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TITLE: Genomic Imprinting of the M6P/IGF2 Receptor: A Novel Breast Cancer Susceptibility Mechanism

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Genomic imprinting is an epigenetic phenomenon in mammals that results in the differential expression of the paternally and maternally inherited alleles of a gene. Imprinted genes normally function to control embryonic growth and development. They also are involved in cancer because their functional haploid state makes them vulnerable to being either inactivated or overexpressed. The M6P/IGF2R has been shown to suppress cancer cell growth and is mutated in a number of human cancers, including those that develop in the lung, liver, colon and breast. These findings are consistent with the M6P/IGF2R functioning normally as a tumor suppressor. We have shown that M6P/IGF2R imprinting and receptor IGF2 binding evolved in an ancestor common to marsupials and eutherian mammals. Although M6P/IGF2R is imprinted in lower eutherian mammals, it is not imprinted in humans, as has been proposed in the literature. Our results with breast cancer and Wilms tumor suggest that a mutational event within intron 10 of the M6P/IGF2R, and not disregulation of imprinting, may have resulted in the aberrant monoallelic expression observed in some humans. Therefore, although our evidence strongly indicates that M6P/IGF2R functions as a tumor suppressor in lung, liver, colon and breast cancer, genomic imprinting at this locus is likely not involved in the etiology of tumorigenesis.
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INTRODUCTION

The mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF2R) gene encodes for a receptor that plays a critical role in regulating the bioavailability of extracellular proteolytic enzymes and growth factors known to be involved in carcinogenesis (1,2). Our recent findings indicate that the M6P/IGF2R also functions as a tumor suppressor gene in liver, breast, and lung cancer (2,3,4,5). We have determined that the frequency of monoallelic M6P/IGF2R expression in breast cancer patients is higher than that of age-matched controls. However, we have also demonstrated that the M6P/IGF2R is not imprinted in humans. Therefore, the observed monoallelic M6P/IGF2R expression in breast cancer is likely not the result of aberrant imprint regulation.

BODY

Genomic imprinting is a non-Mendelian, parent-of-origin specific, epigenetic form of gene regulation that results in monoallelic expression. The M6P/IGF2R is imprinted in both rats and mice, but imprinting at this locus is postulated to be a polymorphic trait in humans (For review see 2,4,6,7,8). Because of this species difference in M6P/IGF2R imprinting, rodents would be predicted to be more sensitive than humans to cancer because only one allele would need to be mutated to inactivate its tumor suppressor function (2,7). Therefore, it is important to better understand the phenomenon of genomic imprinting, and its modification by both genotoxic and "non genotoxic" agents since rodents are used as surrogates for human cancer risk assessment (8,9).

The literature reports of M6P/IGF2R imprinting suggest that this gene may be polymorphically imprinted in humans, with some individuals expressing only the maternal allele and most other individuals expressing both parental alleles. Because of this uncertainty, and also due to our previous finding in Wilms tumor patients with monoallelic upstream M6P/IGF2R expression and biallelic downstream M6P/IGF2R expression, we wished to establish whether the M6P/IGF2R is subject to genomic imprinting. For some genes, imprinting can be age-dependent, with imprinted expression occurring only during early development. Therefore, to determine if the human M6P/IGF2R is imprinted, we utilized multiple organ tissues derived from 12 human conceptuses ranging in gestational age from 55 to 96 days. Using five polymorphisms within the M6P/IGF2R that were discovered in our laboratory (Killian, et al., manuscript in preparation), all 12 conceptuses were shown to express M6P/IGF2R biallelically. This analysis included many individuals that were informative at multiple polymorphic sites, substantiating that the M6P/IGF2R is not subject to genomic imprinting during fetal development. We cannot, however, rule out the possibility that M6P/IGF2R imprinting occurs during embryonic development or in tissues not available for our analysis. However, recent experimental evidence from our laboratory, based on a detailed analysis of the evolution of imprinting of the M6P/IGF2R strongly supports the contention that the M6P/IGF2R is not imprinted in humans.
Genomic imprinting is postulated to have evolved because of a parent-offspring conflict to control fetal growth. This parental “tug-of-war” model predicts that only eutherian mammals would have imprinted genes because of the intrauterine development of their offspring. We tested this postulate by comparing M6P/IGF2R imprinting in monotremes (i.e. echidna and platypus), marsupials (i.e. opossum) and eutherian mammals (i.e. mouse, rat, pig, cow, bat, flying lemur, tree shrew, ringtail lemur and humans). Our findings demonstrate that M6P/IGF2R is not imprinted in the egg-laying platypus and echidna, whereas it is imprinted in the opossum (10). Thus, imprinting evolved in viviparous mammals over 100 million years ago; however, since the opossum lacks a fetal stage of development, invasive placentation and intrauterine fetal growth are not required for genomic imprinting to evolve. The M6P/IGF2R in both the monotremes and didelphid marsupials also lacks the differentially-methylated CpG island in intron 2 previously postulated to be mechanistically involved in imprint control in mice. This demonstrates the existence of alternative mechanisms of M6P/IGF2R imprint establishment and maintenance. Our results also indicate that monotremes and marsupials are not as closely related as predicted by the Marsupionta model; instead, they support the morphology-based Theria hypothesis of mammalian evolution.

We have also shown that although the M6P/IGF2R is imprinted in mice, rats, pigs, cows, and bats, imprinting at this locus was lost approximately 70 million years ago with the evolution of the higher mammalian orders: Dermoptera (e.g. flying lemurs), Scandentia (e.g. tree shrews), and Primates (e.g. ringtail lemurs and humans) (Killian et al., manuscript in preparation). This finding provides compelling evidence that M6P/IGF2R imprinting is not a polymorphic trait in humans since convergent evolution of M6P/IGF2R imprinting would have had to have occurred in humans. This highly unlikely possibility is also supported by our inability to demonstrate M6P/IGF2R imprinting in either fetal or adult human tissues. The lack of M6P/IGF2R imprinting in humans has important ramifications in toxicology because it strongly indicates that although the M6P/IGF2R functions as a tumor suppressor in humans and rodents, rodents are at heightened susceptibility to tumor formation because of the imprinted status and consequent functionally haploid state of the M6p/igf2r.

**Key Research Accomplishments**

- **M6P/IGF2R** is frequently mutated in human breast, liver and lung cancer suggesting it functions as a tumor suppressor gene.

- Monoallelic M6P/IGF2R 3' end gene expression was found in 2/32 (6.3%) of breast cancer patients, suggestive of a chromosomal deletion event or a posttranscriptional mechanism which results in the production of a single or truncated mRNA species.

- We have determined that in Wilms tumor patients, a truncated M6P/IGF2R transcript is produced from one allele and have further mapped the site of truncation to within intron 10 of the M6P/IGF2R gene.

- **M6P/IGF2R** is not imprinted in humans during fetal development.
M6P/IGF2R imprinting (i.e. maternal expression) and receptor IGF2 binding evolved in an ancestor common to marsupials and eutherian mammals. The evolution of this parent-of-origin expression purportedly occurred because of a parental genetic conflict to control offspring growth and development.

The evolutionary loss of imprinting of the M6P/IGF2R in higher mammals (Dermoptera, Scandentia, and Primates) approximately 70 million years ago strongly supports that the M6P/IGF2R is not imprinted in humans.

The finding that the M6P/IGF2R is not imprinted in humans suggests that imprinting disregulation is not a contributing factor in the etiology of breast cancer and Wilms tumor.

Reportable Outcomes

- Murphy, S.K., Wylie, A.A., Jirtle, R.J. Imprinting of PEG3, the human homolog of a gene involved in nurturing behavior. Submitted for publication.
- Personnel active on this project: Susan K. Murphy, Ph.D., J. Keith Killian (graduate student), Catherine A. Nolan, Ph.D. and Andrew A. Wylie, Ph.D.

Conclusions

In conclusion, there is now compelling mutational and functional evidence that the M6P/IGF2R is a tumor suppressor that is frequently inactivated during the early stages of
human cancer formation. *M6P/IGF2R* loss of function not only provides cancer cells with an early growth advantage, but also confers enhanced resistance to radiotherapy treatment. However, the lack of *M6P/IGF2R* imprinting indicates that this gene does not confer susceptibility to cancer because of genomic imprinting. Imprinted genes do provide unique susceptibility loci for cancer and behavioral disorders. Consequently, identifying those genes that are imprinted and the epigenetic mechanisms by which their expression is controlled will greatly enhance our understanding of the molecular mechanisms underlying cancer susceptibility at imprinted gene loci. We will therefore continue our efforts to identify novel imprinted genes and their control mechanisms.

References