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Colorectal cancer

Another important function for an old friend! The role of iron in colorectal carcinogenesis

J R Butterworth

Epidemiological data strongly support a role for dietary and haem iron in colorectal carcinogenesis through multiple pathways

The aetiopathogenesis of colorectal carcinoma (CRC) remains the holy grail for researchers in the field. CRC arises from benign neoplasms and evolves into adenocarcinoma through a stepwise histological progression sequence, proceeding from either adenomas or hyperplastic polyps/serrated adenomas. Genetic alterations are associated with specific steps in this polyp-adenocarcinoma sequence and are believed to drive the histological progression towards colon cancer. Approximately 50% of CRCs are attributed to dietary factors and about 15–20% to genetic factors, including the high risk familial syndromes.¹ Large prospectively collected epidemiological data have suggested that iron may confer an increased risk for CRC.²

WHAT ARE THE MECHANISMS BY WHICH IRON CONFERS AN INCREASED RISK OF CRC?

An apparent dose-response for serum ferritin level and adenoma risk suggests that exposure to dietary iron may be involved in the development of colorectal adenomas (and particularly proximal adenomas).³ Dietary haem iron (through its effect on epithelial proliferation) is associated with an increased risk of proximal colon cancer, especially in women who drink alcohol.⁴ In the dextran sodium sulphate model of mouse colitis, a twofold increase in dietary iron increased iron accumulation

in colonic luminal contents at the colonic mucosal surface and in superficial epithelial cells with a concomitant increase in colitis associated CRC incidence.⁵ High dietary iron decreases tocopherol levels in rat colonocytes, promotes oxidative stress (through generation of lipid peroxides and reactive oxygen species (ROS)) in faeces and colonocytes,^{6–8} and decreases the activity of the colonic antioxidant enzyme manganese superoxide dismutase.⁹ ROS activation of activator protein 1 and nuclear factor κB signal transduction pathways leads to transcription of genes involved in cell growth regulatory pathways.¹⁰

In addition, some studies suggest that possession of HFE gene mutations are statistically associated with increased rates of CRC,¹¹ but other studies have not confirmed this finding.¹² Dietary haem also promotes the development of aberrant crypt foci (ACF),¹³ the earliest identifiable neoplastic lesions in the colon cancer model. However, only a small fraction of ACF evolves to form cancer.¹⁴ Progression from ACF through adenomatous polyp to cancer results from an accumulation of proteomic abnormalities (for example, B-catenin, E-cadherin, inducible nitric oxide synthase, cyclooxygenase (COX-2), and P16(INK4a)); genetic mutations (for example, K-ras, APC, p53); genomic instabilities; microsatellite instability; loss of heterozygosity; and defects in mismatch repair systems.

In summary, dietary iron is taken up by colon cells and participates in the induction of oxidative DNA damage. Its capacity to catalyse the formation of reactive oxygen species is an important risk factor for CRC.

Work conducted by Brookes and colleagues¹⁵ in this issue of *Gut* begins to shed some light on a putative role of iron and the iron cognate proteins in colon carcinogenesis (see page 1449). Since identification of the HFE gene by Feder *et al* in 1996,¹⁶ the last 10 years has seen an unprecedented advance in our understanding of iron physiology. A number of iron related proteins have been identified and their role characterised.¹⁷ Brookes and colleagues¹⁵ have shown that “progression to colorectal cancer is associated with increased expression in iron import proteins and a block in iron export due to decreased expression and aberrant localisation of HEPH (hephaestin) and FPN (ferroportin-1) respectively, resulting in increased intracellular iron which may induce proliferation and repress cell adhesion”.

A major finding of the study was that a difference in the expression of the iron related proteins appeared to be evident only at the carcinoma stage of epithelial cell dedifferentiation. Intuitively, if iron is related to the process of colorectal carcinogenesis then one would have expected to find a gradation of abnormalities from normal colorectal mucosa through dysplasia to carcinoma. However, there was no statistically significant difference between expression of the iron cognate proteins in normal tissue compared with colorectal adenomas with histological high grade dysplasia. It could be inferred from this that expression of these iron proteins is merely an epiphenomena related to accumulation of multiple genetic abnormalities but that iron itself is not involved in any meaningful aetiopathological manner to the process of colorectal carcinogenesis. However, would this be a correct interpretation?

There are a number of pathways by which iron may be involved in epithelial

cell carcinogenesis. Some are outlined below, but there are potentially many more.

- (1) c-Myc induced cell transformation.
- (2) E-cadherin gene silencing.
- (3) Hypermethylation of CpG islands of target genes involved in carcinogenesis.
- (4) Cyclin dependent control of cell cycle.
- (5) CDX2 regulated expression of iron transport proteins.

(1) C-MYC OVER EXPRESSION AND INCREASED FREE CYTOSOLIC IRON

The proto-oncogene c-Myc is over-expressed in a wide variety of human cancers with 80% of breast cancers, 70% of colon cancers, 90% of gynaecological cancers, 50% of hepatocellular carcinomas, and a variety of haematological tumours possessing abnormal Myc expression. Myc proteins act as transcription factors, regulating gene expression. c-Myc protein is capable of repressing the expression of the heavy subunit of the protein ferritin (H-ferritin), stimulating expression of the iron regulatory protein 2,¹⁸ and increasing the expression of transferrin receptor (CD71).¹⁹ These effects combined result in intracellular accumulation of iron. Indeed, c-Myc induced cell transformation requires repression of H-ferritin, implying that intracellular iron concentrations are essential for control of cell proliferation and transformation by c-Myc. Interestingly, c-MYC expression also represses natural resistance associated macrophage protein 1 promoter function leading to an increase in iron in the cytosol.²⁰

(2) E-CADHERIN GENE SILENCING

A striking feature of the work published by Brookes and colleagues¹⁵ in this month's issue of *Gut* is the significant downregulation of E-cadherin mRNA expression following iron loading of the Caco-2 and SW480 cell lines. E-cadherin is a transmembrane glycoprotein that mediates epithelial cell to cell adhesion. Loss of E-cadherin can result in disruption of cell clusters and has been shown to be an independent predictor in disease progression in several cancers. E-cadherin was originally viewed exclusively as a structural protein mediating cell-cell adhesion. However, more recently, its signalling functions have been recognised. Loss or downregulation of E-cadherin releases proteins, such as β -catenin and p120 catenin, from a membrane bound state into the cytoplasm, which are known to regulate transcriptional activity. The

repression effect on E-cadherin may be mediated by the Snail family of transcription factors which are implicated in the differentiation of epithelial cells into mesenchymal cells (epithelial-mesenchymal transition). Functional perturbations of E-cadherin have been associated with the transition from adenomas to invasive carcinomas.²¹ Snail transcription factor appears in the mouse model to be a strong repressor of E-cadherin gene transcription.²² Loss of E-cadherin is considered to be diagnostic of a poor prognosis in CRC and blocking E-cadherin downregulation in tumours may be an important future approach in gene therapy for this disease. To target this molecule is the logical path to prevent the metastasising potential of almost any epithelial tumour.

(3) IRON INDUCED HYPERMETHYLATION OF CPG ISLANDS OF TARGET GENES INVOLVED IN CARCINOGENESIS

Aberrant methylation or hypermethylation is an important epigenetic alteration occurring early in human cancer and resulting in transcriptional silencing. Methylation profile of promoter CpG islands of a number of genes that might play an aetiological role in colon carcinogenesis reveals that genes demonstrating moderate or high methylation intensity include O-6-methylguanine-DNA methyltransferase, hMLH1, and p16(INK4a). The highest methylation intensity is seen in the COX-2, cadherin 13, H-cadherin, p73, and Wilms tumour 1 genes.²³ The role of iron in influencing the methylation status of these genes has yet to be determined. Interestingly, E-cadherin possesses a region from exon 1 to exon 2 which contains a high density CpG island of approximately 1500 base pairs and may be a possible site of aberrant methylation resulting in gene silencing.²⁴ In addition, demethylation of promoter regions within E-cadherin suppresses expression of E-cadherin mRNA, leading to tumour metastasis in certain cancers.²⁵

(4) IRON DEPENDENT CONTROL OF CELL CYCLE PROGRESSION

Iron is also closely involved in cell cycle control, although research into this aspect of iron metabolism is still in its infancy. The best studied molecules include the cyclins and the cyclin dependent kinases (cdks) which associate to form complexes which phosphorylate substrates such as the retinoblastoma protein (rRb) which facilitates cell cycle progression. Treatment of cells with iron chelators has been shown to result in a marked

reduction in expression of cyclins D1, D2, and D3,²⁶⁻²⁸ cdk2,²⁶ cdk1,²⁹ and the hypophosphorylation of pRb.²⁶⁻²⁷ Changes in expression of cdk2 and cyclins D1, D2, and D3 may result in the G1/S arrest observed after iron deprivation.³⁰ The cyclin-cdk2 complexes are modulated by cyclin dependent kinase inhibitors, the most important of which is the p21^{CIP1/WAF1}. Marked transactivation of the WAF1 gene which encodes p21 is also seen after iron chelation and this finding may suggest a potential role for p21 in the G1/S arrest after iron chelation.³¹ Therefore, it is postulated that intracellular iron would promote progression of the cell cycle beyond G1/S by means of rRb or a similar molecule and thus facilitate cellular proliferation.

(5) CDX2 REGULATED EXPRESSION OF IRON TRANSPORT PROTEINS

CDX1 and CDX2 are homeobox transcription factors that demonstrate intestine specific expression and maintain the intestine phenotype in adult tissues.³² They have a key role in regulating proliferation and intestinal cell fate. Cdx2 mutations result in the development of multiple polyps in the proximal colon.³³ In humans, loss of Cdx1 and/or Cdx2 is found in a subset of CRCs.³⁴ Recent work undertaken to define CDX2 regulated genes revealed that CDX2 and the gene for hephaestin (*HEPH*) are strongly correlated in colon cancer cells³⁵ and that the *HEPH* gene is the primary target of CDX2 activity. In addition, the 5' flanking region of the *HEPH* gene contains a CDX2 responsive element. In the colon cancer cell line HT-29, CDX2 activation resulted in modest inhibition of iron uptake rates and marked inhibitory effects on the steady state concentration of intracellular iron. These findings support the hypothesis that activation of *HEPH* expression by CDX2 leads to an increase in iron export from cells. However, further work by Hinoi and colleagues³⁵ did not find a significant change in expression of the iron cognate protein ferroportin with CDX activation. Allelic mutations within the CDX2 gene may be expected to reduce *HEPH* activation and subsequent basolateral membrane ferroportin expression with a subsequent block on cellular iron export and increase in intracellular iron concentrations. Clearly, the role of CDX2 in colon carcinogenesis requires further detailed clarification.

CONCLUSION

Epidemiological data strongly support a role for dietary and haem iron in colorectal carcinogenesis. Although, the

study reported by Brookes and colleagues¹⁵ appears to demonstrate a significant difference in iron staining and protein expression of the iron cognate proteins in colon cancer tissue only, it is highly probable that iron is intimately involved in the process of epithelial cell dedifferentiation in the colon (and by extension in gastric, oesophageal, and pancreatic tissue). The next few years will reveal in more detail the precise role of iron in either initiating or perpetuating this process leading eventually to the develop of carcinoma.

Potentially, iron is involved in the development of colorectal carcinoma through multiple pathways and a number of questions remain unanswered. The work published by Brookes and colleagues¹⁵ is the beginning of another exciting journey in elucidating the role of iron in colon cancer pathogenesis. The field of iron metabolism has enjoyed unprecedented success over the past 10 years since the seminal discovery of the HFE gene by Feder and colleagues.¹⁶ As attention is turned to studying the effects of iron and associated proteins in the colon, it is to be hoped that similar success will result.

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