Reduced expression of the AdipoR1 gene is correlated with venous invasion in colorectal cancer

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Abstract. Serum adiponectin concentrations are negatively correlated with body fat percentage and with the risk of colorectal cancer. However, few studies have examined the relationship between adiponectin receptor expression and colorectal cancer. We measured the expression levels of the AdipoR1 and AdipoR2 genes by quantitative real-time reverse-transcription polymerase chain reaction in 202 paired specimens of cancer tissue and adjacent normal mucosa obtained from patients with colorectal cancer. To evaluate the clinical significance of AdipoR1 and AdipoR2, correlations between the expression of these genes and clinicopathological features were examined. Both genes were expressed in colorectal cancer and in adjacent normal mucosa. The expression levels of the genes were significantly higher in cancer tissue than in normal mucosa (P<0.0001). Reduced expression of the AdipoR1 gene was correlated with venous invasion, but not with any other clinicopathological feature examined. Our findings suggest that reduced expression of the AdipoR1 gene may be a useful predictor of venous invasion.

Introduction

Obesity has been identified as a risk factor for colorectal cancer and several other types of cancer (1-3). Adiponectin, a hormone that is secreted exclusively by adipocytes, has potent insulin-sensitizing effects. Serum adiponectin concentrations are negatively correlated with body fat percentage (4,5). Hypoadiponectinemia is associated with insulin resistance and hyperinsulinemia (4). Serum adiponectin concentrations are negatively correlated with the risk of colorectal cancer (5).

Two types of adiponectin receptors (AdipoR1 and AdipoR2) have been cloned (6). In mice, AdipoR1 is expressed in various organs, including skeletal muscle, the lungs and the spleen, whereas AdipoR2 is predominantly expressed in the liver (6). In humans, AdipoR1 and AdipoR2 are expressed in pancreatic islet cells, macrophages, adipocytes and vascular smooth muscle, as well as in human breast epithelial and breast cancer cells (7-10). AdipoR1 is a receptor for globular adiponectin, whereas AdipoR2 is a receptor for full-length adiponectin (11). AdipoR1 and R2 are thought to mediate increased AMP kinase activity and peroxisome proliferator activated receptor (PPAR)-α ligand activity, fatty acid oxidation and glucose uptake by adiponectin (11).

A recent study showed that adiponectin inhibits the growth of gastric cancer and the development of peritoneal metastasis in vivo through AdipoR1 and AdipoR2 (12). Adiponectin also induces tumor vessel apoptosis and stimulates endothelial cell growth and angiogenesis (13,14).

In this study, we measured the expression levels of the AdipoR1 and AdipoR2 genes in 202 paired specimens of cancer tissue and adjacent normal mucosa obtained from patients with colorectal cancer. To evaluate the clinical significance of AdipoR1 and AdipoR2, we examined the correlation between the expression of these genes and clinicopathological features.

Materials and methods

Patients and samples. Surgical specimens of cancer tissue and adjacent normal mucosa obtained from 202 patients with untreated colorectal carcinoma were studied. The patients underwent surgery at the Gastroenterological Center of Yokohama City University Medical Center and at Kanagawa Cancer Center between 2002 and 2006. Informed consent was obtained from each patient. The ethics committees of Yokohama City University Medical Center and Kanagawa Cancer Center approved the protocol before initiation of the study. All tissue samples were embedded in OCT compound...
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(Sakura Finetechnical Co., Ltd.; Tokyo) and were immediately stored at -80°C until use. No patient had any other malignancies. The specimens were stained with hematoxylin and eosin and examined histopathologically. Sections that consisted of >80% carcinoma were used to prepare total RNA.

Quantitative real-time reverse-transcription polymerase chain reaction. Total RNA isolated from colorectal cancer and adjacent normal mucosa was prepared using Trizol (Gibco, Life Tech, Gaithersburg, MD). Complementary DNA (cDNA) was synthesized from 2 µg of total RNA using an iScript cDNA Synthesis Kit (Bio-Rad laboratories, Hercules, CA). After synthesis, the cDNA was diluted 1:4 with water and stored at -20°C until use. Quantitative real-time PCR was performed with an iQ SYBR Green Supermix Kit (Bio-Rad laboratories). PCR reactions were carried out in a total volume of 15 µl containing cDNA derived from 75 ng of RNA, 0.27 µM of each primer, 7.5 µl of iQ SYBR Green Supermix containing dATP, dCTP, dGTP and dTTP at concentrations of 400 µM each, and 50 U/ml of iTag DNA polymerase. The PCR consisted of 10 min at 94°C, annealing for 30 sec at an appropriate temperature (Table I), and a primer extension for 1 min at 72°C followed by 72°C for 10 min. PCR primer sequences of AdipoR1, AdipoR2 and β-actin, used as an internal control, are shown in Table I.

Immunohistochemical analysis of AdipoR1. Sections (4-µm) were prepared from paraffin blocks, deparaffinized, rehydrated, microwaved for 15 min in 10 mmol/l citrate buffer (pH 6.0), and incubated for 10 min in Peroxidase Blocking Reagent (Dako, Glostrup, Denmark). After incubation in 5% skim milk for 10 min at room temperature, the slides were incubated for 1 h with the primary antibodies at room temperature. The primary antibody used was rabbit anti-human AdipoR1 (raised against amino acid residues 357-375) antiserum (Phoenix Pharmaceuticals Inc., Belmont, CA, USA) at a dilution of 1:500. The secondary antibody was peroxidase-labeled polymer, EnVision+ rabbit (Dako), applied for 60 min at room temperature. The color reaction was developed with 3,3’-diaminobenzidine (Dako), and the slides were counterstained with hematoxylin.

Statistical analysis. Gene expression levels of colorectal cancer were compared with those of the normal adjacent mucosa using the Wilcoxon test. Relationships between gene expression and potential explanatory variables, including age, gender, tumor size, histological type, depth of invasion, lymph node metastasis, location, lymphatic invasion, venous invasion and liver metastasis, were evaluated using the χ² test. Associations between variables were assessed using the Mann-Whitney U test. All statistical analysis was performed using Statview J 5.0 software.

**Table I. PCR primers and conditions.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Annealing temperature (˚C)</th>
<th>Product size (bp)</th>
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<tbody>
<tr>
<td>AdipoR1</td>
<td>5’-CCAGGAAGAAGAGGAGGAGGAG-3’</td>
<td>60.0</td>
<td>166</td>
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<tr>
<td></td>
<td>5’-ATGTCAGATAGCAGTCTTGTC-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AdipoR2</td>
<td>5’-TTCTTTTCTCCTCCTCCTCCT-3’</td>
<td>57.4</td>
<td>199</td>
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<tr>
<td></td>
<td>5’-CTCCCCTCCCTCCTATCC-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>5’-AGTTTGGTTACACCCCTTCTGAC-3’</td>
<td>60.0</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>5’-GCTCGCTCCAACCGACTG-3’</td>
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Figure 1. Comparison of AdipoR1 (A) and AdipoR2 (B) gene expression levels in colorectal cancer tissue (C) and adjacent normal mucosa (N). AdipoR1 and AdipoR2 gene expression levels were higher in cancer than in adjacent normal mucosa (P<0.0001, P<0.0001, respectively).
Comparison of AdipoR1 and AdipoR2 mRNA expression in colorectal cancer tissue and adjacent normal mucosa. AdipoR1 and AdipoR2 gene expression levels were higher in cancer than in adjacent normal mucosa (P<0.0001 and <0.0001, respectively) (Fig. 1).

Relationship between AdipoR1 and AdipoR2 gene expression and clinicopathological features. The expression levels of the AdipoR1 and AdipoR2 genes were categorized as low or high according to their median values. The relationships between the expression of these genes and clinicopathological features were then examined. Reduced expression of AdipoR1 was correlated with venous invasion (P=0.002). AdipoR1 and AdipoR2 expression levels were unrelated to age, gender, tumor location, tumor size, histological type, depth of invasion, lymph node metastasis, lymphatic invasion and liver metastasis (Table II).
Association of AdipoR1 and AdipoR2 gene expression with venous invasion in patients with colorectal cancer. AdipoR1 gene expression levels were higher in the absence than in the presence of venous invasion (P=0.0001) (Fig. 2).

Immunohistochemical analysis of AdipoR1. Upon immunohistochemical analysis, tumor tissue expressing high levels of AdipoR1 mRNA showed positive expression of adiponectin receptor AdipoR1 (Fig. 3).

Discussion
Although many studies have examined the relationship between adiponectin and cancer (5,15-17), the relationship between adiponectin receptors and cancer remains largely unexplored (18). Takahata et al (10) suggested that adiponectin modulates the growth of cancer cells directly through AdipoR1 and AdipoR2. In this study, we examined the expression levels of the AdipoR1 and AdipoR2 genes and

Figure 2: Association of AdipoR1 (A) and AdipoR2 (B) gene expression levels with venous invasion in 202 patients with colorectal cancer. Box boundaries, the 25th and 75th percentiles of the observed values; capped bars, the 10th and 90th percentiles; solid line, median. P-values were calculated by the Mann-Whitney U test. AdipoR1 gene expression was higher in the absence V(-) than in the presence V(+) of venous invasion (P=0.0001).

Figure 3. Immunostaining of AdipoR1 in human colorectal cancer tissue. Immunohistochemical staining of AdipoR1 in surgically resected colorectal cancer tissues (A, x200; C, x400). Negative control (B, x200). Hematoxylin and eosin staining (D, x200). AdipoR1 was positively detected in the cytoplasm of cancer cells expressing high levels of AdipoR1 mRNA.
the relationships between these levels and clinicopathological variables.

First, we compared the relative expression of the AdipoR1 and AdipoR2 genes in colorectal cancer tissue and adjacent normal mucosa. Several studies have previously assessed the expression of AdipoR1 and AdipoR2 in cancer tissue. Takahata et al (10) examined the expression of the AdipoR1 and AdipoR2 genes in four breast cancer cell lines. AdipoR1 and AdipoR2 gene expression was found in all cancer cell lines and in normal breast epithelial cells. More recently, Williams et al (19) studied the expression of AdipoR1 and AdipoR2 in 40 colorectal carcinomas and 12 non-tumor colorectal tissue specimens from patients with colorectal cancer. AdipoR1 was expressed in 95% of the cancer tissue specimens and 8% of the non-tumor colorectal tissue specimens. AdipoR2 was expressed in 88% of the cancer tissue specimens and 0% of the non-tumor tissue specimens. In the present study, we found that the AdipoR1 and AdipoR2 genes were expressed in colorectal cancer tissue, as well as in normal colorectal mucosa.

We went on to examine the correlation between AdipoR1 and AdipoR2 gene expression and clinicopathological features. Several studies have investigated correlations between serum adiponectin concentrations and venous invasion by cancer cells (20,21). To our knowledge, however, no previous study has examined the correlation between the expression of adiponectin receptors and venous invasion. Our study showed that reduced expression of the AdipoR1 gene was correlated with venous invasion in colorectal cancer. These results are partially consistent with the findings of previous studies. Horiguchi et al (22) found no significant association between total adiponectin and venous invasion in renal cell carcinoma. In another study performed by the same group, the serum concentration of leptin, another adipokine, was significantly higher in patients with venous invasion than in those without (20). On the other hand, Ishikawa et al (21) reported that venous invasion and lymph node metastasis tended to be more frequent in gastric cancer patients with low serum adiponectin concentrations than in those with high adiponectin concentrations. Their results suggested that adiponectin may prevent tumor invasion or metastasis.

In conclusion, our results show that reduced expression of the AdipoR1 gene is correlated with venous invasion in colorectal cancer. Our findings suggest that reduced expression of AdipoR1 may be a useful predictor of venous invasion.

References