Maintenance of Genomic Imprinting at the IGF2 Locus in Hepatoblastoma

Stella M. Davies

Divisions of Pediatric Oncology and Bone Marrow Transplantation, University of Minnesota, Minneapolis, Minnesota 55455

Abstract

Genomic imprinting is the parental allele specific expression of genes and has recently been shown to occur in humans. Evidence for a role for genomic imprinting in human cancer comes from the finding of preferential retention of paternal alleles in embryonal tumors undergoing loss of heterozygosity, e.g., Wilms' tumor and osteogenic sarcoma. Recent studies have demonstrated imprinting of the insulin-like growth factor II gene at 11p15 in normal individuals, with the paternally inherited allele expressed and the maternal allele silent. It has been shown that normal imprinting is relaxed, and gene expression is biallelic in a majority of Wilms' tumors which retain heterozygosity at this locus. In this study an intragenic Apal polymorphism is used to examine imprinting of the insulin-like growth factor II gene in hepatoblastoma. Three of 5 tumors studied were heterozygous and hence imprinted. All cases showed monoallelic expression of the insulin-like growth factor II gene, indicating maintenance of normal imprinting at this locus.

Introduction

The phenomenon of genomic imprinting, or parental allele specific gene expression, was first suggested by the observation that both a maternally and a paternally derived genome are absolutely necessary for the normal development of an embryo, suggesting that for at least part of the genome, maternally and paternally inherited genes are not functionally equivalent (1, 2). This hypothesis has been confirmed for the human IGF2 gene, located at chromosome 11p15, by studies which demonstrated monoallelic expression of IGF2 in human fetuses, with only the paternal copy transcribed (3, 4). There are a number of indications that genomic imprinting may be important in human disease. Firstly, a proportion of patients with Beckwith-Wiedemann syndrome show uniparental disomy at 11p15 (5). Secondly, several studies have demonstrated preferential retention of paternal alleles in embryonal tumors such as Wilms' tumor and embryonal rhabdomyosarcoma undergoing loss of heterozygosity at tumor suppressor gene loci, indicating nonequivalence of the two alleles and suggesting a possible role for genomic imprinting in tumorigenesis (6-8).

Patients with Beckwith-Wiedemann syndrome show somatic overgrowth and have an increased relative risk of developing embryonal tumors such as Wilms' tumor, embryonal rhabdomyosarcoma, and hepatoblastoma (9-11). In view of the high incidence of Wilms' tumor in patients with Beckwith-Wiedemann syndrome and the association of both disorders with possible imprinting and abnormalities at 11p15, two recent studies have examined allelic expression of IGF2 in Wilms' tumors (12, 13). Both studies demonstrated biallelic expression of IGF2 in the majority of Wilms' tumors that retained heterozygosity at the IGF2 gene, indicating relaxation of normal imprinting in tumor tissue.

Hepatoblastoma is an embryonal liver tumor of infancy which, like Wilms' tumor, is associated with both Beckwith-Wiedemann syndrome and with allelic loss at 11p15 (10, 14, 15). This study examines allelic expression of IGF2 in hepatoblastoma, using a frequent Apal polymorphism in exon 9 of the IGF2 gene (16). Three of 5 tumors were informative for the polymorphism and all 3 showed monoallelic expression of IGF2, indicating maintenance of normal imprinting at this locus in hepatoblastomas.

Materials and Methods

Tumor Samples. Hepatoblastoma tissue, frozen at -70°C, was obtained from the Cooperative Human Tissue Network, Columbus, OH. Tissue underwent institutional and central review to ensure uniformity of diagnosis. All samples represented tumor resected at initial diagnosis of disease.

Allele Specific Gene Expression. Genomic DNA was extracted using standard techniques. Allele specific gene expression was examined using an Apal polymorphism, essentially as described by Ogawa et al. (13). The strategy used is shown schematically in Fig. 1. Briefly, IGF2 genomic DNA was amplified using primers A (5'-CTTGGACTTTGAGTCAAATGGG-3') and B (5'-GGTGCGACCAAATTCTATCTATTCA-3'), as described by Ogawa et al. (13). Primer B was end-labeled with [32P]ATP using T4 polynucleotide kinase. Polyadenylated RNA was isolated (Fast Track; Invitrogen) and reverse transcribed into cDNA (M-MLV reverse transcriptase, Stratagene) using primer B. IGF2 cDNA was then amplified using primers C (5'-TTCTGGAGAGC-TACTGGCTCA-3') and D (5'-TAC-TGGCTCA-3'). B. As this primer pair spans an intron, DNA specific PCR product (1.4 kilobases) could be detected separately from cDNA specific product (1.12 kilobases) by agarose gel electrophoresis, to exclude the possibility of contamination of RNA (and hence cDNA) with genomic DNA, which would lead to misleading results. In these experiments no 1.4-kilobase genomic DNA band was seen, indicating that there was no DNA contamination of the RNA preparations. PCR was performed using Taq polymerase (Perkin-Elmer) with an initial denaturation of 4 min at 94°C, followed by 35 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 3 min, and 1 cycle of 72°C for 7 min. Amplified gDNA and cDNA PCR products were digested with HindI and then Apal and electrophoresed through 6% denaturing polyacrylamide gels, which were then dried and autoradiographed.

Results

Genomic DNA from 5 hepatoblastomas was amplified by PCR and digested with Apal to identify the presence of the previously described polymorphism in exon 9 of IGF2 (16). Three tumors were found to be heterozygous for the Apal polymorphism and were studied further. Polyadenylated RNA was extracted from these 3 tumors and cDNA was synthesized using M-MLV reverse transcriptase. Genomic and complementary DNAs were then amplified using the primer pairs described in Fig. 1, and PCR products were digested with HindI and Apal. Results are shown in Fig. 2. In each tumor, digestion of genomic DNA-derived PCR product with HindI and Apal yields bands of 256 and 231 base pairs, indicating heterozygosity for the Apal polymorphism (Fig. 2, A-C, Lane 3). When PCR product derived from cDNA...
is digested with Hinf1 and Apal only the 256 base pair band is visible in the 3 cases (Fig. 2, A–C, Lane 6), indicating expression of a single allele and maintenance of normal imprinting in all cases.

Discussion

Study of embryonal tumors of childhood such as retinoblastoma and Wilm's tumor has led to significant advances in our understanding of the molecular basis of these and many other malignancies. However, relatively little is known about the molecular basis of hepatoblastoma, an embryonal liver tumor of infancy. Cytogenetic and molecular studies suggest some fascinating parallels between hepatoblastoma, Wilm's tumor, and embryonal rhabdomyosarcoma, a muscle tumor. Loss of heterozygosity at chromosome 11p15 has been described in each of these tumors, with preferential retention of paternal alleles reported in Wilm's tumors and rhabdomyosarcoma (6, 7, 14, 15). The parental origin of retained alleles has not yet been identified in hepatoblastoma.

Wilm's tumor, rhabdomyosarcoma, and hepatoblastoma all occur with increased frequency in patients with Beckwith-Wiedemann syndrome, a syndrome of generalized somatic overgrowth associated with a predisposition to malignancy (9, 10). Twenty % of patients with Beckwith-Wiedemann syndrome show uniparental disomy at 11p15, linking the disorder to this locus and suggesting that Beckwith-Wiedemann syndrome alters the differential allele expression that normally occurs in this region (5). This hypothesis has been confirmed by the finding, in normal individuals, that only the paternal allele of the human IGF2 locus at 11p15 is expressed and the maternal allele is silent (3, 4). Taken together, these data suggest that the IGF2 locus may participate in a common molecular etiology for Wilm's tumors, hepatoblastoma, and embryonal rhabdomyosarcoma.

Genomic imprinting is the phenomenon of gamete specific modification of the two alleles of a gene in somatic cells, leading to differential allele expression (reviewed in Ref. 17). Genomic imprinting has been shown to occur in 4 endogenous human genes thus far, including IGF2. Two recent studies have examined genomic imprinting of IGF2 at 11p15 in Wilm's tumors (12, 13). Both studies have demonstrated that normal imprinting of IGF2 is relaxed in the majority (69 and 66%) of Wilm's tumors and biallelic expression occurs.

The work described here has examined allelic expression of IGF2 in 3 informative hepatoblastomas and has shown that, in contrast to Wilm's tumors, expression of IGF2 is monoallelic in hepatoblastoma, and normal genomic imprinting is maintained.

While this study involves a small number of tumors, this finding raises an interesting distinction between Wilm's tumor and hepatoblastoma. These data suggest that the IGF2 gene may contribute to tumorigenesis by different mechanisms in these 2 tumors. The pattern of IGF2 gene transcripts in the liver is both tissue and developmental stage dependent (18, 19). Three major IGF2 mRNAs (6, 5.3, and 4.8 kilobases) have been identified. The 6-kilobase transcript is identified chiefly in fetal liver and the 5.3-kilobase transcript is identified chiefly in adult liver. Studies of hepatocellular carcinomas in adults have shown reexpression of fetal IGF2 6-kilobase transcripts in the majority of cases (20). Similar studies of Hep G2, a hepatoblastoma derived cell line, have shown elevated levels of IGF2 mRNA and a fetal pattern of transcripts (15). No data in this regard are currently available for primary hepatoblastoma tumor tissue.

It is possible that elevated levels of IGF2 message are important in the development of hepatoblastoma but are achieved by a mechanism other than relaxation of imprinting. Possible mechanisms include reactivation of a fetal promoter or uniparental disomy with reduplication of an expressed paternal allele and loss of the silent maternal allele. A study by Koufos et al. (15) identified loss of heterozygosity at 11p15 in 2 primary hepatoblastomas and showed that the tumor specific losses of constitutional heterozygosity were effected by nondisjunctional loss of one chromosome with reduplication of the other, rather than deletion, suggesting a possible role for gene dosage in tumorigenesis (15). In support of this, the gene IGF2 is located at 11p15, and IGF2 transcripts have been shown to be elevated in Wilm's tumor, rhabdomyosarcoma, and in Hep G2 cells, a hepatoblastoma derived cell line (21, 22). It is possible that different alterations in IGF2 expression are operative in different tumors. Studies of IGF2 expression in primary hepatoblastomas are currently underway to address this question.
Acknowledgments

I thank Dr. L. Robison for helpful discussions. Tissue used in this study was provided by the Cooperative Human Tissue Network, funded by the National Cancer Institute.

References


Maintenance of Genomic Imprinting at the IGF2 Locus in Hepatoblastoma

Stella M. Davies


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/53/20/4781