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ScienceDirect
Growth Hormone & IGF Research 19 (2009) 126–135
www.elsevier.com/locate/ghir

Expression of insulin-like growth factor system components in colorectal tissue and its relation with serum IGF levels

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Received 15 April 2008; revised 2 July 2008; accepted 5 August 2008
Available online 17 September 2008

Abstract

Context: The insulin-like growth factor (IGF)-system has been implicated in colorectal tumor carcinogenesis. Although both tumor expression levels and serum concentrations of IGF-system components are related to colorectal cancer risk, it is unknown whether IGF levels in tissue and serum are correlated.
Objective: The objective of this study was to determine expression levels of various IGF-system components in different locations of the colorectum, and to investigate whether normal tissue IGF expression levels are correlated with serum IGF-I and IGF-II concentrations.
Design: Biopsies from macroscopically normal mucosa at four locations in the colorectum (ascending, transverse, sigmoid colon, and rectum) and a fasting serum sample were obtained from 48 asymptomatic patients at increased risk of colorectal cancer. Expression levels of IGF-I, IGF-II, IGF-IR, IGF-IIR, and IGFBP-3 messenger RNA (mRNA) in tissue were quantitatively evaluated using real-time RT-PCR. Expression of IGF-IR protein in the ascending colon and rectum tissue specimens was assessed semi-quantitatively by immunohistochemistry. Serum IGF-I and IGF-II concentrations were determined using immunometric assays.
Results: With the exception of IGF-IIR, mRNA levels of all the IGF-system components investigated, as well as IGF-IR protein expression, were significantly higher in the rectum compared with the ascending colon ($p \leqslant 0.001$). Serum IGF-I and IGF-II concentrations did not correlate with any of the parameters studied in colorectal tissues.
Conclusions: Our results indicate that in humans IGF-system components are differentially expressed in the colorectum. Moreover, our findings suggest that local and circulating components of the IGF-system are differentially regulated. However, due to large intra-individual variation in mRNA expression, we cannot formally exclude undetected but existing routes of co-regulation.

Keywords: Insulin-like growth factor; Insulin-like growth factor receptor; Colon; Serum; mRNA

Abbreviations: ALS, acid labile subunit; AU, arbitrary units; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IGF-IR, insulin-like growth factor receptor; IHC, immunohistochemistry; MAPK, mitogen-activated protein kinase; PI3K/Akt, phosphatidylinositol 3′-kinase; RT-PCR, reverse transcriptase polymerase chain reaction.

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doi:10.1016/j.ghir.2008.08.004

1. Introduction

The insulin-like growth factor (IGF)-system has an important role in normal as well as tumor cell growth [1]. IGF-system components are expressed in most tissues, including the colorectum [2]. The liver is the principal source for IGF-I and IGF-II protein in the blood circulation, where the majority of IGFs exist in a ternary complex with IGF binding protein (IGFBP)-3 and the acid labile subunit (ALS). The remainder of the circulating IGFs is associated with other binding proteins or present in a free form ($\leqslant$1%) [3]. Locally produced and circulating IGFs can be released from their binding proteins by IGFBP proteases [4]. These free IGFs can bind to the cellular membrane IGF-I receptor (IGF-IR), resulting in stimulation of proliferation and inhibition of apoptosis through the phosphatidylinositol 3′-kinase (PI3K/Akt) and mitogen-activated protein kinase (MAPK) pathways [4]. The binding of IGF-I or IGF-II to the IGF-IIR leads to degradation of the IGF protein [4].

Local IGF expression is thought to affect colorectal carcinogenesis via an autocrine or paracrine pathway. The IGF-IR and particularly IGF-II have often been found to be overexpressed in human colorectal tumor as compared to normal colorectal tissue [5–7]. Furthermore, IGF-I protein [8] and messenger RNA (mRNA) [9], IGF-II protein [5], and IGF-IR mRNA [9] expression in colorectal tumors have been significantly positively associated with proliferation. High circulating IGF-I and IGF-II concentrations have also been associated with increased risk of colorectal cancer [10]. Furthermore, in vivo studies revealed that in mice with liver deleted IGF-I expression (LID mice), circulating IGF-I concentrations were reduced by 75%, and colorectal tumor development, growth and metastases were decreased [11]. Both lines of evidence suggest an important role of the IGF-system in the development of colorectal cancer.

At present, no data are available on mRNA expression levels of IGF-system components throughout the colon. Moreover, it is unknown whether circulating IGF-I and IGF-II proteins directly affect colorectal tumor growth in humans through IGF-IR binding and activation, whether they influence local tissue expression of IGF-system components, or whether they are reflective of tissue IGF-system component expression and thereby act as a biomarker of tissue IGF-system component bioactivity. We hypothesized that high serum IGF-I and IGF-II concentrations are associated with down-regulation of IGF-IR mRNA and protein expression, and possibly also with down-regulation of other IGF-system components. To investigate this in more depth, we collected normal colorectal tissues obtained from four locations in the colorectum in individuals at increased colorectal cancer risk. We quantitatively evaluated mRNA expression levels of IGF-I, IGF-II, IGF-IR, IGF-IIR, and IGFBP-3 and additionally studied their relationship with serum IGF-I and IGF-II concentrations.

2. Materials and methods

2.1. Study population

Our study population consisted of men (40–75 years of age) and postmenopausal women (50–75 years of age) who were scheduled to undergo a surveillance colonoscopy because of a personal history of histologically confirmed colorectal adenomatous polyps or because of having at least one first degree family member with a history of colorectal cancer. They were selected from medical registries and pathology databases of four hospitals in the Netherlands (the Antoni van Leeuwenhoek hospital in Amsterdam, the Gelderse Vallei hospital in Ede, the Slotervaart hospital in Amsterdam, and the Sint Antonius hospital in Nieuwegein). Exclusion criteria were a history of cancer, familial adenomatous polyposis syndrome, familial Li Fraumeni syndrome, chronic inflammatory bowel disease, diabetes mellitus, acromegaly, significant liver or renal disease, (partial) bowel resection, non-remissive celiac disease, diverticulitis, other severe comorbidity, and laxative abuse. Forty-eight asymptomatic patients (32 males and 16 females) who underwent their surveillance colonoscopy between November 2003 and October 2005 participated in our study. The study protocol was approved by the Medical–Ethical Committees of all participating centers.

2.2. Colorectal tissue specimens

Colonoscopy was performed after whole-gut lavage with 4 l of a macrogol: Klean–Prep (Norgine BV, Amsterdam, the Netherlands) or Coloforte (Ipsen Farmaceutica BV, Hoofddorp, the Netherlands). Colorectal biopsies were obtained with a standard-sized flexible endoscopic forceps and collected from four locations: the ascending colon, transverse colon, sigmoid colon, and rectum. At each location, four biopsies from macroscopically normal mucosa were taken. Two biopsies were snap-frozen in liquid nitrogen and stored at −70 °C until preparation of RNA. The other two biopsies were formalin-fixed and paraffin-embedded for immunohistochemical analyses.

2.3. RNA isolation and RT reaction

Total RNA was extracted from the tissue samples using RNAzolB (Campro Scientific). Total RNA (5 μg) was reverse-transcribed (RT) to generate first strand complementary DNA (cDNA) (total volume