

EDITORIALS

IGF2 Loss of Imprinting: A Potential Heritable Risk Factor for Colorectal Cancer

See article on page 964.

Colorectal cancer (CRC) affects more than 100,000 people in the United States each year, and results in more than 50,000 deaths. Therefore, it would be beneficial to have a diagnostic test for identifying those people with an elevated risk of developing this disease. In this issue of *GASTROENTEROLOGY*, Cruz-Correa et al.¹ provide further support for the novel concept that abnormal insulin-like growth factor 2 (*IGF2*) imprinting in peripheral blood leukocytes (PBL) may provide the basis for an epigenetic blood test to screen individuals early in life to identify those who are highly susceptible to developing CRC.

Progression from the earliest histologically identifiable colorectal lesion, the aberrant crypt focus, to a frank adenocarcinoma is driven by a progressive accumulation of both genetic and epigenetic alterations. Because the histologically distinct stages of tumor development occur in parallel with discrete molecular changes, CRC has become a powerful model system for dissecting out the molecular mechanisms involved in human cancer formation.

Oncogenes and tumor suppressor genes known to promote CRC when mutated include *K-RAS* (Kirsten rat sarcoma viral oncogene homolog), *TP53* (tumor protein p53), and genes involved in transforming growth factor (TGF)- β activation (*M6P/IGF2R* [mannose 6-phosphate/insulin-like growth factor 2 receptor])² and signaling (*TGF β R2* and *SMAD4* [mothers against decapentaplegic homolog 4]).^{3,4} Moreover, the hereditary colon cancer predisposition syndromes, familial adenomatous polyposis and Gardner's syndrome, result from germline mutations in the adenomatosis polyposis coli (*APC*) gene. However, it was clear from early on that epigenetic changes are also required to cause human CRC.

Epigenetic changes in the genome are stable but potentially reversible alterations that do not directly involve the DNA sequence, and are heritable through cell division. Epigenetic modifications include methylation of cytosine located within a CpG dinucleotide, and acetylation, methylation, and phosphorylation of histones.⁵ The first evidence that epigenetics played a role in cancer

was the discovery that the genome of CRC is hypomethylated, relative to that of normal colonic epithelia.⁶ Subsequently, it was shown that coupled with this global hypomethylation of the bulk of the genome is a dense hypermethylation of normally unmethylated CpG islands associated with the promoters of genes involved in cell cycle control (*p16^{INK4a}* [p16 inhibitor of cyclin-dependent kinase 4a]), DNA repair (*bMLH1* [human mutL homolog 1]), and apoptosis (*DAPK* [death-associated protein kinase]).⁷⁻¹⁰

Although tumor-suppressor gene inactivation by promoter hypermethylation occurs frequently in cancer, genome-wide hypomethylation is one of the earliest events to occur in the genesis of CRC.⁸⁻¹⁰ Demethylation of CpG islands can lead to the activation of oncogenes such as *H-RAS* (Harvey rat sarcoma viral oncogene homolog).^{8,9} Demethylation can also reactivate transposable elements, thereby altering the transcription of adjacent genes and disrupting normal gene function by promoting the integration of these parasitic DNA elements into other genomic regions. DNA hypomethylation can also affect nuclear structures other than genes, resulting in chromosomal instability. This would favor mitotic recombination, loss of heterozygosity, and chromosomal aneuploidy. Moreover, the loss of DNA methylation can cause the dysregulation of imprinted genes.

Genomic imprinting results from an epigenetic modification in the germ line that leads to parent-of-origin dependent, monoallelic gene expression in somatic cells.¹¹ The first experimental evidence that mammalian maternal and paternal genomes are not equivalent came from mouse nuclear transplantation experiments.^{12,13} These elegant studies in the mid-1980s showed that diploid androgenotes derived from 2 male pronuclei and gynogenotes formed from 2 female pronuclei failed to develop properly during embryogenesis. Similarly, complete hydatidiform moles in humans, containing only paternal chromosomes, produce primarily placental tissue from which choriocarcinomas can develop, whereas dermoid cysts, containing only maternal chromosomes, produce primarily embryonic tissue from which teratomas can develop.¹⁴

One of the first imprinted genes identified was mouse *Igf2*,¹⁵ and soon thereafter, human *IGF2* was also found

to be expressed only from the paternal allele.⁹ Presently, more than 70 imprinted genes have been identified (<http://www.geneimprint.com>),¹⁶ and it is postulated that 100–500 imprinted genes may exist in the human genome. Marsupials and eutherian mammals are imprinted at the *IGF2* locus, whereas the egg-laying monotremes are not imprinted.^{16,17} Thus, *IGF2* imprinting evolved approximately 150 million years ago in the late Jurassic period with the advent of live birth.

Multiple theories have been proposed to explain the origins of imprinting early in mammalian evolution.^{18,19} According to the most debated of these theories, the “conflict hypothesis,”²⁰ imprinting is viewed not as an adaptation that presently benefits species survival, but rather as a consequence of an ancient reproductive battle between the sexes that involved polyandry, viviparity, and a skewed maternal versus paternal investment in the offspring. According to this provocative theory, imprinting arose because of a genetic tug-of-war between the parents to control the amount of nutrients extracted from the mother by her offspring. The conflict theory predicts that paternally expressed genes promote prenatal growth to benefit offspring fitness whereas maternally expressed genes suppress growth to maximize reproductive performance.²¹

Regardless of which hypothesis correctly describes why imprinting evolved, it is now clear that, because imprinted genes are functionally haploid, they markedly increase our susceptibility to cancer. This was first shown with the discovery that pathologic biallelic expression of *IGF2* occurs early in the genesis of sporadic Wilms’ tumors.⁹ The oncogenic role of *IGF2* loss of imprinting in Wilms’ tumor formation was further substantiated with the finding that the incidence of this juvenile kidney tumor is greatly increased in patients with Beckwith–Wiedemann syndrome in which *IGF2* loss of imprinting arises either in the germline or early in development.⁹ *IGF2* dysregulation in Wilms’ tumor is highly associated with inappropriate maternal methylation of the imprint control region (ICR) upstream of the *H19* gene (Figure 1).^{9,22,23} Biallelic expression of *IGF2* is now known to occur not only in Wilms’ tumor but also in a large number of adult human cancers, including CRC.²⁴

In contrast to Wilms’ tumor, *IGF2* loss of imprinting in CRC unexpectedly involves hypomethylation rather than hypermethylation of the *H19* ICR.²⁵ In addition, the differentially methylated region upstream of *IGF2* exon 3 (DMR0) is hypomethylated (Figure 1), and only this epigenetic alteration is tightly linked with *IGF2* loss of imprinting in CRC.^{25,26} Surprisingly, *IGF2* loss of

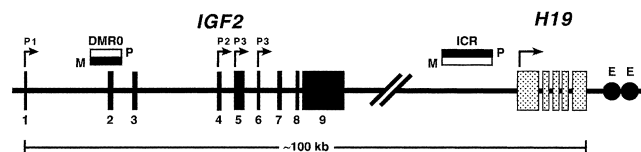


Figure 1. Genomic structure of the human *IGF2/H19* imprinted domain. *IGF2* (9 exons; black boxes) and *H19* (4 exons; stippled boxes) are reciprocally imprinted. *IGF2* is expressed only from the paternal allele, whereas *H19* is expressed only from the maternal allele. Regulation of *IGF2* and *H19* imprinting is controlled by allele-specific methylation at differentially methylated regions (DMR0) and the imprint control region (ICR). Black (methylated) and white (unmethylated) boxes above the genes indicate regions of preferential maternal (M) or paternal (P) CpG methylation. *IGF2* has 4 promoters, P1–P4, that are located 5' to exons 1, 4, 5, and 6, respectively. Enhancers (E) involved in regulating the reciprocal imprinting of *IGF2* and *H19* are also shown.

imprinting is also found in normal endoderm-derived colonic mucosa of patients with CRC,^{26,27} and even in 10% of people who are healthy.²⁶ Moreover, *IGF2* loss of imprinting is present in mesoderm-derived PBL, and abnormal *IGF2* imprinting in this tissue is highly correlated with both a familial and personal history of CRC.²⁶ Taken together, these findings suggest that the epigenetic alteration responsible for abnormal *IGF2* imprinting in these patients is an early event in embryogenesis.

In this issue of *GASTROENTEROLOGY*, Cruz-Correa et al.¹ add to these intriguing findings by assessing whether *IGF2* loss of imprinting in PBL is associated with known environmental risk factors for CRC. A total of 172 individuals were examined for *IGF2* loss of imprinting in PBL. Individuals with colorectal neoplasia (adenomas/cancer) were found to have a 5.1-fold increased risk of *IGF2* loss of imprinting in PBL than people without colorectal tumors. In contrast, tobacco smoking; alcohol consumption; nonsteroidal antiinflammatory agent use; and the nutrient ingestion of calcium, folate, selenium, and fat were not correlated with *IGF2* loss of imprinting. These findings provide compelling support for the postulate that abnormal *IGF2* imprinting in PBL is not environmentally acquired during adulthood, but rather occurs early in embryonic development. Consequently, detection of this epigenetic perturbation could possibly be performed early in life, allowing for cancer-preventive measures to be started in high-risk patients before the early stages of CRC are first visually evident.

The most obvious unanswered question remaining is whether *IGF2* loss of imprinting in PBL results from an inherited genetic mutation, and/or an epigenetic alteration induced by an environmental perturbation early in embryogenesis.²⁹ Furthermore, it is important to know if the frequency of *IGF2* loss of imprinting in PBL depends

on factors such as (1) ethnicity; (2) geographical location; (3) the smoking, drinking, and nutritional habits of the birth mother; and (4) birth weight. It is necessary to also determine if abnormal imprinting of *IGF2* is present in other normal tissues, and, if so, whether or not it predisposes an individual to cancer in those tissues. It would even be fascinating to determine if other imprinted genes are deregulated in individuals with *IGF2* loss of imprinting, particularly other reciprocally imprinted genes, such as *DLK1* (delta-like 1 homolog) and (maternally expressed gene 3) *MEG3*.³⁰ Finally, it will need to be determined if the incidence of other pathologies such as obesity, diabetes, cardiovascular disorders, and even behavioral disorders are higher in individuals with abnormal *IGF2* imprinting than in people who do not have this epigenetically induced perturbation in imprinting regulation.

The role of epigenetics and imprinting in carcinogenesis has long been in the shadow of human cancer genetics⁹; however, the compelling findings of this study have the potential to reverse this trend. It now appears possible to identify individuals early in life who are at high risk of developing sporadic CRC by screening the general population for epigenetic changes that result in *IGF2* loss of imprinting. Thus, it may someday be possible not only to reduce their risk of CRC by increasing colonoscopy surveillance but also to directly modify their cancer risk with the use of dietary and/or therapeutic agents developed to reverse the epigenetic alterations that resulted in abnormal *IGF2* imprinting. Because imprinting regulation often varies markedly between species,^{31–33} the best model for these important future studies remains the human.

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Functional Gastrointestinal Disease: Has the Genomic Era Arrived?

See article on page 971.

Functional gastrointestinal disorders (FGIDs) are common, disabling, poorly understood, and bereft of curative therapy. Within the broad canvas that is functional gastrointestinal disease are found all of those patients with a symptom or, more usually, symptoms apparently originating from the gut, yet in whom conventional endoscopic, radiologic, serologic, manometric, and pathologic studies fail to uncover any abnormality of diagnostic significance. For some time, clinicians have recognized a tendency for some of the more common of the many and varied functional symptoms to aggregate into 2 major “clusters” which, in turn, tend to focus on either the upper or lower gut. These 2 primary groupings of FGIDs are more commonly recognized as functional dyspepsia (FD) and irritable bowel syndrome (IBS), respectively. Though some have counselled against such an approach, insisting that unexplained symptoms should be recognized as such and no more,¹ the syndromic approach to FGIDs, supported, in part, by clinical studies and expert opinion, has held sway and has led to the recognition and acceptance of both FD and IBS as valid targets for investigative and therapeutic research.² FGIDs have, in essence, become “respectable” and one can now admit openly to a clinical or research interest in FD or IBS without attracting condescending glances and even opprobrium from fellow academics, funding authorities and peer-reviewed journals, alike.

Even more tantalizing for the newly accepted “functionalists” have been recent descriptions of disrupted physiology, subtle pathologic abnormalities and even genetic predisposition in FGIDs; are these disorders

functional after all? On the physiological front, both FD and IBS have been associated with abnormalities of motor function,³ visceral hypersensitivity,³ autonomic function,⁴ and cerebral perception. Reports of the precipitation of both of these functional disorders by infectious agents are now extant in the literature.^{5–7} Especially impressive is the association between IBS and prior bacterial gastroenteritis; the natural history, predisposing factors and functional and pathologic correlates of this particular relationship are now well documented.^{6,7} Equally provocative are very recent reports, of firstly, inflammatory cell activation, in the colon, among unselected IBS patients, regardless of the nature of onset or symptom predominance,⁸ and, secondly, of epithelial and myenteric plexus inflammation, in the jejunum, in a group of patients with severe IBS⁹; taken together, these studies suggest an immunological basis for IBS.¹⁰ Our current knowledge of mucosal immunology and its interactions with the gut flora, as well as experimental models of immune-motor and immune-sensory interactions have, in turn, provided a conceptual framework within which one can construct direct links between a luminal trigger, a mucosal immune response and symptom-generating alterations in enteric myoneural function.^{11–13} As in all other hypotheses of infectious initiation of chronic unexplained disease, a genetic predisposition is assumed to lay the groundwork and thus explain why not all exposed develop the disorder under study. Evidence for such a predisposition, indeed, exists for some FGIDs^{14–16} and is now further supported by the study from Holtmann et al. reported in this issue of *GASTROENTEROLOGY*.¹⁷ Taken to its limits, the infection/inflammation hypothesis would appear, therefore, and for the first time, to square the circle in IBS and FGIDs?