Loss of Imprinting of IGF2: A Common Epigenetic Modifier of Intestinal Tumor Risk

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Abstract

Epigenetic alterations in cancer occur at least as commonly as genetic mutations, but epigenetic alterations could occur secondarily to the tumor process itself. To establish a causal role of epigenetic changes, investigators have turned to genetically engineered mouse models. Here, we review a recent study showing that a mouse model of loss of imprinting (LOI) of the insulin-like growth factor II gene (Igf2), which shows aberrant activation of the normally silent maternal allele, modifies the risk of intestinal neoplasia caused by mutations of the adenomatous polyposis coli (Apc) gene. This increased risk corresponds to the apparent increased risk of colorectal cancer in patients with LOI of IGF2. The model suggests that preexisting epigenetic alterations in normal cells increase tumor risk by expanding the target cell population and/or modulating the effect of subsequent genetic alterations on these cells, providing a novel idea for cancer risk management. (Cancer Res 2005; 65(24): 11236-40)

Introduction

Epigenetics is the study of cellular information other than the DNA sequence itself, which is heritable in cell progeny and involves modification of DNA or its associated proteins. Epigenetic changes include DNA methylation, a covalent modification of cytosine, and post-translational modifications of histone tails, such as acetylation, methylation, and phosphorylation (1). Epigenetic alterations have been increasingly recognized as an important mechanism in tumorigenesis since their discovery in human tumors in 1983 (2, 3). Genomic imprinting is an epigenetic modification of a specific parental chromosome in the gamete or zygote, leading to parental origin-specific differential expression of the two alleles of a gene in somatic cells of the offspring (3).

The insulin-like growth factor-II gene (IGF2), an imprinted gene with parental allele expressed and maternal allele silenced, is an important autocrine growth factor in tumors due to its mitogenic and antiapoptotic functions mediated by the type I receptor (IGF1R; refs. 4, 5). Increased expression of IGF2 is found frequently in a wide variety of malignancies, including colorectal, liver, esophageal, and adenocortical cancer, as well as sarcomas (6). Paracrine signaling by IGF2 also plays a role in tumors including breast cancers, as abundant expression of IGF2 is found in stromal fibroblasts surrounding malignant breast epithelial cells (7).

Although the downstream effectors are similar in signaling by insulin, IGF1, and IGF2, the receptors are different, with insulin acting through the insulin receptor, and IGF1 and IGF2 acting through IGF1R (5). These signaling pathways are reviewed elsewhere (8, 9) and are briefly summarized here. IGF1R tyrosine kinase, when activated by ligand binding, phosphorylates several substrates, such as the insulin receptor substrates IRS-1 and IRS-2. Phosphorylated IRS recruits the phosphatidylinositol 3-kinase (PI3K), resulting in increase of phosphatidylinositol-3,4-diphosphate and phosphatidylinositol-3,4,5-triphosphate in the cytoplasmic membrane and in activation of phosphoinositide-dependent kinases and protein kinase B/AKT. AKT phosphorylates a wide range of target proteins, including glycogen synthase kinase 3β (GSK3β), mouse double minute 2 (MDM2), bcl-associated death promoter (BAD), and mammalian target of rapamycin (mTOR). GSK3β degrades cyclin D1, and inactivation of GSK3β by phosphorylation leads to accumulation of cyclin D1. MDM2 binds and degrades p53, and phosphorylation of MDM2 increases its translocation to the nucleus and p53 degradation. BAD is a member of the BCL-2 family, and phosphorylation of BAD prevents its positive regulation of cell apoptosis. mTOR regulates protein translation through activation of p70 S6 kinase (S6K), translational enhancer of mRNAs with 5′-polypyrimidine tracts, and through inhibition of eIF4E binding protein-1 (4EBP1), translational repressor of mRNAs with 5′-CAP sequences, such as cyclin D1 and MYC. This PI3K/AKT pathway is implicated in gene expression, cell survival, and growth signals. Activation of receptor tyrosine kinase also triggers another pathway. Growth factor receptor-bound protein 2 (GRB2) binds to the receptor through its Src homology domain SH2 and forms a complex with guanine nucleotide exchange factor SOS through its SH3 domains, resulting in activation of Ras, Raf, and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK). MEK phosphorylates MAPK/ERK, leading to translocation of MAPK/ERK to the nucleus and activation of various target proteins, such as ETS domain transcription factor ELK1 and MYC. This Raf/MEK/ERK pathway is implicated in cell proliferation (8, 9).

Loss of imprinting (LOI) of IGF2, or aberrant activation of the normally silent maternally inherited allele, was first described in Wilms tumor, a childhood kidney cancer (10, 11), and later in many common adult malignancies, including colorectal cancer (3, 12). What was unusual about LOI in colorectal cancer was that when it was present in tumors, it was also present in adjacent normal mucosa, albeit more commonly in patients with colorectal cancer (12). A systematic analysis of 172 patients presenting to a colonoscopy clinic showed a 4.7-fold increased likelihood of LOI among patients with past or present colorectal neoplasia, and a 5.2-fold increased likelihood of LOI among
patients with a positive family history of colorectal cancer among first-degree relatives. This increased apparent risk was with LOI in peripheral blood lymphocytes, which when present also occurred in the colonic mucosa, suggesting that a potentially genetically determined stringency in the maintenance of imprinting was familial and associated with colorectal cancer risk (13). The association of LOI with colorectal neoplasia has subsequently been confirmed by two groups. The first study showed a 5-fold increased likelihood of LOI in normal colonic mucosa with colon adenoma formation (14). The second was an independent replicate in a mixed racial/ethnic population, which showed a 5-fold increased likelihood of LOI in normal peripheral blood lymphocytes with the presence of colorectal polyps or cancers (15).

One of the hallmarks of epigenetic changes in cancer is that they lead to a change in the level of expression of the affected gene rather than a DNA sequence change. Proving that an epigenetic change plays a causal role is difficult, because the epigenetic alterations could arise secondarily in the tumor. To circumvent this problem, investigators have developed mouse models to test the epigenetic hypothesis. For example, a mouse carrying a hypomorphic mutation for DNA methyltransferase I (Dnmt1) shows that DNA methylation is a modifier of tumorigenesis. Hypomethylation reduces the frequency of intestinal tumors in Min mice with a mutation in the adenomatous polyposis coli (Apc) gene (16), as well as in Mlh1-deficient mice (17). However, the Dnmt1 hypomorphic mice show increased lymphomagenesis either alone or when crossed with Mlh1-deficient mice (17, 18).

Mouse Model of Loss of Imprinting of Igf2 in Intestinal Neoplasia

LOI in human tumors leads to only a 2- to 3-fold increase of IGF2 levels in both normal and tumor tissues (14, 19). Previous models of Igf2 in cancer involved a substantial overexpression of Igf2, which might act independently in tumorigenesis (20, 21). Our goal was to reproduce as physiologically as possible the double dose of IGF2 expression caused by LOI.

### Table 1: Genotype/Imprinting Status/In Vivo Analysis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Imprinting status</th>
<th>Maternal allele</th>
<th>Paternal allele</th>
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<tbody>
<tr>
<td>H19+/+</td>
<td>LOI(−)</td>
<td>Igf2</td>
<td>DMR H19</td>
</tr>
<tr>
<td>H19−/+</td>
<td>LOI(+)</td>
<td>neo</td>
<td>DMR H19</td>
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#### Crosses

<table>
<thead>
<tr>
<th>Cross</th>
<th>Genotypes</th>
<th>Tumors</th>
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<tbody>
<tr>
<td>♀ H19+/− x ♂ Apc+/Min</td>
<td>H19+/− Apc+/Min</td>
<td>+</td>
</tr>
<tr>
<td>♀ H19+/− x ♂ Apc+/Min</td>
<td>H19+/− Apc+/−</td>
<td>++</td>
</tr>
<tr>
<td>♀ H19+/− x ♂ Apc+/Min</td>
<td>H19+/− Apc−/+</td>
<td>−</td>
</tr>
<tr>
<td>♀ H19+/− x ♂ Apc+/Min</td>
<td>H19+/− Apc+/+</td>
<td>−</td>
</tr>
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Figure 1. Mouse model of LOI of Igf2 in intestinal neoplasia. Top left, in LOI(−) mice (H19+/+), the maternal H19 allele is expressed (red arrow) with its differentially methylated region (DMR) unmethylated (white circles), and the maternal Igf2 allele is silenced. Also in LOI(−) mice, the paternal H19 allele is silenced with its differentially methylated region methylated (red circles), and the paternal Igf2 allele is expressed (blue arrow). In LOI(+) mice (H19−/+), the maternal H19 and differentially methylated region are replaced with neo, and Igf2 is activated. Top right, female mice heterozygous for the H19 deletion were crossed with male Min mice (heterozygous for an Apc mutation) to produce the four different genotypes shown. Tumors were analyzed in LOI(−) and LOI(+) Min mice, and normal tissues were analyzed in LOI(−) and LOI(+) mice with and without Apc mutation. Data from ref. 22. Bottom, a model of the effect of LOI on small intestine. In LOI(−) mice, the progenitor compartment (yellow within the crypts) and the differentiated compartment in the villi (brown), with the Paneth cells (white). In LOI(+) mice, the progenitor compartment is larger and also altered epigenetically (orange). Finally, adenomas (red) arise from this altered epithelium but are capable of differentiation given their progenitor cell origin.
Our mouse model was designed by crossing female H19 deletion mice with male ApC Min mice (ref. 22; Fig. 1A-B). When deletion of H19 and its upstream differentially methylated region is inherited from mother, the offspring shows activation of the normally silenced allele of Igf2 (i.e., biallelic expression of Igf2 by LOI; ref. 23; Fig. 1A). In Min mice, intestinal tumors are developed when a mutation of the Apc gene is inherited on one allele, and Min is considered a good model of human colorectal cancer because it involves a genetic mutation of the gatekeeper gene in human colorectal cancer (24).

When we crossed the female H19 deletion mice with male Min mice, we found the desired 2-fold increase of Igf2 in the normal intestinal tissue and tumors of LOI(+) mice compared with LOI(−) mice (22). The numbers of adenomas, surface area of adenomas, and number of adenomas per unit area of intestine in LOI(+) Min mice were increased 2.2-fold (P < 0.0001), 2.5-fold (P < 0.001), and 1.9-fold (P < 0.0001), respectively, compared with LOI(−) Min mice (22). These data provide strong experimental evidence to support the data from human studies (13, 14), linking LOI of Igf2 to colorectal cancer risk.

In addition to the increase in tumor incidence in this model, LOI(+) mice showed a shift of maturation of intestinal epithelium to a more undifferentiated state (Fig. 1C). This was apparent both in the length of intestinal crypts, the location of the stem cell compartment in intestinal epithelium, as well as in the number of cells with positive staining for progenitor cell markers, such as Musashi1 and Twist (22). We also found a similar shift in maturation of intestinal epithelium using mice with a mutation in the H19 imprinting regulatory region rather than H19 deletion (22). Consistent with an effect of LOI on normal epithelium, the ratio of microadenomas to macroadenomas was not changed comparing LOI(+) Min mice with LOI(−) Min mice; that is, the rate of tumor initiation rather than tumor progression was increased (22).

Expansion of a progenitor cell population induced by LOI has also been shown in human tissues. Patients with LOI showed a statistically significant increase in staining with progenitor cell markers in their colon (22). In addition, patients with Beckwith-Wiedemann syndrome, an overgrowth syndrome predisposing to Wilms tumor of the kidney, show focally expanded nephrogenic precursor cells termed perilobar nephrogenic rests (PLNR; ref. 25). Similar PLNR are also found in about half of the kidneys of patients with sporadically occurring Wilms tumors, specifically those with LOI of IGF2, and the PLNR themselves also undergo LOI (19). The Wilms tumors with LOI show an approximate doubling of IGF2 expression (19), similar to that induced in the intestine in our mouse model. Thus, an epigenetically induced doubling of IGF2 seems to have the capacity to alter the balance between progenitor and differentiated cells in diverse tissues, increasing the risk of malignancy several fold.

In addition to epigenetic mechanisms, genetic changes [e.g., inhibition of Bmp (26) and inactivation of Mac2 (27) or p27 (28)] may alter this balance (Table 1). Inhibition of Bmp, a genetic model of hereditary juvenile polyposis, causes abundant ectopic crypt formation (26). Inactivation of Mac2 or p27<sup>−/−</sup> causes intestinal tumors, associated with increased cell proliferation and decreased apoptosis in normal intestinal epithelium, and decreased goblet cell differentiation (27, 28). Thus, expansion of progenitor cell populations may be a common mechanism increasing cancer risk, and there may be multiple ways to get to this end point.

A second potential mechanism by which LOI might affect intestinal tumor risk is by modulating the effect of APC mutation. APC is a cytoplasmic protein that interacts with both GSK-3β and β-catenin, promoting degradation of β-catenin. Mutation of APC increases the β-catenin and subsequent relocation of β-catenin to the nucleus, where accumulated β-catenin forms a complex with TCF4, causing increased transcriptional activation of target genes, such as MYC, leading to increased cell proliferation (29).

In vitro transformation caused by deregulated MYC expression requires exogenous growth factors, such as IGF2, IGFl, and platelet-derived growth factor. Up-regulation of MYC induces apoptosis in the absence of serum, but addition of any one of these growth factors suppresses MYC-induced apoptosis in vitro (30). IGF2 also causes relocation of β-catenin to the nucleus in vitro and activates transcription of target genes of the β-catenin/TCF4 complex (31). Thus, the effects of APC mutation, such as activation of β-catenin pathway and MYC-induced transformation, might be modulated by increased expression of IGF2. Thus, LOI of IGF2 may increase cancer risk both by expanding the progenitor cell

<table>
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<tr>
<th>Mouse model</th>
<th>Target gene (reference)</th>
<th>Frequency in normal human tissue</th>
<th>Dedifferentiation phenotype</th>
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<tr>
<td>Genetic mouse models</td>
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<tr>
<td>Noggin transgene</td>
<td>Bmp inhibition (26)</td>
<td>Rare</td>
<td>Ectopic crypt formation</td>
</tr>
<tr>
<td>Mac2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Mac2 inactivation (27)</td>
<td>Rare</td>
<td>Elongation of crypt</td>
</tr>
<tr>
<td>p27&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>p27 inactivation (28)</td>
<td>Rare</td>
<td>Decreased goblet cell differentiation</td>
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<tr>
<td>Epigenetic mouse model</td>
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<tr>
<td>H19&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>Igf2 LOI (22)</td>
<td>Common</td>
<td>Elongation of crypt</td>
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Table 1. Genetic and epigenetic mouse models of intestinal tumors involving altered differentiation in the normal epithelium
commonly associated with colorectal cancer in the human.

**Conclusion**

We have proposed that epigenetic alterations, in addition to serving as a surrogate of genetic change, can precede the development of cancer and increase the probability of cancer when genetic changes arise (32). LOI in colorectal cancer is an example of this model, in that LOI of IGF2 in normal cells is commonly associated with colorectal cancer in the human population (12–15) and plays a causal role in increasing the risk of intestinal neoplasia in a mouse model (22). Another potential example is p16INK4a promoter methylation in some normal human breast epithelial cells, which when cultured acquire tumor-related properties, such as chromosomal alterations and cyclooxygenase-2 overexpression (33).

This epigenetic model suggests a novel and important approach to cancer prevention. One might attempt to down-modulate the increased signaling caused by LOI of IGF2 in patients before their developing neoplasms. It has been shown that modification of IGF1R signaling reduces cancer cell growth (e.g., by addition of neutralizing antibodies (34), an excess amount of binding proteins (35), antisense RNA (36), or selective inhibitors of IGF1R (37, 38)). Downstream molecules in the IGF1R signaling pathway, such as AKT and mTOR, might also be a therapeutic target, as rapamycin, an mTOR inhibitor, and its ester.

Whether any of these drugs can also modify the effect of a double dose of IGF2 is unknown. However, given the high prevalence of LOI, even modest reversal of its effects could have a significant effect on cancer prevention, similar to the use of statins to lower lipid in averting heart disease (40).

Because a limited number of patients have informative polymorphisms to determine the LOI status of IGF2, we may need better markers to predict cancer risk other than IGF2 LOI itself (e.g., target genes of IGF2 signaling or genes identified by genome-wide expression analysis comparing LOI− and LOI+ samples). An interesting question is why a double dose of IGF2 can affect cell differentiation so significantly. Insight is provided by a recent experiment in which mouse parthenogenotes were derived. Previously, this had been impossible, as the embryos form complete ovarian teratomas (i.e., disorganized relatively well differentiated tumors containing diverse tissue types). When one maternal pronucleus was derived from the same H19 deletion mouse strain (23) we used in our intestinal neoplasia model, fully developed mice could be obtained (41). Thus, it seems that IGF2 is a dose-dependent titrator of tissue development, consistent with the altered progenitor-differentiated cell balance we observed. Careful analysis of dose-dependent changes in IGF2-mediated signal transduction may clarify this question (e.g., by observing phosphorylation and translocation of molecules in the IGF1R signaling pathway).

Such work, in addition to its importance for cancer research, may lead to an answer to the question of why one of the two alleles has been silenced through evolution to play a role in growth regulation.

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**References**


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