

Short Communication

Positive Correlation of Insulin-Like Growth Factor-II with Proliferating Cell Index in Patients with Colorectal Neoplasia

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Abstract

Background: Insulin-like growth factor-II (IGF-II) stimulates cell proliferation and is considered a potential risk factor for colorectal cancer. Tumor levels of IGF-II seem to positively correlate with colorectal cancer cell proliferation. This investigation examined the association of circulating IGF-II to the proliferating cell index (PCI) of tumor and matched normal mucosa in patients with colorectal neoplasia.

Methods: Circulating IGF-II level (ng/mL) was determined in the peripheral blood plasma by ELISA. The proliferating cells in tumor or matched normal mucosa were immunohistochemically stained using the primary antibody against Ki-67. Computer image analysis was used and PCI was expressed as the percentage of Ki-67-positive cells/total counted cells.

Results: Sixty-four patients were evaluated; 45 had colorectal neoplasia (27 males/18 females; mean age, 66.8 ± 11.8 years) and 19 had hyperplastic polyps (6 males and 13 females;

mean age, 58.4 ± 14.4 years). Among patients with colorectal neoplasia, blood IGF-II levels were positively correlated with PCI in the matched normal mucosa ($r = 0.46$, $P < 0.05$) but not in the tumor. In patients with hyperplastic polyps, blood IGF-II levels were not correlated with the PCI in the polyps. Blood IGF-II levels were higher in colorectal cancer patients with Dukes' C/D stage ($P < 0.01$) or with positive lymph nodes ($P < 0.01$).

Conclusion: Circulating IGF-II positively correlated with PCI in normal colonic mucosa of patients with colorectal neoplasia, suggesting that IGF-II may have a role in initiating the carcinogenic pathway by stimulating cell proliferation. Blood IGF-II was increased in advanced colorectal cancer, indicating that it might enhance colorectal cancer progression and be a useful marker of poor prognosis. (Cancer Epidemiol Biomarkers Prev 2005;14(7):1819-22)

Introduction

Colorectal cancer is a common cause of cancer death in Western countries, and the second leading cause in North America, accounting for over 57,000 deaths annually (1). Unfortunately, most patients who develop colorectal cancer are asymptomatic and have no known risk factors. Identification of frequent alterations in patients with colorectal cancer and understanding the possible role on oncogenesis could improve cancer screening with resultant mortality reduction.

During the past decade, extensive research showed that the insulin-like growth factor (IGF) system has an essential role in regulating cell growth, differentiation, apoptosis, and transformation (2) of colorectal cancer via paracrine and autocrine mechanisms. IGF-II, an important member of the IGF system, is elevated in circulating blood and highly expressed in patients with colonic polyps and/or colorectal cancer (3-7). Furthermore, IGF-II expression is significantly correlated with

several clinicopathologic and prognostic features of colorectal cancer (5, 8, 9). *In vitro* and animal studies reveal that IGF-II efficiently stimulates proliferation of colorectal cancer cells (10) and positively correlates with tumor growth (11, 12). However, whether elevated IGF-II blood levels also play an important role on the cytokinetics of the colorectal tumor and matched normal colonic mucosa in colorectal cancer patients remains unknown.

We hypothesized that elevated circulating IGF-II levels correlated with cell proliferation of the tumor and matched normal mucosa in patients with colorectal neoplasia. Consequently, we investigated the association of circulating IGF-II with proliferating cell index (PCI) in tumor and matched normal colonic mucosa in patients with colorectal neoplasia. Additionally, the level of circulating IGF-II was examined according to colorectal cancer neoplasia grade.

Materials and Methods

Patients. Qualified candidates were identified from the patients who underwent colonoscopy and/or colorectal surgery at Cleveland Clinic Florida from January to December 2003 and were diagnosed with hyperplastic polyp, adenoma, and/or colorectal cancer. Of these patients, 64 were prospectively recruited, with 80% participation/consent rate. All had a pathologic diagnosis. Patients with the following conditions were excluded from the study: (a) younger than 18 years old, (b) received or receiving

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Table 1. Characteristics of patients with colorectal adenoma or carcinoma

	Adenoma	Carcinoma			
		Dukes A/B	Dukes C/D	Lymph node -	Lymph node +
Colonic	24	8	3	9	2
Rectal	4	3	3	3	3
Total	28	11	6	12	5

preoperative cytotoxic agents, (c) with concomitant medical conditions that may alter IGF-II levels, including malnutrition, diabetes mellitus, metabolic/endocrine diseases, or hepatitis, and (d) unable or unwilling to sign informed consent. The demographic, medical, and pathologic data were retrieved from the patient's medical records and pathology report. The study was approved by the Institutional Review Board of Cleveland Clinic Florida.

Circulating IGF-II Measurement. Two milliliters of blood was drawn from the patients' peripheral vein and placed in a coded tube with EDTA. The blood plasma was collected by centrifuge at 1,500 rpm for 40 minutes and stored at -80°C freezer until IGF-II analysis. The IGF-II level was determined by an ELISA kit (Diagnostic Systems Laboratories, Inc., Webster, TX). According to the standard protocol from the manufacturer, plasma samples were analyzed and the blood IGF-II concentration (ng/mL) were determined by using a Multiskan-RC microplate reader (Labsystems, Helsinki, Finland) and Delta SOFT JV software (BioMetallics, Princeton, NJ).

Proliferating Cell Index. Formalin-fixed and paraffin-embedded tumor tissue blocks were retrieved from the Pathology Department. Three 4- μm -thick tissue sections were cut from each block for immunohistochemical staining. Tumor as well as the matched normal mucosa (2-5 cm from the tumor) were immunocytochemically stained using a primary antibody, goat anti-human Ki-67 antibody, and immunocytochemical staining kit (DAKO, Carpinteria, CA). The staining was done according to the standard protocol from the company. As negative control, the primary antibodies were replaced with nonimmune normal goat serum or with preabsorbed antiserum; appropriate tissue controls were used as noted above.

PCI was determined by using a computer-assisted image analysis system, which is composed of an Optronics Engineering DEI-750D digital output camera (Optronics, Goleta, CA), Hewlett Packard Vectra VE81 Pentium III-500 MHz computer (Hewlett Packard), and the software of Scion Image for Windows (Scion Corporation, Frederick, MD). Twenty randomly chosen microscopic fields (image magnification, $\times 63$) of tumor or matched mucosa were blindly analyzed by two qualified investigators (R. Zhao and M. Berho). To ensure that the same positive staining was not analyzed twice, consecutive sections were not used. By use of a threshold setting, all nuclei and the positively stained nuclei of entire mucosa crypt epithelial or carcinoma cells were automatically identified and counted, respectively. The PCI was presented as a percentage of the positive number of nuclei in the total number of observed nuclei. A minimum of 500 cells from each sample (normal mucosa and tumor) was required. Areas of necrosis, fibrosis, and damaged structure were avoided during analysis.

Statistic Analysis. Data were presented as mean and SD. Linear correlation, Student's *t*, and ANOVA tests were used for statistical analysis by using SPSS 10.0 software, with the level of statistical significance set at $P < 0.05$.

Results

A total of 64 patients were recruited for this investigation, 45 had adenomas and/or colorectal cancer (27 males and 18 females, age: 66.8 ± 11.8 years) and 19 patients had hyperplastic polyp (6 males and 13 females, age: 58.4 ± 14.4 years). The baseline characteristics of patients with colorectal adenoma or carcinoma are listed in Table 1.

Among the 45 patients with colorectal adenoma and/or colorectal cancer, circulating IGF-II levels were positively correlated with PCI in the matched normal mucosa ($r = 0.46$, $P < 0.05$; Fig. 1). However, circulating IGF-II levels did not correlate with PCI in the tumor ($r = 0.14$, $P = 0.38$). Similarly, among the 19 patients with hyperplastic polyp, circulating IGF-II levels were not correlated with PCIs in the polyps ($r = 0.30$, $P = 0.22$).

IGF-II was significantly higher in patients with rectal adenoma and/or carcinoma (638.97 ± 173.66) than in patients with colonic tumor (493.80 ± 170.78 , $P < 0.05$). Among 17 patients with adenocarcinoma, IGF-II level was higher in Dukes C/D patients (686.62 ± 208.77) and in patients with positive lymph nodes (712.84 ± 222.09) than in Dukes A/B patients (445.83 ± 110.53 , $P < 0.01$) or in patients without positive lymph nodes (454.97 ± 110.04 , $P < 0.01$), respectively (Fig. 2). There was no statistically significant difference of sex, age, and body mass index between these compared subgroups.

Discussion

IGF-II; a 67-amino-acid polypeptide is considered an important autocrine molecule in colorectal carcinogenesis. In most human tissues, the *IGF-II* gene displays monoallelic paternal expression with the maternal allele silenced from genomic imprinting (13, 14). This study revealed that the circulating IGF-II level was significantly correlated with the PCI of matched normal mucosa cells in patients with colorectal adenoma and/or colorectal cancer, suggesting an important role for this compound in regulating the proliferation of normal cells. Previous studies showed that IGF-II has proliferative and antiapoptotic actions (15). IGF-II growth promoting and antiapoptotic effects are mediated by binding to the IGF-I receptors (IGF-IR) located along the crypt villous axis in normal colonic mucosa with highest expression in crypt cells (13, 16-18). Furthermore, the neoplastic conversion of colorectal cells is associated with overexpression of IGF-II gene and protein (19). These findings strongly suggest that circulating IGF-II stimulates

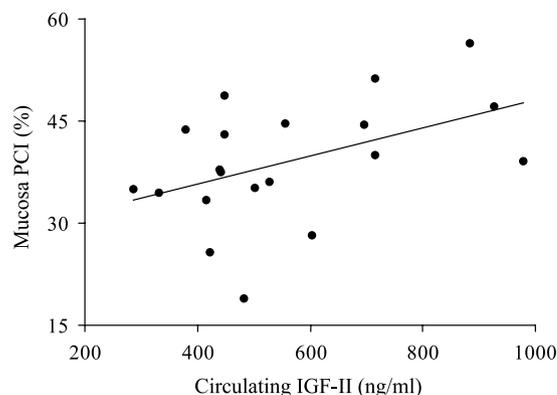


Figure 1. Correlation of circulating IGF-II with PCI in matched normal mucosa ($r = 0.464$, $P < 0.05$).

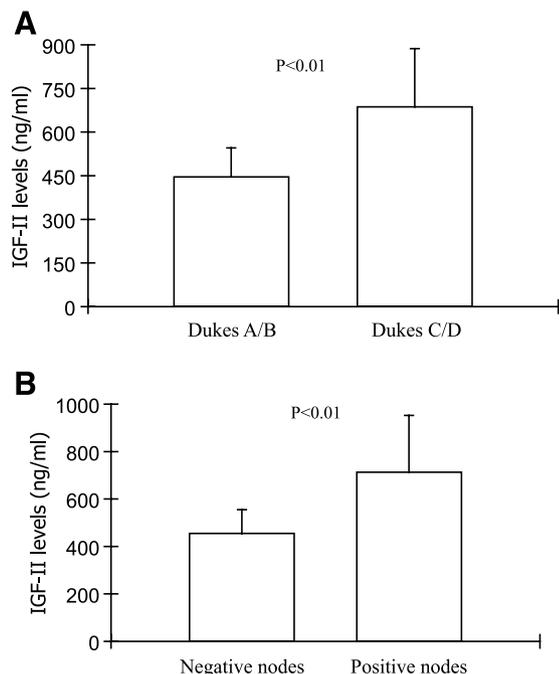


Figure 2. IGF-II levels in patients with various stages of colorectal cancer (A) and with or without positive lymph nodes (B).

the cell proliferation of normal colorectal mucosa via its specific receptor, IGF-IR, and potentiates neoplastic transformation.

IGF-II and IGF-IR are overexpressed in colorectal tumor (5-8) and the coexpression of IGF-II mRNA with the Ki-67 proliferation marker was reported in hepatocellular carcinoma (20, 21). In contrast, the IGF binding proteins (IGFBP-2 and IGFBP-4), which limit the availability of intratumoral IGFs, are decreased in colon cancer tissues (22). These observations suggest that the IGF-II levels should positively correlate with the PCI of tumor cells. However, we found no positive correlation between the circulating IGF-II levels and the PCI in colorectal adenoma, colorectal cancer, or hyperplastic polyp. Presently, it is unclear why IGF-II levels correlated with PCI in normal, but not cancer tissue. Several possibilities may explain our observations. First, IGF-II regulates tumor cell proliferation more likely via an autocrine pathway, so IGF-II overexpressed in tumor cells may be much more important than that in the circulation. Second, as a member of the IGF axis, the effects of IGF-II depend not only on absolute level but also on interaction among the members of the system. Studies on the proliferation of colorectal tumors assessing all elements of the IGF system will help elucidate this issue. Third, once cells become neoplastic, proliferation might be primarily regulated by oncogenes and/or cytokines other than IGF-II.

Several investigators have reported that IGF-II levels are elevated in the serum and overexpressed in the tumor of patients with colorectal adenoma and/or carcinoma (3-8) and that IGF-II levels are related to the colorectal cancer risk (13, 23). Moreover, the IGF-II level positively correlates with tumor stage (5, 9) and worse prognosis (9). The present study showed a similar result with circulating IGF-II levels significantly higher in colorectal cancer patients with positive lymph nodes and advanced diseases (Dukes C/D). Taken together, our observations indicate that IGF-II might enhance the progression of colorectal cancer, and have potential as a biomarker for colorectal cancer screening, prognosis, prevention, and treatment. The underlying

mechanisms of IGF-II variation in colorectal cancer patients are still unclear. Studies on IGF-II gene expression and regulation, such as promoter methylation and genomic imprinting status (24), will be critical in answering these questions.

Although this investigation showed that circulating IGF-II levels positively correlated with PCI in matched normal mucosa among patients with colorectal neoplasia, there are several limitations to consider. To further elucidate the association between IGF-II and normal mucosa PCI, and potential effect on colorectal carcinogenesis, studies with a relatively larger sample size, including individuals without colorectal neoplasia, will be required. In addition, as the normal colonic mucosa evaluated in this study was in close proximity with the neoplastic lesions, evaluation of more distant sites will be important to validate the observed associations.

In summary, circulating IGF-II positively correlated with PCI in normal colonic mucosa of patients with colorectal adenoma and/or colorectal cancer, revealing that IGF-II may have a role in initiating the carcinogenic pathway by stimulating cell proliferation. The absence of any correlation between IGF-II and PCI in tumor suggests that other autocrine and paracrine factors rather than circulating IGF-II are operative. Increased circulating IGF-II levels in advanced colorectal cancer indicate that IGF-II may enhance tumor progression and be a useful marker of poor prognosis.

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