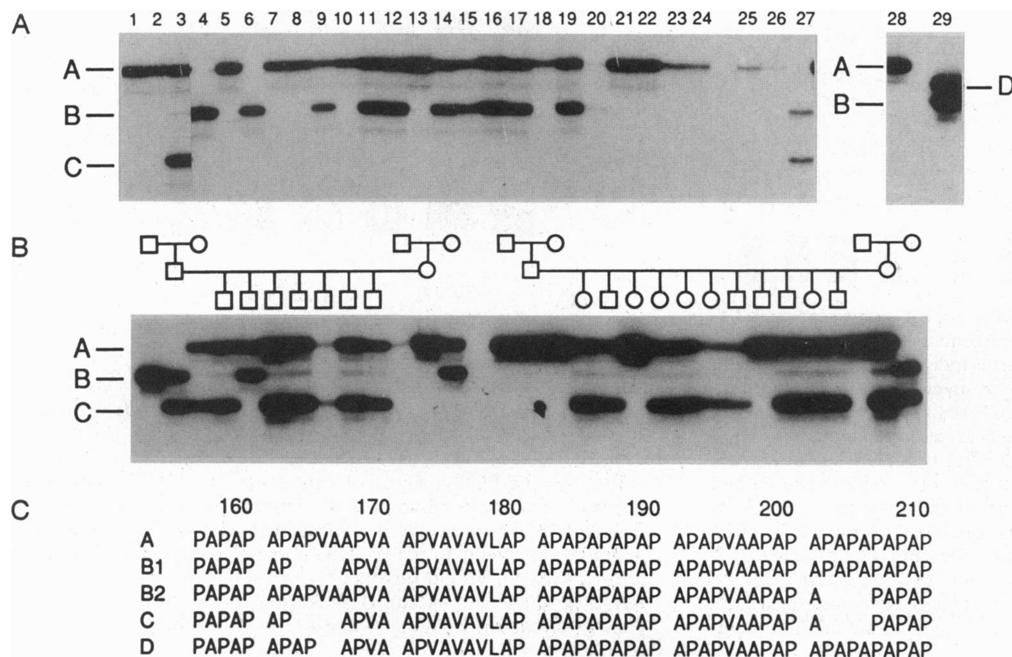

FIG. 1. Polymorphic nature of the PAPA repeat region of human p57^{KIP2}. (*A*) PCR analysis of the PAPA repeat region in a human population. Samples in lanes marked 1–27 were derived from Centre d'Etude du Polymorphisme Humain (CEPH) families. Samples 28 and 29 are from non-CEPH normal individuals. (*B*) PCR analysis of the PAPA repeat region in a normal family. The pedigrees shown represent CEPH families. (*C*) Amino acid sequence variations of the PAPA repeat region observed in the polymorphisms shown in *A*.

tween genomic amplification versus cDNA amplification; thus, genomic DNA contamination in the RT-PCRs could be easily ruled out.

DNA from 17 fetuses was analyzed for the p57^{KIP2} polymorphism, and 6 heterozygotes were identified. All 6 showed preferential expression of a single allele in most tissues, suggesting that the gene is imprinted in normal human development (Fig. 3, Table 1). In one case, maternal DNA from the decidua allowed assignment of the preferentially expressed allele as being of maternal origin.

The one exceptional tissue was brain, which showed comparable expression of the two alleles in both informative cases for which this tissue was available. The difference in expression levels of the two alleles in brain was ≈2-fold. Other tissues also showed some degree of expression of the other (presumably paternal) allele, although the degree of expression of that allele was much lower than seen in brain (Fig. 3, Table 1) and in many samples was virtually undetectable. Low-level expression of the (presumed) paternal allele was ≈1/20th of that of the corresponding maternal allele in all other nonbrain tissues, which was confirmed by reconstitution experiments at various dilutions of the two alleles (J.S.T., unpublished data).

To determine conclusively the parental origin of the expressed p57^{KIP2} allele, normal kidneys from Wilms tumor patients were examined, and, when possible, parental DNA was obtained from blood samples. From 53 Wilms tumor patients (and one hepatoblastoma patient), 15 informative normal kidneys were obtained for RT-PCR analysis. In all 15 samples, preferential expression of one p57^{KIP2} allele was observed, as found in the fetal samples (Fig. 4). Low-level expression of the paternal allele was observed in some normal kidney samples; however, the expression level of the maternal allele exceeded that of the paternal allele by an average ratio of 9:1. Of these 15 samples, parental identity of the expressed allele was determined directly from parental DNA of five of the patients (Fig. 4 *A* and *B*; Table 1), which verified maternal expression of p57^{KIP2}. Maternal expression of the p57^{KIP2} gene was determined for an additional sample on the basis of flanking markers on 11p15 (patient 19, Table 1), and it was inferred in the case of four other patients on the basis of presumed maternal loss of heterozygosity in the corresponding tumor (indicated in the legend to Table 1). Thus, maternal-specific expression of p57^{KIP2} gene was verified in 11 different normal individuals (7 direct, 4 inferred; 10 postnatal kidneys, 1 fetal specimen).

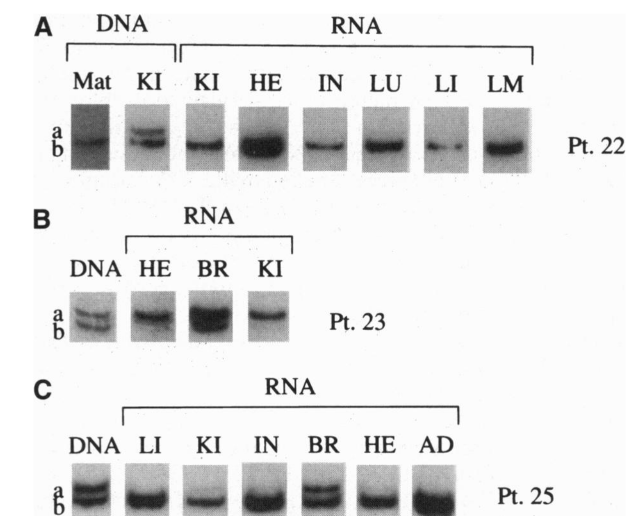

FIG. 3. RT-PCR analysis of expression of p57^{KIP2} from fetal tissue samples. mRNA samples isolated from heterozygous fetal tissue samples were analyzed by RT-PCR as described in *Materials and Methods* and as outlined in Fig. 2. Each set includes PCR of genomic DNA isolated from fetal tissues, along with RT-PCRs of RNA from the indicated tissues. KI, kidney; HE, heart; IN, intestine; LU, lung; LI, liver; LM, limb; BR, brain; AD, adrenal. A and B alleles are shown on the left of each panel. (*A*) Patient 22 (Table 1). Mat refers to maternal DNA isolated from accompanying decidua. (*B*) Patient 23 (Table 1). (*C*) Patient 25 (Table 1).

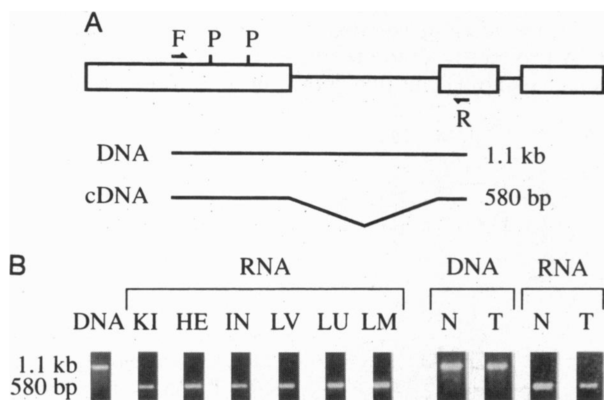

FIG. 2. Amplification of the PAPA repeat polymorphism. (*A*) Schematic representation of the p57^{KIP2} gene. Boxes indicate the three exons, with single lines between the boxes indicating the introns. F and R indicate primers hF19 and hRT1, respectively, utilized for both typing of tissue samples and for RT-PCR reactions. P indicates *Pvu* II sites used for digestion of PCR products as described in *Materials and Methods.* Below the gene is a map indicating the relative sizes of PCR products generated with either a genomic or a reverse-transcribed RNA template. (*B*) PCR products from either genomic DNA or reverse-transcribed RNA (cDNA), isolated from fetal tissues (first seven lanes) or from a tumor/normal kidney pair (last four lanes). KI, kidney; HE, heart; IN, intestine; LV, liver; LU, lung; LM, limb; N, normal kidney; T, Wilms tumor. Note that lack of genomic-sized PCR products in the cDNA lanes indicates that no genomic DNA contamination was present in the RT-PCRs.

Imprinting of p57^{KIP2} Gene in Wilms Tumors. Alteration of imprinting patterns has been observed previously in embryonal tumors. Specifically, the maternal copy of *IGF2* and *H19* have been found to undergo a conversion to a paternal

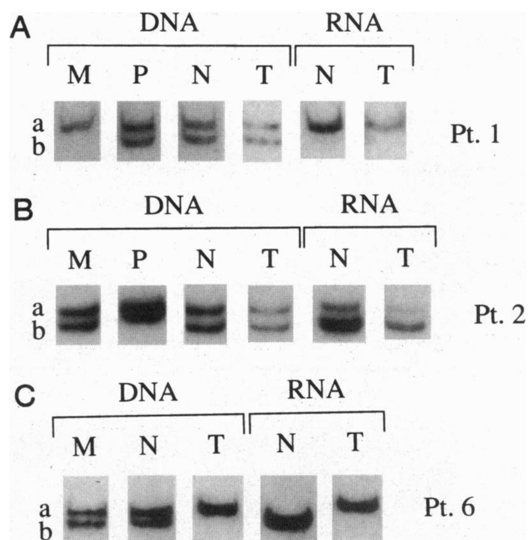

FIG. 4. RT-PCR analysis of expression of p57^{KIP2} from Wilms tumors and corresponding normal kidneys. mRNA from tumor and normal kidney was analyzed as described in Figs. 2 and 3. PCR of maternal (M) and paternal (P) DNA isolated from blood, DNA from normal kidney (N) and tumors (T), and RT-PCR reactions (labeled RNA) are indicated. A and B alleles are shown on the left of each panel. (*A*) Patient 1 (Table 1; non-LOH). (*B*) Patient 2 (non-LOH). (*C*) Patient 6 (LOH).

Table 1. Allele-specific expression of $p57^{KIP2}$ in normal tissues and embryonal tumors

Patient	Tissue	DNA	RNA
1	Normal kidney	A/B	$A_{mat}/-$
	Wilms tumor	A/B	$A_{mat}/-$
2	Normal kidney	A/B	$-/B_{mat}$
	Wilms tumor	A/B	$-/B_{mat}$
3	Normal kidney	A/B	
	Wilms tumor	A/B	$A_{mat}/-$
4	Normal kidney	A/C	
	Wilms tumor	A/C	A/C
5	Normal kidney	A/B	
	Wilms tumor	−/B	−/B
6	Normal kidney	A/B	−/B
	Wilms tumor	A/−	A/−
7	Normal kidney	A/B	−/B
	Wilms tumor	A/B	−/B
8	Normal kidney	A/B	−/B
	Wilms tumor	A/−	A/−
9	Normal kidney	A/B	A/−
	Wilms tumor	A/B	A/−
10	Normal kidney	A/B	−/B
	Wilms tumor	A/B	−/B
11	Normal kidney	A/B	
	Wilms tumor	A/B	$A_{mat}/-$
12	Normal kidney	A/B	
	Wilms tumor	A/B	−/B
13	Normal kidney	A/B	
	Wilms tumor	A/−	A/−
14	Normal kidney	A/B	−/B
	Wilms tumor	A/B	A/B
15	Normal kidney	A/B	$-/B_{mat}$
	Wilms tumor	A/−	$A_{pat}/-$
16	Normal kidney	A/B	$A_{mat}/-$
	Wilms tumor	A/B	$A_{mat}/-$
17	Normal kidney	A/B	−/B
	Wilms tumor	A/−	A/−
18	Normal kidney	A/B	−/B
19	Normal kidney	A/B	$A_{mat}/-$
20	Normal kidney	A/D	$A_{mat}/-$
	Wilms tumor	A/D	$A_{mat}/-$
21	Normal liver	A/B	A/−
	Hepatoblastoma	A/B	A/−
22	Fetal kidney	A/B	$-/B_{mat}$
	Fetal heart	A/B	$-/B_{mat}$
	Fetal intestine	A/B	$-/B_{mat}$
	Fetal lung	A/B	$-/B_{mat}$
	Fetal liver	A/B	$-/B_{mat}$
	Fetal limb	A/B	$-/B_{mat}$
23	Fetal heart	A/B	A/−
	Fetal brain	A/B	A/B
	Fetal kidney	A/B	A/−
24	Fetal lung	D/B	−/B
	Fetal gut	D/B	−/B
	Fetal kidney	D/B	−/B
	Fetal adrenal	D/B	−/B
	Fetal liver	D/B	−/B
	Fetal heart	D/B	−/B
25	Fetal liver	A/B	−/B
	Fetal kidney	A/B	−/B
	Fetal intestine	A/B	−/B
	Fetal brain	A/B	A/B
	Fetal heart	A/B	−/B
	Fetal adrenal	A/B	−/B
26	Fetal lung	A/B	−/B
	Fetal spinal cord	A/B	−/B
	Fetal intestine	A/B	−/B
	Fetal liver	A/B	−/B
	Fetal kidney	A/B	−/B
27	Fetal intestine	A/B	−/B
	Fetal kidney	A/B	−/B
	Fetal heart	A/B	−/B
	Fetal adrenal	A/B	−/B
	Fetal ovary	A/B	−/B

Analysis of $p57^{KIP2}$ expression from informative individuals based on the PAPA repeat polymorphism. Relevant tissue is indicated in the second column. $p57^{KIP2}$ alleles determined from DNA typing are indicated in the third column. A dash indicates loss of heterozygosity in the tumor. Expression of alleles from RT-PCR analysis is indicated in the column marked RNA. In the cases where parental identity of the alleles could be determined on the basis of parental DNA informativity, the term "mat" is marked next to the expressed allele to designate it as of maternal origin, or "pat" to designate paternal origin. Parental identity of alleles from patients 1, 2, 3, 11, 15, 16, and 22 were determined directly from informativity of the $p57^{KIP2}$ PAPA polymorphism from parental DNA. Parental identity of patient 19 was determined by informativity of flanking polymorphic markers on 11p15. All other LOH tumors without designations are assumed to be maternal LOH.

epigenotype, resulting in biallelic expression of *IGF2* and loss of expression of *H19* (16, 17). Given these observed changes in tumors and the potential connection between $p57^{KIP2}$ and cancer, we examined both non-LOH and LOH Wilms tumors to determine if imprinting of $p57^{KIP2}$ gene was altered.

Twelve non-LOH Wilms tumors informative for $p57^{KIP2}$ were identified and examined for the imprinting status of $p57^{KIP2}$ gene. As found for the normal kidney samples, monoallelic expression was observed in nearly all tumors, with maternal identity verified in six of the tumors (Fig. 4 *A* and *B*; Table 1). Some expression of the paternal allele was also observed in these tumors, with an average maternal/paternal expression ratio of 6:1 (comparable to the corresponding normal kidneys). Of the 12 tumors, 10 displayed a normal pattern of imprinting compared to normal kidney, while 2 of the tumors showed nearly equal biallelic expression of $p57^{KIP2}$, with a <2-fold difference in expression between the two alleles, suggesting LOI. While LOI of *IGF2* and *H19* is common in Wilms tumors (≈70%), LOI of $p57^{KIP2}$ appears to be a relatively rare event. One non-LOH hepatoblastoma was also examined, and normal imprinting was observed.

A prediction of the imprinted tumor-suppressor gene model is that LOH, which preferentially affects the maternal allele, would result in loss of expression of the gene (3). However, since $p57^{KIP2}$ gene is incompletely silenced by imprinting, we did not expect to see complete loss of expression in maternal LOH tumors. In six Wilms tumors informative for the $p57^{KIP2}$ polymorphism and showing LOH, preferential expression of the maternal allele was observed in the normal tissue, but maternal allele expression was absent in the tumor samples (Fig. 4*C*). However, expression of the paternal allele was observed in these tumors, indicating, as expected, that loss of the normally expressing maternal allele does not result in complete loss of $p57^{KIP2}$ expression. Since the RT-PCR assay employed was not quantitative, we are not able to draw a conclusion concerning the level of expression from the paternal chromosome.

DISCUSSION

In summary, the $p57^{KIP2}$ gene is imprinted in humans, the first cell cycle-regulatory gene found to be regulated by this mechanism. While the maternal allele shows preferential expression, the paternal allele is also expressed at low levels in most tissues, but at levels comparable to the maternal allele in developing brain and some embryonal tumors. Low-level

expression of the paternal allele may reflect incomplete repression of that copy, although conceivably a small subpopulation of cells within a tissue may not be imprinted.

The identification of imprinting of a cyclin-dependent kinase inhibitor is a surprising result. If p57^{KIP2} is a bona fide tumor suppressor or growth regulator, monoallelic expression would seem to increase the risk of developmental malformation and cancer. Perhaps p57^{KIP2} is most important during embryogenesis, thereby making the temporal window for mutation small. It may contribute only marginally to maintenance of the nonproliferative state in adult tissues and thus not be a significant suppressor of growth. Alternatively, it is possible that its levels are tightly controlled and that the weak expression of the paternal allele may be enhanced when the maternal allele is lost in adult tissues, allowing it to escape the effects of imprinting to some degree. These experiments will be best addressed when mice lacking p57^{KIP2} are available. The parental specificity of the imprint, with expression of the maternal allele of p57^{KIP2}, is consistent with the general paradigm that imprinted genes expressed from the maternal allele tend to inhibit cell growth and may have arisen to mediate maternal–fetal conflicts for nutrients, as proposed by Haig and Graham (18).

The localization of the p57^{KIP2} gene to 11p15, its maternal-specific expression, and its biochemical potential for growth regulation match the properties predicted for a tumor suppressor involved in embryonal tumors and BWS. We have found that the p57^{KIP2} gene lies within 100 kb of a cluster of five maternal balanced chromosomal rearrangement breakpoints from BWS patients (19), and its expression in BWS might be influenced by these breakpoints. It is also of interest that p16, another cyclin-dependent kinase inhibitor involved in some familial melanomas (20), shows decreased expression and increased methylation in some tumors in which it is not mutated (21). Thus, it will be important to determine whether other cdk inhibitors are also imprinted.

Third, these results, combined with our localization of p57^{KIP2} gene on an 11p15 cosmid contig (19), indicate that there is a 450- to 500-kb domain extending from p57^{KIP2} gene to *H19*, containing multiple imprinted genes. This is supported not only by the relative proximity of these genes, but also by the fact that *IGF2* and *H19*, like p57^{KIP2} gene, are not imprinted in some brain tissues (22). Not all genes within this domain are necessarily imprinted, as observed in the Prader–Willi syndrome region of 15q11-12 (23), but it seems likely that other imprinted genes within this domain will be identified that may contribute to embryonal tumors and BWS.

Finally, these data suggest that establishment of an imprint may be coordinately regulated throughout the entire domain, as suggested by similar tissue-specific expression and imprinting patterns of *IGF2*, *H19*, and p57^{KIP2} gene (ref. 11 and this report), while loss of imprinting may not necessarily affect the entire region. LOI of *IGF2/H19* involves an epigenetic switch of the maternal allele to a paternal epigenotype (16). We find that LOI of *IGF2/H19* is not necessarily linked to the LOI of the p57^{KIP2} gene [patients 1, 2, and 11 show a LOI of *IGF2* (6, 16) but not of the p57^{KIP2} gene]. Thus, while imprint establishment may occur across 11p15 by a common mechanism (analogous to X-chromosome inactivation), LOI may reflect a breakdown in imprint maintenance, which may be regulated independently for each gene.

Note Added in Proof. After submission of this manuscript, imprinting of p57^{KIP2} in mouse was reported (24).

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