

MINIREVIEW

Genomic Imprinting and Cancer

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Although we inherit two copies of all genes, except those that reside on the sex chromosomes, there is a subset of these genes in which only the paternal or maternal copy is functional. This phenomenon of monoallelic, parent-of-origin expression of genes is termed genomic imprinting. Imprinted genes are normally involved in embryonic growth and behavioral development, but occasionally they also function inappropriately as oncogenes and tumor suppressor genes. The evidence that imprinted genes play a role in carcinogenesis will be discussed in this review. Additional information about imprinted genes can be found on the Genomic Imprinting Website at: (<http://www.geneimprint.com>). © 1999 Academic Press

INTRODUCTION

Genomic imprinting is a non-Mendelian inherited epigenetic form of gene regulation that results in monoallelic expression. In contrast to the random allele inactivation that occurs for example at the *Xist* locus [1], the expressed allele for imprinted genes is dependent upon parental inheritance [for reviews see 2–4]. Thus, genomic imprinting is a phenomenon where the expression of a gene in this generation is dependent upon whether it resided in a male or female the past generation. Epigenetic events such as DNA methylation at CpG sites control the imprinting of genes [4]. Therefore, factors other than the sex of the parent could even modify the imprint process, thereby resulting in a Lamarckian-like inheritance of acquired traits. Such potential imprint-altering factors could include the parental level of nutrition, stress, and exposure to chemical and physical agents.

The existence of imprinted genes first became apparent when nuclear transplantation experiments demonstrated that diploid androgenotes derived from two

male pronuclei, and gynogenotes formed from two female pronuclei failed to develop properly during embryogenesis [5, 6]. Similarly, in humans complete hydatidiform moles which contain only paternal chromosomes produce primarily placental tissue, while dermoid cysts which contain only maternal chromosomes produce primarily embryonic tissue [7, 8]. These findings demonstrated that the mammalian genome contains autosomal genes that are only expressed from either the maternal or paternal allele. There are now more than 20 human imprinted genes identified ranging from growth factors to untranslated RNA, and it is postulated that 100 to 500 imprinted genes may exist [for review see 9].

The first endogenous imprinted gene identified was *Igf2* [10]. In 1991 De Chiara *et al.* [10] discovered that homozygous *Igf2*-null mice were approximately 40% smaller than wild-type mice when they were born, consistent with the known growth effects of *Igf2*. Importantly, the dwarfing phenotype was also observed in heterozygous mice, but only when the mutated allele was inherited from the father. This demonstrated that the *Igf2* gene is imprinted and expressed only from the paternal allele. *IGF2* is also imprinted in human tissues with the notable exception of the adult liver where expression is biallelic because of promoter switching after birth [11].

The second imprinted gene discovered was the maternally expressed *mannose 6-phosphate/insulin-like growth factor 2 receptor (M6p/Igf2r)* [12]. The *M6p/Igf2r* maps to the *Tme* locus on mouse chromosome 17 [12], and is the gene responsible for this maternal lethal effect [13]. The *M6p/Igf2r* encodes for a receptor that binds both M6P-containing glycoproteins and *Igf2* through independent binding sites [for review see 14]. The primary function of this receptor is the intracellular trafficking of phosphomannosyl glycoproteins from the Golgi apparatus to the lysosomes, and the internalization of *Igf2* and other extracellular ligands to the lysosomes for degradation [14]. *Igf2* signaling is not mediated by *M6p/Igf2r*, but rather it occurs principally

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through the *Igf1* receptor and the insulin receptor [15, 16].

Thus, the bioavailability of *Igf2* is controlled by a receptor that is also imprinted but expressed only from the maternal allele. The reciprocal imprinting of the *Igf2* and *M6p/Igf2r* genes suggested that the evolution of genomic imprinting may have resulted from a parent-offspring conflict to control fetal growth [17]. This parental "tug-of-war" model postulated by Haig [17] predicts that paternally expressed genes promote prenatal and postnatal growth while maternally expressed genes are growth suppressors. The identification of additional imprinted genes and their function will be required to determine whether this provocative model is adequate to explain the evolutionary pressure that resulted in the creation of genes that are functionally haploid [18].

Imprinted genes are not only important in prenatal [10, 19] and postnatal [20, 21] growth control, but also in behavioral development. People with Angelman syndrome, a congenital disease that evidence suggests is caused by the inactivation of the maternally expressed *UBE3A* (*ubiquitin protein ligase 3A*) gene, are severely retarded in addition to having ataxia, tremulousness, sleep disorders, seizures, and being hyperactive [for review see 9, 22]. *PEG1/MEST*, a member of the α/β -hydroxylase fold family, is a paternally expressed gene that maps to human chromosome 7q32 [23]. *Peg1/Mest*(+/-)-deficient mice are viable and fertile; however, they exhibit growth retardation and increased lethality [24]. Interestingly, the females that inherit the mutated allele from their fathers also have a decreased reproductive fitness because of an abnormal nurturing behavior. It is presently unknown whether *PEG1/MEST* inactivation has a similar effect on maternal nurturing behavior in humans or what effect gene inactivation has in males. Furthermore, the *M6P/IGF2R* has been identified as the first putative "IQ gene," implicating imprinted genes in the development of cognitive ability [25]. Parent-of-origin inheritance effects suggest that imprinted genes are also a genetic determinant in autism [26], bipolar affective disorder [27, 28], and schizophrenia [29], to name only a few [30]. These results demonstrate that imprinted genes play a prominent role in behavioral genetics.

IMPRINTED ONCOGENES AND TUMOR SUPPRESSOR GENES

Imprinted genes are normally involved in embryonic growth and behavioral development; however, occasionally because of inappropriate expression, they also function as oncogenes and tumor suppressor genes. Loss of heterozygosity (LOH) or uniparental disomy (UPD) at an imprinted locus may result in the deletion

of the only functional copy of an imprinted tumor suppressor gene [9, 31]. Alternatively, loss of imprinting (LOI) or UPD at an imprinted locus may result in an increased expression of an imprinted proto-oncogene. Furthermore, mutational inactivation of an imprint control center could cause aberrant expression of multiple imprinted proto-oncogenes and/or tumor suppressor genes since imprinted genes often occur in chromosomal domains [32, 33]. Imprinted genes now implicated in human carcinogenesis include: *IGF2*, *WT1*, *p57^{KIP2}*, *p73*, *NOEY2*, and *M6P/IGF2R* [9].

Aberrant genomic imprinting and its role in cancer are best exemplified by studies on Wilms' tumor, a sporadic and familial childhood kidney tumor that arises from metanephric blastemal cells. Direct genetic evidence linking tumorigenesis and aberrant imprinting was identified when 70% of Wilms' tumors were found to have biallelic expression of *IGF2* [34–36], a gene that encodes for a growth factor known to be oncogenic when overexpressed [37, 38]. Inactivation of the reciprocally imprinted *H19* gene was also present in a number of these cases [36] suggesting that LOI at the *IGF2* locus in Wilms' tumor could result from loss of *H19* expression [39, 40]. This postulate is supported by the finding that *H19*-null transgenic mice show biallelic expression of *IGF2* [41]. The coupling of biallelic *IGF2* gene expression with *H19* inactivation is even observed in phenotypically normal kidney tissue surrounding Wilms' tumors [42]. Thus, *H19* inactivation and the biallelic expression of *IGF2* appear to be linked and occur early in tumor development. Deregulation of *IGF2* imprinting has now been shown to occur in over 20 different tumor types, demonstrating its fundamental mechanistic importance in carcinogenesis [9].

Another imprinted gene involved in Wilms' tumor formation is *WT1*, a tumor suppressor located at human chromosome 11p13 [43]. *WT1* is biallelically expressed in the kidney, heart, lung, liver, and intestine, but is expressed largely or exclusively from the maternal allele in fetal brain [44]. It is also imprinted in 40% of preterm placenta [44, 45]. Since the imprint status is not correlated with gestational age of the placenta [45], imprinting of the *WT1* gene in the placenta is a polymorphic trait. Imprinting at the *WT1* locus is also polymorphic in fibroblasts and lymphocytes, but the paternal rather than the maternal allele is expressed [46]. These findings suggest the interesting possibility that polymorphic imprinting of the *WT1* tumor suppressor gene could result in both tissue- and individual-dependent susceptibilities to cancer.

The maternally expressed cyclin-dependent kinase inhibitor, *p57^{KIP2}*, maps to human chromosome 11p15.5 [47, 48]. Approximately 10% of Beckwith-Wiedemann syndrome patients have *p57^{KIP2}* mutations, but *p57^{KIP2}*

has not been found to be mutated in tumors [49, 50]. The maternal allele of $p57^{KIP2}$ is selectively lost in 85% of lung cancers with 11p15 deletions [51]; however, in Wilms' tumors with maternal loss of $p57^{KIP2}$, the normally silent paternal allele is expressed [52]. This suggests that $p57^{KIP2}$ is not a tumor suppressor, at least in Wilms' tumor. Since the imprinting of $p57^{KIP2}$ is incomplete in humans with paternal expression occurring even in some tissues, the putative tumor suppressor function of $p57^{KIP2}$ needs to be further clarified.

NOEY2 is a recently identified member of the *RAS* superfamily with high homology to both *RAS* and *RAP* [53]. It maps to human chromosome 1p31 and is expressed only from the paternal allele. LOH at this locus is observed in 41% of ovarian and breast cancers, and the paternally expressed allele is preferentially deleted. Furthermore, transfection of *NOEY2* into breast and ovarian tumor cells that normally lack expression suppresses growth. Thus, *NOEY2* appears to be an imprinted tumor suppressor gene whose function is frequently abrogated in ovarian and breast cancers.

p73 is an imprinted, maternally expressed gene that encodes for a protein sharing considerable homology with the tumor suppressor *p53* [54]. It maps to human chromosome 1p36, a region containing a putative neuroblastoma tumor suppressor gene expressed predominantly from the maternal allele. The frequent loss of *p73* in neuroblastomas coupled with the demonstration that its overexpression inhibits growth suggested that *p73* is a tumor suppressor gene [55]. Additional studies with a variety of tumors, however, were unable to demonstrate either preferential loss of the expressed maternal allele or somatic mutations in the remaining allele. These findings suggest that *p73* is not the putative imprinted tumor suppressor present at this chromosomal location [56–62].

Monoallelic expression of *p73* has recently been demonstrated in normal lung and kidney tissue, whereas expression is biallelic in the tumors that develop in these tissues [63, 64]. The high frequency of LOI and imprint switching at the *p73* locus in lung cancer and renal cell carcinomas suggest that *p73* is involved in tumorigenesis through the activation of the silent allele and overexpression of wild-type *p73*. Consequently, *p73* may function as an oncogene rather than as a tumor suppressor gene as originally proposed. It would be ironic if both *p53* and *p73* were initially described to have an oncogenic function opposite to that which it possesses.

The *M6P/IGF2R*, at human chromosome location 6q26, is inactivated in a variety of tumors at the earliest stage of transformation [65–68]. It is mutated in 60% of dysplastic liver lesions and hepatocellular carcinomas (HCCs) of patients with or without hepatitis virus (HV) infection [65, 66, 68, 69]. The *M6P/IGF2R* is

also mutated in rat liver tumors induced with the genotoxic agent, diethylnitrosamine [70]. The gene contains a poly-G region that is a common mutational target in colon, gastric and endometrial tumors with mismatch repair deficiencies and microsatellite instability [71–73]. Moreover, the *M6P/IGF2R* is mutated in human gliomas that do not contain mutations in the *transforming growth factor β type II receptor* or *Bax* genes [73], and in 30% of human breast tumors [67]. Thus, the *M6P/IGF2R* has been shown to be frequently mutated in a number of different cancers.

Although gene imprinting is often conserved between mammalian species, the imprint status of the *M6P/IGF2R* in humans and rodents is strikingly different. The *M6p/Igf2r* is imprinted in mice [12] and rats [70], but imprinting at this locus appears to be a polymorphic trait in humans, with most individuals having biallelic expression [74–76]. The existence of individuals with an imprinted *M6P/IGF2R* tumor suppressor suggests that they may have increased susceptibility to tumor development because of aberrant imprint control. This postulate is supported by Xu *et al.* [77] who recently reported partial imprinting of the *M6P/IGF2R* in 50% of Wilms' tumor patients. Furthermore, only 1 hit rather than 2 hits would be required to inactivate the tumor suppressor function of the *M6p/Igf2r* in mice. This may in part explain why mice are more sensitive to tumor formation than humans. It also suggests that transgenic mice with biallelic expression of the *M6p/Igf2r* may be better human surrogates for carcinogen risk assessment than those presently employed.

The precise molecular mechanism for genomic imprinting of the *M6P/IGF2R* is not completely defined. Methylation of a CpG-rich region in intron 2 (region 2) of the expressed maternal allele carries the imprint signal for this gene in mice [78, 79], and the imprinting box in this region has also now been identified [80]. This region appears to function as the promoter of an antisense transcript that originates only from the repressed paternal allele. This indicates that a form of expression competition may regulate imprinting of the *M6p/Igf2r* gene in mice [79]. Region 2 of the human *M6P/IGF2R* also contains parent-of-origin methylation, but gene expression is biallelic [81, 82]. Consequently, humans and mice appear to possess an altered ability to "read" the *M6P/IGF2R* imprint marks.

M6P/IGF2R inactivation is an early event in liver carcinogenesis, occurring in the initiation rather than the progression stage of transformation (Fig. 1) [68]. Clonal expansion of normal-appearing, preneoplastic hepatocytes with a single *M6P/IGF2R* allele inactivated often occurs in patients chronically infected with hepatitis virus. These precancerous hepatocytes have an enhanced risk of developing into tumors because

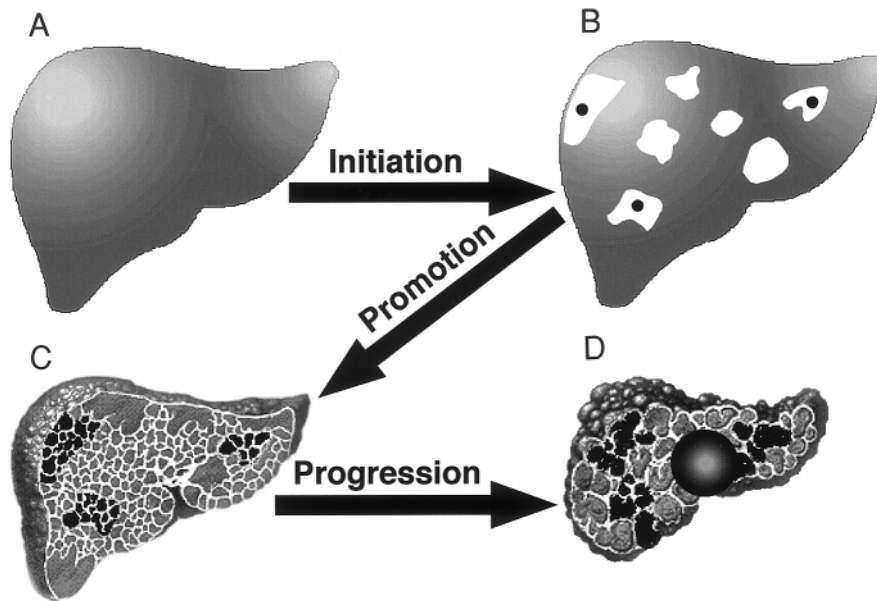


FIG. 1. Oncogenesis model of hepatocellular carcinoma (HCC) development in patients with liver cirrhosis. (A) Normal human liver. (B) Chronic hepatitis virus infection and/or alcohol abuse results in hepatocyte loss (white areas), and the formation of preneoplastic hepatocytes in which a single allele of the *M6P/IGF2R* tumor suppressor gene is inactivated (●). (C) The preneoplastic, *M6P/IGF2R*-mutated hepatocytes preferentially regenerate and/or survive, forming clonal lesions (black areas) in the cirrhotic liver. (D) These clonal regions of preneoplastic hepatocytes (black areas) continue to expand as liver cirrhosis progresses. Approximately 60% of HCCs (large sphere) ultimately arise from this clonally expanded population of preneoplastic, *M6P/IGF2R*-mutated hepatocytes; both alleles of the *M6P/IGF2R* are commonly inactivated in the HCCs.

they ultimately give rise to more than 60% of human HCCs [65, 66, 68]. This suggests that a primary “initiation event” in human liver carcinogenesis is the inactivation of a single allele of the *M6P/IGF2R* gene. The “promotion event” in the transformation process is the clonal expansion of these phenotypically normal, *M6P/IGF2R*-mutated preneoplastic hepatocytes at high risk of completely losing the tumor suppressor function of this gene. All other oncogenic events observed in dysplastic to neoplastic liver lesions occur in the progression stage of transformation. The inactivation of the *M6P/IGF2R* is also an early event in breast cancer [67, 83], but it is unknown whether gene inactivation results in clonal growth in the breast as it does in the liver.

In conclusion, genomic imprinting is an epigenetic form of gene regulation that results in the expression of only one parental allele. Imprinted genes not only play an important role in embryogenesis and behavioral development but are also mechanistically involved in carcinogenesis. Because imprinted genes are functionally haploid, imprinted tumor suppressor genes and proto-oncogenes are particularly vulnerable to inactivation and activation, respectively. The imprinting of genes also varies between species, individuals, tissues, cells, and stage of embryonic development. Therefore,

the overall effect of genomic imprinting on cancer susceptibility and penetrance is potentially great.

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