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Invited Review

Genomic imprinting and carcinogenesis

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Summary. The Mendelian inheritance is based on the fundamental rule in which mammalian genes are expressed equally from two homologous biparental alleles. Recently a small number of genes have been identified to show an exception to this rule in that homologous alleles can function differently in somatic cells depending on whether they come from the mother or the father. This intriguing biological phenomenon is called as genomic imprinting which does not conform classical Mendelian inheritance and has potentially far reaching implications for genetics, evolution, developmental biology and pathology including cancer. The gene encoding insulin-like growth factor 2 (*IGF2*) harbors at 11p15.5 and serves as paradigm for an imprinted gene. The *IGF2* gene has been demonstrated to be imprinted with the paternal allele expressed and the maternal being silent which is evolutionally conserved between mice and human. Loss of imprinting (LOI) of *IGF2* has been demonstrated in a dozen of tumor types including Wilms tumor (WT) with a promise of many more to come. The LOI of *IGF2* may induce increased or deregulated *IGF2* expression which could initiate the onset of WT. Thus the LOI of *IGF2* may provide a novel mechanism of gene activation and play a role in the development of a wide range of tumors. This review also discusses other imprinted genes on 11p15 which may have a role in WT or other diseases. Finally molecular mechanisms of genomic imprinting are discussed.

Key words: Genomic imprinting, Carcinogenesis, Loss of imprinting, Wilms tumor, Insulin-like growth factor 2

1. Introduction

The concept that both maternal and paternal genomes are essential for complete and undisturbed development came from the observations of aberrant development of embryos after experimental induction of parthenogenesis in mice (McGrath and Solter, 1984; Surani et al., 1984). Development of gynogenetic (diploid maternal, equivalent to benign cystic teratoma of the ovary in human) or androgenetic (diploid paternal, equivalent to complete mole in the gestational trophoblastic disease in human) embryos were severely disturbed. The former were relativley normal in size and appearance up to the 25 cell stage but showed poorly developed extraembryonic membrane whereas the latter showed reciprocal phenotype.

Mouse genetic experiments have been used to demonstrate that duplication of certain chromosomes from one parent with loss of the homologue from the other parent, or uniparental disomy, result in embryonic lethality (Cattanach and Beechey, 1995). This has allowed the assignment of imprinted or non-imprinted regions of the mouse genome. A recently developed method is the restriction landmark genome scanning which allows to analyze allele specific differences in CpG methylation pattern. This allows speculation that the total number of imprinted genes in the whole genome are less than 200 (Hayashizaki et al., 1994). A dozen of imprinted genes, so far, are identified in human which are shown in Table 1. These imprinted genes have been shown to play an important role during prenatal development. Accumulative evidence indicate that aberrant imprinting of these genes is also implicated in the aetiology of an increasing number of diseases, including cancer (Hall, 1990). This review will primarily focus on the development of Wilms tumor (WT) in view of genomic imprinting.

2. Genomic imprinting and gene activation

2.1. Loss of imprinting (LOI)

2.1.1. Increased *IGF2* expression in WT

WT is a malignant tumor of the kidney and the most common abdominal solid tumor of childhood, occurring at an estimated frequency of 1 in 10,000 live births. Distinctive histological features are characterized by its striking similarities to embryonic nephrogenesis and by diversity of cell types, tissue patterns and degrees of differentiation (Fig. 1A).

Over the several years our major goal is to understand molecular mechanisms which underlie the

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Table 1. Human imprinted genes identified.

LOCUS	CHROMOSOME	ACTIVE ALLELE	GENE FUNCTION	REFERENCES
IGF2	11p15.5	Pat	Growth factor	Ogata et al., 1993; Rainier et al., 1993;
H19	11pt 5.5	Mat	IGF2 controller	Zhang and Tycko, 1992
P57KIP2	11p15.5	Mat	Cell cycle repressor	Hatada et al., 1996a
KVLQT1	11p15.5	Mat	Potassium channel	Lee et al., 1997
WT1	11p13	Mat	Tumor suppressor	Jinno et al., 1994
SNRPN	15q11.2-q12	Pat	Spliceosome component	Leff et al., 1992
ZNF127	15q11.2-q12	Pat	Transcription factor	Glenn et al., 1997
PAR1	15q11.2-q12	Pat	Unknown	Glenn et al., 1997
PAR5	15q11.2-q12	Pat	Unknown	Glenn et al., 1997
IPW	15q11.2-q12	Pat	Unknown	Wevrick and Francke, 1994
IGF2R	6q25-q27	Mat	iGF2 antagonist	Ogawa et al., 1993

development of WT. One of the intriguing findings in WT is the increased expression of the insulin-like growth factor 2 gene (*IGF2*) (Reeve et al., 1985; Wilkins et al., 1989; Yun et al., 1993b). Increased *IGF2* expression has been reported in a variety of embryonal tumors, suggesting its wider role in childhood tumors (Yun, 1993a,b; Sharifah and Yun, 1994; Sharifah et al., 1995a,b). *IGF2* transcripts were 32-64 fold more abundant in WT than in normal kidney (Fig. 1B). Whether or not the increased *IGF2* expression is a cause or effect of the development of WT remains to be clarified. However, cumulative evidence strongly suggests that *IGF2* might act as a transforming gene in WT. The evidence includes: (a) the *IGF2* gene product, a 67 amino acid secreted polypeptide, displays mitogenic properties *in vitro*, a characteristic anticipated for a

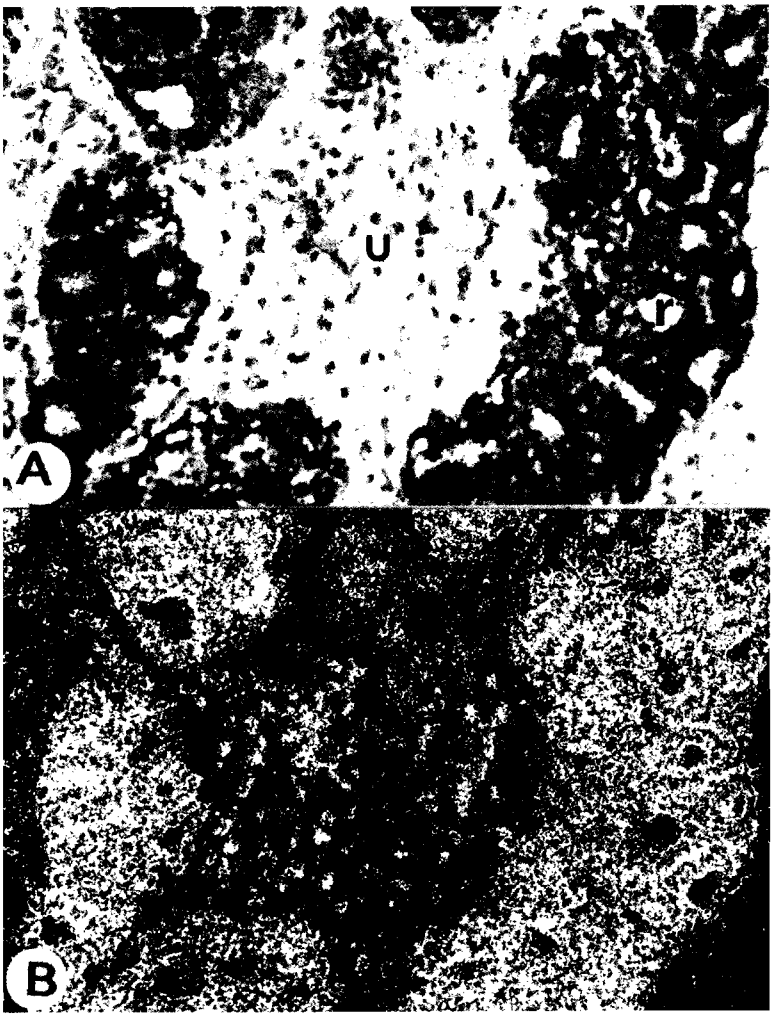

Fig. 1. A. A bright-field micrograph of a typical triphasic WT histology. u: undifferentiated blastema; b: blastema; r: renal vesicle-type epithelial differentiation. x 135. **B.** A dark-field micrograph of *in situ* mRNA hybridization for *IGF2* transcripts in WT. x 135

putative transforming gene product (Humbel, 1990); (b) high levels of *IGF2* transcripts are present in the undifferentiated cells of both fetal kidney and WT (Wilkins et al., 1989; Yun et al., 1993b); (c) antibodies binding to IGF1 receptors, through which *IGF2* protein is thought to exert its mitogenic activity, inhibit growth of WT derived cell lines, while antibodies against the *IGF2* protein itself inhibit the growth of rat fetal kidney *in vitro* (Gansler et al., 1989); (d) the human *IGF2* gene is located at 11p15.5 a region of the human genome thought to encode a second gene involved in the development of WT (Coppes et al., 1993); and (e) the pattern of *IGF2* expression in WT is different from that in fetal kidney (Yun et al., 1993b). These evidence indicate that *IGF2* expression in WT does not simply reflect the embryonal nature of the tumor but is rather significantly altered, suggesting a role as a transforming growth factor in Wilms tumorigenesis (Yun et al., 1993b).

2.1.2. Genomic imprinting of the *IGF2* gene

The term 'genomic imprinting' has been used to describe a number of different observations to fall into 4 broad classes, namely: (a) differential phenotypic effects of parental alleles; (b) monoallelic expression; (c) allele specific methylation; and (d) monoallelic genomic changes (Surani, 1993). In all cases, the allele inherited from fathers behave differently from those inherited from mothers: even though they may be genetically identical, they are epigenetically different. In humans, the phenomenon of genomic imprinting is implicated in the aetiology of an ever-increasing number of diseases, such as Huntington's chorea, myotonic dystrophy, fragile X syndrome, Beckwith-Wiedemann syndrome (BWS), Prader-Willi syndrome (PWS) and Angelman syndrome (AS) including cancer (Hall, 1990; Caskey et al., 1992).

Evidence which strongly suggest that the human *IGF2* gene could be imprinted came from mouse genetic studies. Using a mouse *IGF2* knockout model, DeChiara et al. clearly demonstrated monoallelic *IGF2* expression exclusively transcribed from the paternal allele in all tissue examined except for the leptomeninges and the choroid plexus where the expression is biparental (DeChiara et al., 1991). Whether or not the human *IGF2* gene was also imprinted had to wait until a Japanese group reported an *ApaI* (*HaeIII*) polymorphic site in exon 9 of the human *IGF2* gene (Fig. 2), whereby enabling to carry out allele-specific *IGF2* expression analyses using reverse transcriptase-polymerase chain reaction (RT-PCR) combined with ApaI restriction fragment length polymorphism (RFLP) analyses (Tadokoro et al., 1991). Using this technique we were first among two groups which demonstrated that *IGF2* is expressed exclusively from the paternal allele (Fig. 3), indicating that the parental imprinting of *IGF2* is evolutionally conserved from mouse to man (Ogawa et al., 1993; Rainier et al., 1993). Furthermore, we also reported that in 40-50% of WTs with retention of heterozygosity (ROH) at the *IGF2* locus, normal imprinting is relaxed and gene expression is biallelic (Fig. 3), namely, loss of imprinting (LOI) (Feinberg, 1993). Subsequently LOI of *IGF2* has been detected in a number of childhood tumors which include rhabdomyosarcoma (RMS) (Scrable et al., 1989; Zhan et al., 1994), Ewing sarcoma (Zhan et al., 1995), hepatoblastoma (Rainier et al., 1995), congenital mesoblastic nephroma (Becroft et al., 1995) and clear cell sarcoma of the kidney (Sohda et al., 1997).

LOI of *IGF2* is also reported in common adult tumors including lung (Suzuki et al., 1994), breast (McCann et al., 1996; Yballe et al., 1996; Soejima et al., 1998) cervical cancer (Douc-Rasy et al., 1996) and gestational trophoblastic diseases such as mole and choriocarcinoma (Walsh et al., 1995). These data suggest that LOI of *IGF2* may offer a novel mechanism for carcinogenesis, where *IGF2* may be re-activated and result in a transforming growth factor.

2.1.3. Promoter specific *IGF2* gene imprinting

The human *IGF2* gene is a complex transcription unit that contains 4 different promoters (P1-P4) (Fig. 2). The *IGF2* gene promoters are subjected to developmental and tissue-specific regulation of expression (Sussenbach et al., 1993). The differential promoter usage between the hepatoma cell line, Hep3B and the

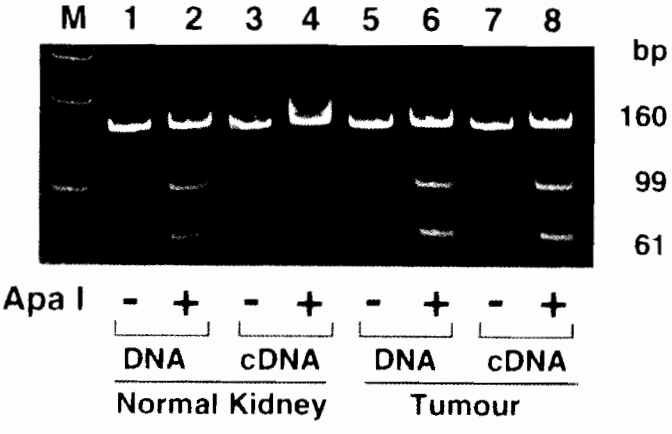

Fig. 3. *IGF2* allele-specificity analysis of WT and kidney. Lanes 1, 2 and 3, 4 show genomic DNA PCR and cDNA PCR with (+) or without (-) Apa1 digestion, respectively from the normal kidney tissue adjacent to the tumor. The normal kidney demonstrates monoallelic *IGF2* expression (lane 4). A same analysis is carried out in WT tissue (lane 5-8) which demonstrate biallelic *IGF2* expression (lane 8).

Fig. 2. A schematic drawing of the *IGF2* gene on 11p15.5. Shaded exons show *IGF2* protein coding region. Note 4 different promoters (P1-P4). An ApaI (HaeIII) polymorphic site is present in exon 9.

colon carcinoma cell line, SW631 has been reported (Schneid et al., 1993). These results indicate that regulation of these promoters plays an important role in the control of *IGF2* gene expression and *IGF2* synthesis. It is demonstrated in the adult liver that *IGF2* gene imprinting is promoter specific (Vu et al., 1994; Taniguchi et al., 1995; Yun et al., 1998). In the early human embryo *IGF2* transcripts expressed from the promoter P1-P4 are exclusively derived from the paternal allele (Fig. 4A). As development proceeds expression from the P1 promoter becomes biallelic while those from P2-P4 remain biallelic (Fig. 4B). These studies suggest that there may be a local cis-acting imprinting switch between the promoter P1 and P2-P4 (Vu et al., 1994). The promoter specific *IGF2* gene imprinting has also been investigated in WT (Taniguchi et al., 1995a,b). In WT with *IGF2* LOI, all transcripts from the promoter P1-P4 are biallelic (Fig. 4C) whereas, in WT without LOI those are monoallelic (Fig. 4D). In addition WT with 11p maternal loss of heterozygosity (LOH) is usually associated with duplication of paternal chromo-some which leads to monoallelic *IGF2* expression from P1-P4 with double gene dosages (Fig. 4E).

2.1.4. Role of *IGF2* LOI in cancer

An important question to be addressed is how LOI of *IGF2* contributes to tumorigenesis. Whether WT with LOI of *IGF2* expresses higher levels of *IGF2* transcripts than WT without LOI remains to be determined. This interesting question has been addressed in Ewing sarcoma and breast cancer by Helman et al. who report that LOI of *IGF2* is not associated with increased *IGF2* expression (Zhan et al., 1994, 1995). The authors suggest that LOI may not be involved in the regulation of *IGF2* expression and may be related to genetic or epigenetic abnormalities in tumors independent of *IGF2* expression.

Regarding a role of LOI of *IGF2* in tumorigenesis we have reported an interesting BWS case (Matsumoto et al., 1994) which is an overgrowth syndrome and chracterized by the EMG triad (exomphalus, macroglossia and gigantism) and predisposition to a variety of cancer. This BWS child developed RMS and renal cell carcinoma (RCC). Karyotypes of peripheral lymphocytes and RMS cells were normal. DNA analyses showed maternal LOH at 11p15 region in RMS but not in RCC. *In situ* mRNA hybridization demonstrated a high level of *IGF2* transcripts in RMS but not in RCC. Allele-specific analyses demonstrated LOI of *IGF2* in RCC and in peripheral lymphocytes, whereas RMS showed monoallelic *IGF2* expression because of maternal LOH. These data have important implications. a) The finding of LOI of *IGF2* in RCC and peripheral lymphocytes is consistent with reports that BWS

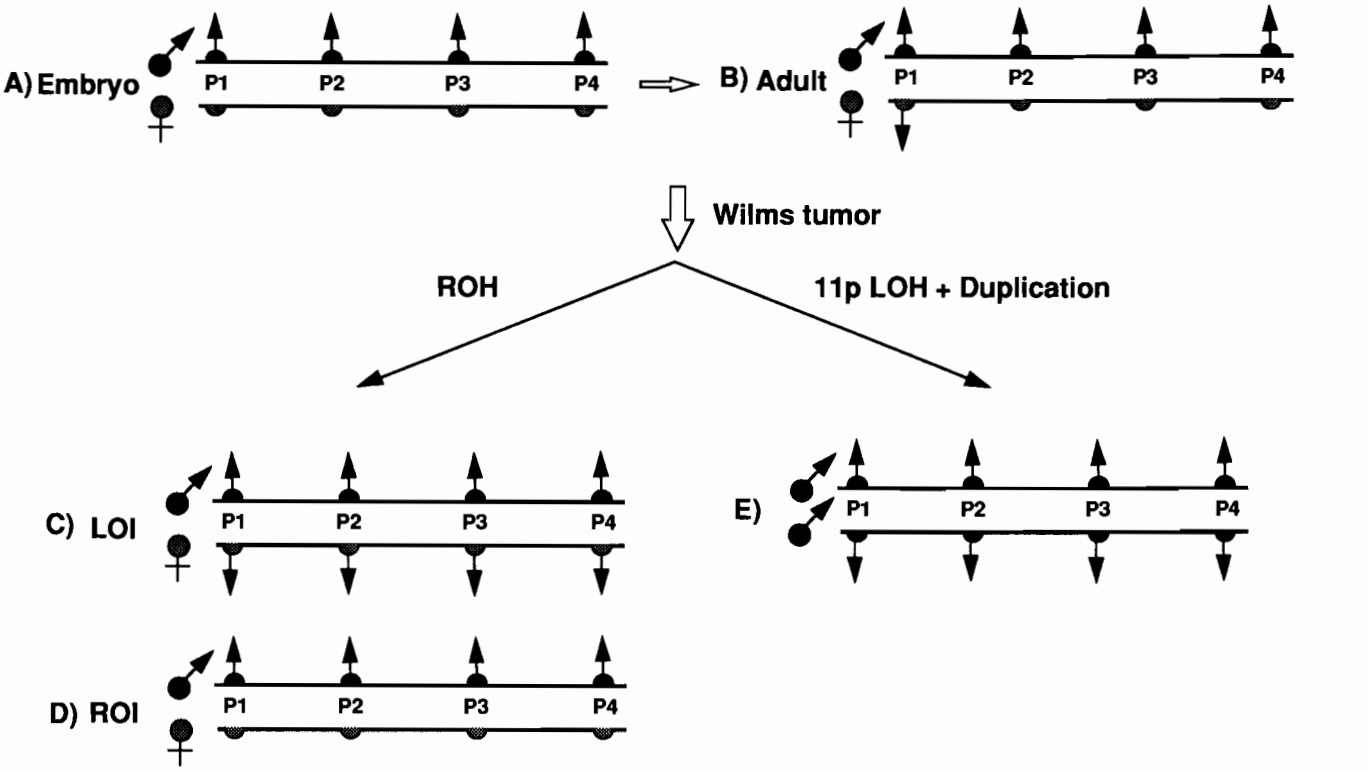

Fig. 4. Promoter-specific *IGF2* gene expression in early fetus, adult and WT (modified from Taniguchi et al., 1995). ROH: retention of heterozygosity; LOH: loss of heterozygosity; LOI: loss of imprinting; ROI: retention of imprinting.

Table 2. Preferential parental allelic alteration in cancer (modified from Freinberg, 1993).

CANCER	CHROMOSOME	LOCUS	ALTERATION	ALLELE AFFECTED	REFERENCE
Wilms tumor	11p15	?	LOH	Mat	Schroeder et al., 1989
Rhabdomyosarcoma	11p15	?	LOH	Mat	Scrable et al., 1989
Osteosarcoma	13q14	*RB*	LOH	Mat	Toguchida et al., 1989
Bilateral retinoblastoma	13q14	*RB*	LOH	Mat	Dryja et al., 1989
Unilateral retinoblastoma	13q14	*RB*	LOH	Mat	Leach et al., 1990
AML	7q31-q36	?	LOH	Pat	Katz et al., 1992
CML	9q, 22q	*ABL, BCR*	Translocation	Pat *ABL*, Mat *BCR*	Haas et al., 1992
Neuroblastomas	1p36	?	LOH	Mat	Caron et al., 1993
Neuroblastoma	2p24	*N-MYC*	Amplification	Pat	Cheng et al., 1993

children are often associated with LOI of *IGF2* (Weksberg et al., 1993). However, *in situ* hybridization clearly demonstrated almost no *IGF2* transcripts in RCC, which strongly suggests that LOI itself may not induce increased *IGF2* expression. On the other hand, abundant *IGF2* transcripts were detected in RMS which showed monoallelic *IGF2* expression because of maternal LOH at 11p15 region. Since the karyotype in RMS was normal, there had to be duplication of paternal chromosome at 11p15 region, indicating a double dosage of the *IGF2* gene, of which status is similar to LOI of *IGF2* (Fig. 4E). b) Therefore, it is conceivable to speculate that LOI of *IGF2* may significantly contribute to tumorigenesis when tumors contain transcriptionally active the *IGF2* gene. When the *IGF2* gene is not active in tumors, whether LOI or ROI of *IGF2* may not be important. The latter case most likely fit adult tumors including lung, breast and ovarian cancer in which the levels of *IGF2* transcripts are very low. This speculation concurs with previous reports which showed no correlation between LOI of *IGF2* and its expression level (Zhan et al., 1995; Yballe et al., 1996). c) A next question is then what types of tumor contain the active *IGF2* gene. These tumors would most likely be embryonal-type tumors whose precursor cells at the developmental stage produce high levels of *IGF2* transcripts, such as metanephric blastema (WT), skeletal muscle (RMS), fetal hepatocytes (hepatoblastoma) and so on.

3. Genomic imprinting and gene inactivation

3.1. Preferential loss of heterozygosity (LOH)

The Knudson two-hit hypothesis (Knudson and Strong, 1972) offered a theoretical model where tumor suppressor genes would play a vital role in cancer. The discovery of the RB gene served as a paradigm of a tumor suppressor gene which is inactivated by two events, namely mutation of one allele followed by loss of the second functional allele in somatic cells (Dryja et al., 1989). Thus tumor tissue becomes homozygous for DNA markers located on the chromosome that harbors the first mutation, namely LOH. LOH studies using polymorphic DNA markers indicate the presence of tumor suppressor genes of particular loci involved in the onset of tumors.

In sporadic tumors most of which thought to be a consequence of somatic mutations, the maternal and the paternal gene would be expected to have an equal chance of carrying the mutations. However there have been accumulative evidence indicating that this expectation is not a case. In most sporadic cases of both WT (Schroeder et al., 1989) and osteosarcoma (Toguchida et al., 1989), it is the parental chromosome that is retained in the tumor, the maternal one being lost. The preferential parental allelic deviation of LOH is also demonstrated in other tumors (Table 2), strongly suggesting that genomic imprinting may have a role in the onset of tumors. If a tumor suppressor gene is subject to genomic imprinting, a single event, namely, loss of maternal active allele might be a sufficient alteration at this locus to predispose to tumor. Therefore only one hit is required.

Alternatively, it could be possible that imprinting status of a tumor suppressor gene is polymorphic among human populations as has been suggested by Sapienza (1990). Under this circumstance some may show biallelic expression of a tumor suppressor gene whereas others show monoallelic expression. In this setting, the latter would be more susceptible to tumor than the former.

3.2. Candidate imprinted tumor suppressor genes in WT

LOH at 11p13 and 11p15 are detected in approximately 20% and 40% of WTs, respectively, which suggest that there are at least two WT loci on 11p: (a) *WT1* at 11p13, associated with the WAGR (WT, aniridia, genitourinary abnormalities, mental retardation) syndrome (Call et al., 1990; Gessler et al., 1990); (b) a second major gene at 11p15.5, associated with BWS. (Reeve et al., 1984, 1989). Here candidate tumor suppressor genes which are located at 11p and subject to imprinting will be described.

3.2.1. *WT1*

A tumor suppressor gene cloned from 11p13 is *WT1*. *WT1* has been demonstrated to be mutated in 5-10% of sporadic WTs, which is much lower than anticipated (Little et al., 1992). Sequence analysis indicates that *WT1* protein contains four zinc fingers and shares

homology with early growth response (EGR) protein, suggesting a role as a DNA-binding transcription control factor (Call et al., 1990; Gessler et al., 1990). *In vitro* binding studies and cotransfection CAT assays have confirmed that *WT1* protein does bind to the consensus EGR-1 or PDGF-α binding sequence to repress transcription from an associated promoter (Rauscher et al., 1993). In accordance with the finding, we have shown that, in fetal kidney, the expression of *WT1* is reciprocal to that of *IGF2*, suggesting that *WT1* protein may have a role in repressing *IGF2* gene transcription (Yun et al., 1993a; Grubb et al., 1994).

Initially *WT1* has been speculated to be a possible candidate for the imprinted tumor suppressor gene. It has been, however, demonstrated that this is not a case since *WT1* is biallelically expressed not only in kidney but also in WT (Huff et al., 1990; Little et al., 1992). We have, however, demonstrated that *WT1* is imprinted in a tissue specific manner where pre-term placenta and brain show monoallelic expression from the maternal allele while kidney shows biallelic expression (Jinno et al., 1994). Therefore, although *WT1* does not seem to be involved in the onset of WT as an imprinted gene, the possibility where aberrant imprinting of *WT1* may play a role in other types of tumors exists and should be tested.

3.2.2. *H19*

The *H19* gene is one of the first genes shown to be imprinted and codes for a spliced and polyadenylated RNA which is highly expressed in a large variety of fetal tissue at a stage when cells are differentiating (Bartolomei et al., 1991; Poirier et al., 1991). Because of the apparent lack of evolutionally conserved open reading frames, the protein-coding potential of *H19* RNA is uncertain. In part for this reason it has been proposed that this gene may act directly at the level of its RNA, perhaps through the formation of ribonucleoprotein particle (Brannan et al., 1990). *H19* maps to chromosome 11 in close proximity of the *IGF2* locus (Fig. 5). The *H19* gene shows a similar spatial pattern of expression to that of *IGF2* with the particular exception of the choroid plexus and leptomeninges where *IGF2* is biallelically expressed and *H19* is suppressed (DeChiara et al., 1991; Ohlsson et al., 1994). These genes are reciprocally imprinted with *H19* expression being maternal and *IGF2* paternal (Jinno et al., 1995). Based on these observations of the *H19* and *IGF2* genes, an "enhancer competition model" has been proposed as a mechanism of monoparental expression of the genes (Bartolomei et al., 1993), which will be described in the next section.

H19 RNA overexpression in G401 cells (a cell line from a malignant rhabdoid tumor of the kidney) has been shown to reduce tumor growth *in vivo* (Hao et al., 1993) but the authors did not exclude the possibility that a high levels of *H19* RNA, which is not normally expressed in these cells, may as many ectopically proteins do exert nonspecific cytostatic effects on cells. There appears no reports, so far, which demonstrate mutations or deletions of the *H19* gene itself and the promoter region of *H19* in WT (personal communication). These data suggest that, although *H19* plays a role in the control of *IGF2* expression, it may not be a tumor suppressor gene implicated in WT.

3.2.3. *P57^{KIP2}*

p57^{KIP2} (KIP2) is a cyclin-dependent kinase inhibitor which induces cells to arrest in G1 (Lee et al., 1995; Matsuoka et al., 1995). Recent experimental data show that *KIP2* is imprinted and expressed preferentially from the maternal allele except in the brain (Hatada et al., 1996a; Matsuoka et al., 1996). Since this gene was mapped to the centromeric region of 11p15.5 (Matsuoka et al., 1995), *KIP2* may be a candidate tumor suppressor gene in WT (Fig. 5). Available evidence have been conflicting but may not be mutually contradictory. Data suggesting *KIP2* as a tumor suppressor gene include: 1) A nonsense mutation of *KIP2* maternal allele in Cdk inhibitory domain has been reported in a patient of BWS which predisposes WT (Hatada et al., 1996b). 2) *KIP2* expression has been reported to be reduced in some WTs (Chung et al., 1996; Matsuoka et al., 1996) or virtually absent in all WTs (Thompson et al., 1996). 3) *KIP2* overexpression is known to arrest cells in G1 (Matsuoka et al., 1995). On the other hand, data which do not support include: 1) In WTs with LOH, *KIP2* is expressed from the remaining paternal allele (Matsuoka et al., 1996; Chung et al., 1996). 2) In the variety tumors analyzed by PCR-SSCP analysis and DNA sequencing, no mutation has been detected in *KIP2* (Kondo et al., 1996; Orlow et al., 1996). 3) *KIP2* expression has been reported to show little correlation with tumor suppression in a G401 hybrid cell assay which detects *KIP2* transcripts in both tumorigenic and non-tumorigenic G401 hybrid lines (Reid et al., 1996). Our data using RT-PCR and *in situ* hybridization analyses suggested that although *KIP2* may play a role in differentiation of fetal kidney and WT, it may not likely be a tumor suppressor gene implicated in WT.

3.2.4. *KVLQT1*

The fourth imprinted gene which has been recently cloned from the region between *IGF2* and *p57^{KIP2}* on

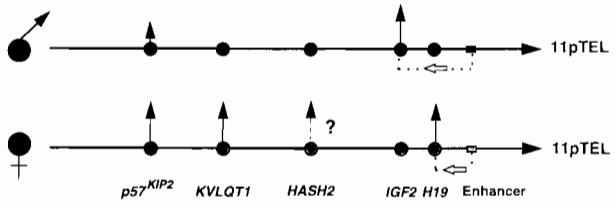

Fig. 5. Different imprinted genes on 11p15. Enhancer competition model predicts that enhancers induce paternal *IGF2* and maternal *H19* gene reciprocal monoparental expression.

11p15.5 is *KVLQT1*, a gene encoding for a putative potassium channel (Fig. 5). The deletion-insertion mutation (1244, -7 +8) of this gene has been identified in patients of Jervell and Lange-Nielson (JLN) syndrome which is an inherited autosomal recessive disease characterized by congenital bilateral deafness associated with QT prolongation, syncopal attacks due to ventricular arrythmia and a risk of sudden death (Neyroud et al., 1997). *KVLQT1* has been shown to be preferentially monoallelically expressed from the maternal allele in all tissue except for the heart where it is biallelically expressed (Lee et al., 1997). *KVLQT1* transcripts appear to consist of 4 different isoforms among which isoform 1 is expressed in all tissue except for the heart where isoform 1 and 2 are expressed. It is intriguing that this gene encompasses chromosomal break points observed in patients of BWS which is distinct from JLN syndrome. How defects in a potassium channel leading to cardiac arrhythmia also produce such overgrowth abnormalities remains to be seen. Mannens and Wilde speculate that a region critical to BWS contains an imprinting control center, similar to the recent data on the PWS/AS region on 15q11-q12, whose disruption would lead to relaxation of other imprinted genes on 11p (Mannens and Wilde, 1997).

3.2.5. *HASH2* (human homologue of *Mash2*)

Mash2 has recently been shown to be imprinted with maternal expression in mice (Guillemot et al., 1995). The expression appeared to be restricted in the placenta and is required for the development of trophoblasts. Although a full length of cDNA of *HASH2* has not been cloned yet, our recent data using *in situ* mRNA hybridization demonstrated *HASH2* transcripts in the ectodermal tissue including brain, spinal cord, retina, choroid plexus and skin, and placental trophoblasts, suggesting some other additional role in human (manuscript in submission). Our data also indicate that *HASH2* is not imprinted in placentae. A role of *HASH2* in WT and kidney is not known.

4. Other cancer showing allele-specific deviation

4.1. Preferential ABL/ BCR *translocation*

In 15 patients with Philadelphia chromosome-positive chronic myeloid leukemia, Haas et al., using unique specific chromosome band polymorphisms, demonstrate that the translocated *ABL* gene on chromosome 9 is preferentially of paternal origin whereas the *BCR* gene on chromosome 22 (Philadelphia chromosome) is frequently of maternal origin (Table 2) (Haas et al., 1992). The data therefore provide evidence that imprinting phenomena may play an important role in acquired tumour-specific chromosome rearrangements. The simplest explanation of the observation is that both the *ABL* and *BCR* genes are imprinted. *BCR* would then be expressed from the maternal but not from the paternal allele. Thus even if translocation were to occur randomly with respect to the two homologues, the maternal 22 translocation would be selected because of the proliferative advantage of certain stem cell compartments in which *BCR/ABL* exerts its effect. Conversely, the preferential involvement of paternal chromosome 9 suggests that the *ABL* gene may be expressed form the paternal but not from the maternal allele (Reik, 1992).

4.2. Preferential N-MYC *gene amplification*

Neuroblastoma is a childhood neural crest tumor. One of the common genetic alterations encountered in neuroblastoma is *N-MYC* gene amplification which is found in 25-30% of the patients and is associated with rapid disease progression and a fatal outcome. Cheng et al. reported that amplified *N-MYC* gene is derived from the paternal allele in 12 out of 13 neuroblastoma cases (Table 2) (Cheng et al., 1993). The results suggest that *N-MYC* is imprinted with the parental allele expressed and the maternal allele being silenced.

5. Mechanism of genomic imprinting

5.1. DNA methylation

A central question in imprinting is how the transcriptional machinery of cell discriminates between the maternal and paternal alleles. It has been assumed that the alleles must be marked differently, presumably during gametogenesis when the maternal and paternal genomes are in separate compartments. One promising candidate for such a mark is the methylation of CpG dinucleotides in DNA. (Bestor and Coxon, 1993; Efstratiadis, 1994; Tycko, 1994) This covalent modification of DNA has been implicated in the perpetuation of the imprinting state in X chromosome inactivation, on the basis of the observation that genes on the inactive X chromosome are more highly methylated than their counterpart on the active X (Lyon, 1992). In addition a set of transgenes in mice exhibit parent-of -origin differences in DNA methylation, and in at least one instance, the methylation acts to silence the expression of the transgene. In all but one of these transgenic mouse lines, the transgenes becomes methylated after passage through the female germline. (Sasaki et al., 1989)

5.2. Enhancer competition model for H19 *and* IGF2 *expression*

While bearing in mind that methylation may play a role as an epigenetic marking in distinguishing parental genomes, an enhancer competition model was proposed to explain mechanistic links between *H19* and *IGF2* monoparental reciprocal expression based on the observation that *H19* and *IGF2* showed essentially identical patterns of expression (Bartolomei et al., 1993;

Leighton et al., 1995). According to the model, the two genes utilize and compete for the same cis-acting enhancers. When the *H19* promoter is methylated on the paternal chromosome, the *H19* gene is silenced, thereby permitting *IGF2* expression. When the *H19* promoter is not methylated, the *H19* gene competes successfully for the enhancers and thereby excludes expression of *IGF2*. Thus the methylation state of the *H19* promoter serves as a developmental switch between *H19* and *IGF2* expression. This model appears to fit very well in the endodermal tissue such as liver or in the endo-mesodermal mixed tissue including gut, kidney and lung. In other tissue such as placenta, skeletal muscle and brain, the model does not fit, suggesting the presence of another factors which are involved in the control of *H19* and *IGF2* expression.

In WT biallelic *IGF2* expression has been reported to be associated with reduced *H19* expression, which is consistent with the model (Steenman et al., 1994; Taniguchi et al., 1995a,b). We have, however, recently reported that the early stage of placentae showed biallelic *H19* and monoallelic *IGF2* expression in same placental trophoblasts, which is not consistent with the model (Jinno et al., 1995). The discrepancy between these works may derive from which tissue or tumor is employed in the analyses.

5.3. Imprinting center and the pathogenesis of PWS/AS

Prader-Willie syndrome (PWS) and Angelman syndrome (AS) are two distinct neurogenic imprinted disorders, which map to chromosome 15q11-q13. PWS is caused by the absence of activity of a paternally expressed gene or genes whereas AS is caused by the absence of a maternally expressed gene. Dittrich et al. recently have analyzed PWS/AS mutations which affect the germline imprinting process which allows identification of a region known as the imprinting center (IC) (Dittrich et al., 1996). They propose a model in that the IC consists of an imprintor and an imprint switch initiation site. The imprintor encodes the novel, alternatively spliced, non-coding 5' exons (BD transcripts) which is expressed from the paternal chromosome only and act in cis on the switch initiation site which is comprised of the SNRPN promoter or its exon 1. According to their model, paternally inherited mutations in the IC prevents the XY germline from carrying out the maternal to paternal switch and results in retention of the maternal epigenotype. Offspring inheriting this unswitched chromosome from their farther will have two maternal genotypes and therefore PWS. In the XX chromosome, IC mutations prevent the paternal chromosome from acquiring a maternal epigenotype and offspring therefore may have a paternal epigenotype on their maternally inherited chromosome and likewise exhibit AS.

6. Evolutional role of genomic imprinting

A number of hypotheses have been put forward to explain why higher mammals provide allele specific silencing, all of which are at this point completely speculative. Many other organisms from a wide range of phyla are fully capable of parthenogenetic reproduction. The answer may lie in a fact that mammalian evolution may be linked to that of the placenta. It has been suggested that imprinting might function to ensure that species continued to propagate by sexual reproduction. (Solter, 1988). Hall suggests that genomic imprinting evolves to enable mammalian mothers to tolerate the implantation of a foreign conceptus and at the same time restrain its growth (Hall, 1990). Varmuza and Mann suggest the ovarian time bomb theory that imprinting protects female mammals from the potential ravages of gestational trophoblastic diseases (Varmuza and Mann, 1994). Haig propose the genetic conflict hypothesis based on the observations that loci expressed from only the paternally-inherited genome promote the growth of the placenta which would ensures a larger and stronger individual offspring for father's interest whereas those from the maternally-inherited genomes inhibit the growth of the placenta which reduces fetal growth and increases the survival potential of the mother and her subsequent offspring (Haig, 1993). Another possible rationale is that imprinting may serve as a built-in safeguard against potential deleterious consequences of chromosomal losses such as tumor suppressor genes which result in malignant transformation (Sapienza, 1990).

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