Presence of Iron in Colorectal Adenomas and Adenocarcinomas

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Background/Aims: Iron overload reportedly increases the risk of colorectal neoplasms, but the distribution of tissue iron in a colorectal neoplasm remains controversial. In this study, we attempted to determine the significance of tissue iron in colorectal adenomas and adenocarcinomas. **Methods:** This study investigated 138 colorectal neoplasms (54 adenocarcinomas, 25 adenomas with high-grade dysplasia, and 59 adenomas with low-grade dysplasia) that were removed by surgical or endoscopic resection in Konkuk University Hospital between August 2005 and August 2006. Adjacent normal colon tissues and colorectal neoplasms were stained with Perls' Prussian blue to reveal ferric compounds. **Results:** Positive Perls' staining was evident in 35.2% (19/54) of the adenocarcinomas and 22.6% (19/84) of the adenomas, and in only 2.2% (3/138) of the samples of adjacent normal colon tissue ($p < 0.001$). Iron appears to reside exclusively in the stroma and outside the gland, rather than in the epithelial cells. Iron expression was strong in larger ($p = 0.004$) and pedunculated ($p < 0.001$) adenomas, and in all types of adenocarcinomas regardless of their size, shape, and location. **Conclusions:** The frequent presence of iron in the stroma of large adenomas, pedunculated adenomas, and adenocarcinomas indicates that iron deposition is a secondary phenomenon to intralesional hemorrhage rather than a consequence of epithelial-cell carcinogenesis. (*Gut and Liver* 2008;2:19-22)

Key Words: Iron; Colorectal neoplasm; Adenoma; Adenocarcinoma

INTRODUCTION

Iron stores increase the risk of both colorectal adenoma

and cancer.¹⁻³ There are several pathways by which iron could be involved in epithelial cell carcinogenesis: (i) c-Myc-induced cell transformation, (ii) E-cadherin gene silencing, (iii) hypermethylation of CpG islands of target genes involved in carcinogenesis, (iv) cyclin-dependent control of the cell cycle, and (v) CDX2-regulated expression of iron transport proteins.³ The progression to colorectal cancer is associated with an increased expression of iron-import proteins and a reduction in iron export due to decreased expression and aberrant localization of HEPH (hephaestin) and FPN (ferroportin-1), respectively, resulting in increased intracellular iron that may induce proliferation and repress cell adhesion.⁴ In addition, increased iron-binding proteins in human colorectal adenomas and carcinomas suggest that they are related to the increased requirement of iron for the turnover of rapidly dividing cells.⁵

It has been suggested that iron is associated with both the initiation and promotion phases of carcinogenesis, and that there might be an iron toxicity to produce free radicals in colonic neoplasms.⁶ In a mouse model, a two-fold increase in dietary iron increased iron luminal accumulation at the colonic mucosal surface and in superficial epithelial cells, with a concomitant increase in the incidence of colorectal cancer associated with colitis.⁷ However, several questions on the role of iron in the development of colorectal carcinoma remain unanswered, and the distribution of tissue iron in colorectal neoplasms remain controversial. Whether the presence of iron in the colon tissue directly indicates tumorigenesis requires clarification, since iron is evident in all tissues with a sufficient blood supply, and the blood supply to colorectal polyps is higher in large polyps, pedunculated polyps with a thick stalk, and malignant lesions of the colorectal

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region.⁸ In this study, we attempted to determine the significance of tissue iron in colorectal adenomas and adenocarcinomas.

MATERIALS AND METHODS

1. Materials

The study enrolled 121 consecutive patients who underwent colectomy or colon polypectomy in Konkuk University Hospital between August 2005 and August 2006. Patients with inflammatory bowel disease, infectious colitis, severe cardiopulmonary dysfunctions, or other acute inflammatory diseases were excluded from the study. We examined 77 men and 44 women with a median age of 62.7 years and an age range of 32-88 years. A total of 54 colon cancers, 59 colon adenomas with low grade dysplasia (LGD), and 25 colon adenomas with high grade dysplasia (HGD) were obtained from the patients, since 8 patients had 2 lesions, 3 patients had 3 lesions, and 1 patient had 4 lesions.

Data on patient age and sex as well as the location, size, shape, and histological type of neoplasm were recorded. The size of each retrieved polyp was determined as the largest distance between the two lateral limits of the tissue. All of the patients provided written informed consent prior to undergoing the procedure. This study was approved by the institutional review board of Konkuk

University School of Medicine, which confirmed that the study was in accordance with the ethical guidelines of the Helsinki Declaration.

2. Histological examination and Perls' Prussian staining

A total of 138 tissue-array blocks were obtained from each case of formalin-fixed, paraffin-embedded neoplastic tissues without visible tumor necrosis. Samples of adjacent normal colonic mucosa were also obtained. Adjacent normal colon tissues and colorectal neoplasms were stained by Perls' Prussian blue to reveal iron compounds in the ferric state. Serial 4- μ m-thick sections cut from formalin-fixed and paraffin-embedded tissues were mounted on glass slides deparaffinized in xylene and dehydrated in ethanol. Perls' acid ferrocyanide reaction was used to reveal iron compounds in the ferric state. Nuclear fast red was used for counterstaining.

3. Statistical analysis

A p value of less than 0.05 was considered statistically significant. Continuous variables are presented as median values (range) and compared by using the Kruskal-Wallis test. Ordered categorical variables were analyzed by using Chi-square test or Fisher's exact test.

RESULTS

1. Presence of iron in adenomas

The status of iron expression in the 84 colorectal adenomas is summarized in Table 1. Positive Perls' staining

Table 1. Iron Expression in 84 Colorectal Adenomas

	Positive Perls' staining (n=19)	Negative Perls' staining (n=65)	p-value
Size (cm ² , range)	2.38 (1.19-3.56)	0.83 (0.43-1.24)	0.001*
Grade of dysplasia			1.000 [†]
Low	13	46	
High	6	19	
Pathology			0.332 [†]
Tubular	13	53	
Tubulo-villous	5	8	
Villous	1	4	
Shape			<0.001 [†]
Sessile	3	28	
Protruded	3	26	
Pedunculated with stalk	13	11	
Location			0.445 [†]
Cecum and A-colon	2	14	
T-colon and flexures	1	9	
D-colon and S-colon	12	31	
Rectum	4	11	

NS, not significant; A-colon, ascending colon; T-colon, transverse colon; D-colon, descending colon; S-colon, sigmoid colon.

*Kruskal-Wallis test.

[†]Chi-square test.

Fig. 1. Photomicrograph of Perls' staining of a tubular adenoma. Blue-stained iron deposits are evident in the intervening stroma of the dysplastic glands and in the surrounding inflammatory cells and mesenchymal cells of the enlarged vessel (arrow) ($\times 100$).

was evident in 22.6% (19/84) of the adenomas (Fig. 1), with iron expression being more frequent in larger ($p=0.004$) or pedunculated ($p<0.001$) adenomas (Fig. 2). The iron expression did not differ significantly between adenomas with LGD and adenomas with HGD.

2. Presence of iron in adenocarcinomas

Positive Perls' staining was evident in 35.2% (19/54) of the adenocarcinomas (Fig. 3) with iron being more frequently expressed in all types of adenocarcinomas regardless of their size, shape, and location (Table 2).

Fig. 2. Perls' staining according to the shape of the colonic adenomas. Black and white dots indicate positive and negative Perls' staining, respectively. Positive Perls' staining is more common in pedunculated polyps with a stalk ($p<0.001$).

Fig. 3. Photomicrograph of Perls' staining of an adenocarcinoma. Blue-stained iron deposits are evident in the infiltrated inflammatory cells and mesenchymal cells around the invasive carcinoma glands (arrow). Vascular proliferation is evident noted in the surrounding stroma of invasive carcinoma glands ($\times 100$).

3. Presence of iron in normal colon tissue

Only 2.2% (3/138) of the samples of adjacent normal colon tissue exhibited positive Perls' staining ($p<0.001$), indicating that most of the normal colonic mucosa samples exhibited no iron expression.

DISCUSSION

In the present study, iron expression was more frequent in large adenomas, pedunculated adenomas, and adenocarcinomas. Our study shows that iron accumulation in adenomas can be predicted on the basis of the size and shape of the polyp, but not from the age and sex of the subject, or the location and pathology of the polyp. Iron was present in adenocarcinomas regardless of their size and shape, which suggests that its presence differ significantly between adenoma and adenocarcinoma of the colon.

To the best of our knowledge, only two studies have detected iron in colorectal neoplasms.^{9,10} Both studies found that iron was absent in colon adenomas, while it was present in colorectal carcinomas regardless of the degree of differentiation, location and stage of the cancer. The levels of iron and ferritin did not vary with the location of the neoplasm and its stage of development.¹⁰ Although the mechanism by which iron could induce car-

Table 2. Iron Expression in 54 Colorectal Adenocarcinomas

	Positive Perls' staining (n=19)	Negative Perls' staining (n=35)	p-value
Size (cm ² , range)	17.56 (8.96-26.16)	22.28 (14.63-29.93)	0.431*
Depth of invasion			0.237 [†]
Mucosa or submucosa	6	5	
Proper muscle	1	6	
Pericolic adipose tissue	12	22	
Serosa or visceral peritoneum	0	2	
Shape			0.510 [†]
Polypoid or fungating	7	8	
Ulcerofungating	10	21	
Ulceroinfiltrative	2	6	
Location			0.201 [†]
Cecum and A-colon	1	8	
T-colon and flexures	1	1	
D-colon and S-colon	10	10	
Rectum	7	16	

NS, not significant; A-colon, ascending colon; T-colon, transverse colon; D-colon, descending colon; S-colon, sigmoid colon.

*Kruskal-Wallis test.

[†]Fisher's exact test.

cinogenesis is not fully understood, these studies suggested that iron may be a carcinogenic factor, possibly facilitating tumor growth and modulating local immune response.⁹ The authors suggested that iron is a carcinogenic factor involved in both the initiation and promotion of cancer, thus supporting that excess intracellular iron causes oxidative injury.

However, in the current study, iron appeared to reside exclusively in the stroma and outside the gland, but not in the epithelial cells. In addition, we found positive staining for iron also in large adenomas and pedunculated adenomas. These findings indicate that the iron deposition is a secondary phenomenon of rapid growth with hemorrhage or a secondary phenomenon of increasing nutritional requirements for iron in a developing neoplasm. It is more likely that our result represents residual iron from intralesional hemorrhage, especially given the known propensity of large polyps and cancers to bleed with higher incidence of fragile neovascular blood vessels.^{8,11}

In conclusion, presence of iron in the stroma but not in the epithelial cells, and the frequent presence of iron in large adenomas, pedunculated adenomas, and adenocarcinomas indicate that the iron deposition is a secondary phenomenon to rapid growth with hemorrhage rather than a consequence of epithelial cell carcinogenesis.

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REFERENCES

1. Nelson RL, Davis FG, Sutter E, Sobin LH, Kikendall JW, Bowen P. Body iron stores and risk of colonic neoplasia. *J Nat Cancer Inst* 1994;86:455-460.
2. Wurzelmann JI, Silver A, Schreinemachers DM, Sandler RS, Everson RB. Iron intake and the risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 1996;5:503-507.
3. Butterworth JR. Another important function for an old friend! The role of iron in colorectal carcinogenesis. *Gut* 2006;55:1384-1386.
4. Brookes MJ, Hughes S, Turner FE, et al. Modulation of iron transport proteins in human colorectal carcinogenesis. *Gut* 2006;55:1449-1460.
5. Tuccari G, Rizzo A, Crisafulli C, Barresi G. Iron-binding proteins in human colorectal adenomas and carcinomas: an immunocytochemical investigations. *Histol Histopathol* 1992;7:543-547.
6. Nelson RL. Dietary iron and colorectal cancer risk. *Free Radio Biol Med* 1992;12:161-168.
7. Seril DN, Liao J, Yang CS, Yang GY. Systemic iron supplementation replenishes iron stores without enhancing colon carcinogenesis in murine models of ulcerative colitis: comparison with iron-enriched diet. *Dig Dis Sci* 2005;50:696-707.
8. Dobrowolski S, Dobosz M, Babicki A, Glowacki J, Nalecz A. Blood supply of colorectal polyps correlates with risk of bleeding after colonoscopic polypectomy. *Gastrointest Endosc* 2006;63:1004-1009.
9. Labropoulou EP, Datsis A, Kekelos S, et al. The presence and significance of iron in neoplasia of the colorectum. *Tech Coloproctol* 2004;8:S211-213.
10. Kucharzewski M, Braziewicz J, Majewska U, Gozdz S. Iron concentrations in intestinal cancer tissue and in colon and rectum polyps. *Biol Trace Elem Res* 2003;95:19-28.
11. Nakama H, Zhang B, Kamijo N. Sensitivity of immunohistochemical fecal occult blood test for colorectal flat adenomas. *Hepatogastroenterology* 2004;51:1333-1336.