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mRNA Expression of IGF-1 and IGF-1R in Patients with Colorectal Adenocarcinoma and Type 2 Diabetes

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Background and Aims. Increasing studies show that messenger RNA (mRNA) levels of local IGF-system are overexpressed in cancer tissue of patients with colorectal cancer (CRC). However, the influence of type 2 diabetes (T2DM) on the expression of insulin-like growth factor-1 (IGF-1) and IGF-1 receptor (IGF-1R) mRNA in colorectal cancer tissue and adjacent non-cancerous tissue (ANCT) is unknown. The aim of this study was to assess mRNA expression of IGF-1 and IGF-1R in paired samples of cancer tissue and ANCT between colorectal adenocarcinoma (CA) patients with and without T2DM.

Methods. To quantify the levels of IGF-1 and IGF-1R mRNA in CA, we analyzed the expression of IGF-1 and IGF-1R mRNA levels in paired samples of cancer tissue and ANCT in CA patients with and without T2DM using real-time reverse transcription-polymerase chain reaction (RT-PCR).

Results. mRNA levels of IGF-1 and IGF-1R were significantly higher in cancer tissue compared with its ANCT in CA patients with and without T2DM. Compared with the CA group, significantly higher levels of IGF-1 and IGF-1R mRNA were observed in cancer tissue in CA with T2DM group. No significant differences were observed in the role of cancer locations, Dukes stages and diabetes duration on mRNA expression of IGF-1. After adjusting for age, gender and Dukes stages, multivariate analysis indicated IGF-1 mRNA level was a risk factor for prognosis ($p < 0.05$).

Conclusions. Our results support the hypothesis that IGF system plays an important role in CRC. Further larger studies are needed.

Key Words: Insulin-like growth factor-1, Insulin-like growth factor-1 receptor, Messenger RNA, Colorectal adenocarcinoma, Type 2 diabetes.

Introduction

The insulin-like growth factor (IGF) system plays an important role in regulating cell growth, differentiation, and apoptosis, which has been demonstrated to promote the development of cancer (1). IGF system has been implicated in colorectal tumor carcinogenesis (2). This family consists of three ligands (insulin, IGF-1, and IGF-2), six receptors (insulin receptor [IR] alpha [fetal], IR beta [adult], IGF-1 receptor [IGF-1R], IGF-2R, hybrid IGF-1R/IR alpha, hybrid IGF-1R/IR beta), and up to seven IGF binding proteins (IGFBP1-7) (3). IGF-1 is produced in most tissues, particularly liver (4). In IGF-responsive tissues, IGF-1 ligands can be delivered through the circulation from the liver (an endocrine source), but IGF-1 can also be locally produced through autocrine or paracrine mechanisms (5). More than 75% IGF-1 is confined to the vascular compartment as a 150 kDa ternary complex with the acid labile subunit (ALS) and IGFBP-3, the most abundant circulating IGFBP (6). The relation between IGF-1 and IGFBP-3 can be modified further by specific IGFBP proteases, which degrade IGFBP-3 into smaller fragments that have a decreased affinity for IGF-1 and hence increase IGF-1 bioavailability (7). The free form of circulating and locally produced IGF-1 activates signaling via the phosphatidylinositol-3-kinase (PI3K)-Akt and RAS/RAF/mitogen-activated protein kinase (MAPK) pathways (8) that enhance the proliferation and survival of colorectal cancer cells (9).

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Colorectal cancer (CRC) is the third most common cancer in men (663,000 cases, 10.0% of the total) and the second in women (570,000 cases, 9.4% of the total) worldwide (10). More than 95% of colorectal cancers are adenocarcinomas (11). Expression of IGF-1 and IGF-1R has been shown to increase in CRC (12). Results from Renehan's meta-analysis suggested that higher serum IGF-1 levels are associated with increased risk of CRC (13). Tissue IGF-1 bioactivity is determined not only by serum (endocrine) IGF-I, but also by locally (autocrine or paracrine) produced IGF-1. Local expression of IGF-1 messenger RNA (mRNA) in the CRC had been significantly positively associated with proliferation (14). High-level IGF-1R expression in colorectal cancer tissue is initiated by an abnormality of stem cell programmed differentiation in the aberrant crypt focus (ACF) (15). ACFs of the colon are possible precursors of colon adenoma and cancer (16). However, it is worth noting that the results of previous studies (2,12,15,17−20) that examined expression of local IGF system components in colorectal tissue are inconsistent.

Possible common mechanisms for a biological link between diabetes and colorectal cancer include hyperinsulinemia, hyperglycemia, and inflammation (21). The IGF-1-hyperinsulinemia theory suggests that high insulin levels may act via IGF-1R and by decreasing IGFBPs with a consequent increase in the biological activity of circulating and local IGF-1 (22). Elevated levels of insulin and free IGF-1 promote proliferation of colon cells and lead to a survival benefit of transformed cells, ultimately resulting in colorectal cancer (23). However, the influence of type 2 diabetes (T2DM) on expression of IGF-1 and IGF-1R mRNA in colorectal cancer tissue and adjacent non-cancerous tissue (ANCT) is unknown. Therefore, mRNA levels of IGF-1 and IGF-1R in paired samples of cancer tissue and ANCT in colorectal adenocarcinoma (CA) patients with and without T2DM was evaluated using real-time reverse transcription-polymerase chain reaction (RT-PCR). In addition, we assessed the influence of locations, Dukes stages, and diabetes duration on mRNA expression of IGF-1 and IGF-1R in cancer tissue and ANCT between CA patients with and without T2DM. We also discussed the association between the expression levels of IGF-1 and IGF-1R mRNA and survival.

Materials and Methods

Study Subjects

We selected subjects who were diagnosed with CA in Tianjin Union Medical Center from January 1, 2009−December 31, 2012. The subjects consisted of 40 CA patients with T2DM and 40 CA patients without diabetes, matched by age and gender. T2DM was defined according to the most recent clinical practice recommendations by the American Diabetes Association (ADA) (24). Date of cancer diagnosis, date of detection of metastases, date of first relapse, and date of last follow-up (or date of death) were requested. The date of last follow-up updating for all patients was June 30, 2013.

Subjects were excluded if they had type 1 diabetes, ulcerative colitis, familial adenomatous polyposis syndrome, chronic inflammatory bowel disease, hereditary CRC syndromes, previous bowel resection, and preoperative chemotherapy. This study was approved by the Ethics Committee of Tianjin Union Medical Center.

Colorectal Tissue Specimens

Cancer tissue and ANCT specimens were obtained from patients during surgical therapy of CA. ANCT was taken at a distance of at least 5 cm from cancer tissue. Paired samples of colorectal cancer tissue and ANCT were provided immediately after surgical removal. All specimens were immediately frozen in a −80°C ultra-low temperature freezer until preparation of RNA.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from colorectal cancer tissue and ANCT samples by using the TranZol Up Kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. Quality of total RNA is generally checked by the ratios of OD260/280 and OD260/230. The presence of protein contaminants or light scattering by any precipitates decreases the ratios of OD260/280 and OD260/230, respectively (25). Ratio OD260/280 1.8−2.0 is considered good. A low ratio might indicate protein contamination. A ratio >2.1 might indicate RNA degradation. Ratio OD260/230 >1.8 is considered good. A low value might indicate organic contamination.

Total RNA (3 μg) was reverse-transcribed to generate first-strand complementary DNA (cDNA) (total volume 20 μl) by using TransScript Reverse Transcriptase [M-MLV, RNase H-] Kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. After 10 min reverse transcription for annealing of the random hexamers at 25°C, the reverse transcription polymerase chain reaction was performed at 42°C for 30 min, followed by inactivating the reverse transcriptase at 85°C for 5 min. The resulting cDNA was analyzed immediately by real-time RT-PCR.

Real-time RT-PCR

Real-time RT-PCR was used to quantify the levels of IGF-1 and IGF-1R mRNA in the paired samples of colorectal cancer tissue and ANCT. Primers for these reactions were designed using Primer Express software (Gene Runner and Primer Premier 5.0) and verified using NCBI-BLAST (National Center for Biotechnology Information-Basic Local Alignment Search Tool) (Table 1).

Primers were chosen in two adjacent exons to avoid DNA contamination and amplification of the homologous insulin and insulin receptor genes. Real-time RT-PCRs were carried out using the CFX96TM Real-Time System (Bio-Rad, Hercules, CA) with SYBR Premix Ex Taq (Takara Bio, Shiga,