Loss of Imprinting of the Insulin-like Growth Factor 2 Gene and Risk of Colorectal Cancer

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The insulin-like growth factor 2 (IGF2) gene is the first gene discovered to be imprinted and expressed exclusively from the paternal allele in both humans and mice. Similarly, IGF2 is the first imprinted gene displaying loss of imprinting (LOI) in human cancers. LOI of IGF2 results in activation of the normally silent maternal allele, with increased IGF2 expression and possible increased cancer risk. LOI occurs in colonic cancer tissues, matched normal tissues, and peripheral blood lymphocytes. Human studies have found a significant, independent, positive association between LOI of IGF2 and personal and family history of colorectal neoplasia. Furthermore, animal studies using a murine model of LOI of IGF2 support an increase in intestinal neoplasia risk and abnormal colonic mucosal differentiation. LOI of IGF2 appears to be associated with a human colorectal cancer phenotype involving younger age at diagnosis, more advanced disease, right-side colonic location, and poorly differentiated or mucinous carcinoma.

Genomic Imprinting and Loss of Imprinting
Genomic imprinting is an epigenetic modification of a gene, or the chromosome on which it resides, that is present in the gamete or zygote and leads to differential expression of the two parental alleles of the gene in somatic cells of the offspring [3]. The normally imprinted genes currently identified can be classified into three groups based on their function: they are regulators of embryonic growth, placental growth, and adult metabolism. Morison et al. [4] collated a census of known imprinted genes and listed 83 transcriptional units, of which 29 are imprinted in both humans and mice. Some, such as TP73 and CDKN1C, are considered tumor suppressor genes, and others, such as IGF2, are considered tumor promoter genes.

Loss of genomic imprinting (LOI) is an epigenetic alteration in cancer cells that involves activation of a normally silent allele of a growth-promoting gene or silencing of the normally expressed allele of a growth-inhibiting gene [3]. LOI is generally absent in normal tissue. In cancer, LOI was first reported in the IGF2 gene in Wilms’ tumor, in which the switch from monoallelic (paternally active and maternally silenced) to biallelic expression was noted. LOI of other genes, including ARH1, GLK1/GTL2, PEG1 [5–7], p73, and H19 [8,9], has also been observed in cancer. IGF2, an important member of the IGF family, is a tumor promoter that stimulates cell proliferation via an autocrine mechanism and prevents chemical-induced apoptosis [10]. Elevated circulating IGF2 levels and increased expression of IGF2 are observed in patients with colonic adenomas, adenocarcinomas, or both and have been significantly correlated with clinicopathologic features and prognosis of cancer [11–15]. The IGF2 gene is located on chromosome 11p15, which is normally imprinted—maternally silenced and paternally transcribed [16]. In addition to Wilms’ tumor, LOI of IGF2 is found in various adult malignancies, including leukemia and colorectal, prostate, and lung cancer [17•]. The frequency of LOI of IGF2 varies from 12% to 100% in different tumors [17•].

Introduction
Colorectal cancer (CRC) is a major public health concern in all developed countries. Despite decades of advances in the treatment and prevention of colorectal cancer, this malignancy remains a substantial cause of death, with poor 5-year survival rates. In 2007, CRC was the fourth most common cancer in incidence and second in mortality for both sexes in the United States [1]. Hereditary syndromes secondary to germline genetic mutations account for only 5% to 10% of all CRC cases [2]. A positive family history of CRC remains the most important risk factor for the tumor. Epigenetic, genetic, or posttranscriptional factors may be important in CRC carcinogenesis, and the identification of other such factors could improve prevention of this malignancy and reduce mortality.
Mechanisms of LOI of IGF2

IGF2, located on chromosome 11p15, is close to H19, another downstream imprinted gene. Consequently, the two imprinted genes may share common mechanisms for maintaining normal imprinting status. Two epigenetic mechanisms are thought to be involved in the regulation of IGF2 gene imprinting status.

First, the differentially methylated region (DMR) upstream to exon 3 of IGF2 was found to be hypomethylated in LOI patients compared with those normally imprinted [18]. In CRC cell lines, hypomethylation can be induced artificially by double knockout of the DNA-methyltransferase 1 gene (DNMT1) and the DNA-methyltransferase 3β gene (DNMT3B); LOI is found only in the hypomethylated cell lines [18]. These findings indicate that the DMR methylation status in the IGF2 gene is crucial for maintaining normal imprinting status (Fig. 1) [19]. Aberrant hypomethylation on the maternal allele can lead to the activation of the normally silenced IGF2 gene, resulting in LOI.

The other epigenetic mechanism for maintaining normal imprinting relies on the methylation status of the “imprinting control region” (ICR) located between the IGF2 and H19 genes [19]. As shown in Figure 1, the ICR on the normal maternal allele is unmethylated, allowing binding of the ICR with CCCTC-binding factor (CTCF), an 11-zinc finger protein. This forms a chromatin barrier insulating the IGF2 promoter from transcriptional enhancers located distally from the H19 gene. In contrast, on the paternal allele the ICR is methylated, preventing binding of the ICR with CTCF and allowing the IGF2 promoter to be activated by transcriptional enhancers located distal to the H19 gene. Therefore, the IGF2 gene is active for transcription only on the paternal allele. When LOI occurs, the methylation status of the ICR on the maternal allele is changed to that of the paternal allele (hypermethylated), allowing the IGF2 genes from both alleles to be actively transcribed.

Based on the study by Cui et al. [18], it is inferred that LOI of IGF2 in CRC appears to be caused mainly by hypomethylation of the IGF2 DMR. Understanding the underlying mechanisms of LOI of IGF2 in colorectal carcinogenesis may lead to novel strategies for cancer prevention and treatment via epigenetic approaches.

LOI of IGF2 and Colorectal Cancer

Animal studies

To investigate the mechanisms by which LOI of IGF2 contributes to intestinal carcinogens, Sakatani and collaborators [20••] developed a murine model by deleting the DMR upstream of the H19 gene. Deletion of the DMR leads to biallelic expression (LOI) of the IGF2 in the offspring, when the deletion is maternally inherited. The investigators crossed LOI of IGF2 mice with Min mice (with germline mutation in the adenomatous polyposis coli [APC] gene). The LOI-positive mice showed doubling in IGF2 mRNA levels not associated with age or Min status, as well as doubling in the amount of IGF2 protein [20••]. LOI-positive mice developed about twice as many adenomas in both the small intestine and the colon as did the LOI-negative mice. Furthermore, LOI-positive mice had longer intestinal crypts (site of epithelial stem cell renewal). The same researchers also evaluated a small number of patients with known LOI status and reported that LOI-positive individuals had a shift toward a less differentiated colonic mucosa as assessed by progenitor cell markers. These results suggest a cellular mechanism by which epigenetic alterations in normal cells may affect cancer risk by altering the balance of differentiated and undifferentiated cells. This epigenetically mediated shift in normal tissue to a less differentiated state may increase the target cell population for subsequent genetic alterations.

Clinical epidemiologic studies

There have been several reports of the association of LOI of IGF2 with CRC. Cui et al. [21] noted that LOI of IGF2 occurred in 44% of CRC patients. Other laboratories have also reported rates of LOI ranging from 33% to 87% [22–24].

In a pilot cross-sectional study, we examined the association of LOI of IGF2 in peripheral blood lymphocytes (PBL) and in normal colonic mucosa of 172 individuals undergoing colonoscopic examinations. We reported that individuals with a personal history of CRC had a 22-fold (95% CI, 3.5–153.4) increased likelihood of expressing LOI of IGF2 in PBL, compared with healthy controls [25]. The likelihood was increased fivefold (95% CI, 1.7–16.9) in individuals with a family history of CRC. A