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Research Article

Expression of *AdipoR1* and *AdipoR2* and Serum Level of Adiponectin in Gastric Cancer

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Keywords
AdipoR1 · *AdipoR2* · Adiponectin serum level · Gastric cancer

Abstract
Background: Cancer is one of the major causes of death worldwide and the third leading cause of death in Iran. One of the proteins that are considered having anticancer effects is the adiponectin hormone. Adiponectin leads to programmed cell death, prevents cell growth and proliferation, and increases the expression levels of *BCL2*. ***Aim:*** The aim of this study was to assay the expression of adiponectin receptors (*AdipoR1* and *AdipoR2*) genes in gastric cancer patients. ***Materials and Methods:*** In this case-control study, 42 gastric cancer patients and 52 volunteers as healthy controls were enrolled. Total RNA was extracted. cDNA was synthesized by the reverse transcription method, and expression analysis was performed by real-time PCR. The serum level of adiponectin was also measured by ELISA. ***Results:*** The expression of both *AdipoR1* and *AdipoR2* was significantly higher than the control group ($p = 0.02$). Serum adiponectin was significantly lower in gastric cancer cases when compared with normal controls ($p = 0.03$). ***Conclusion:*** We found that expression level of *AdipoR1* and *AdipoR2* is strongly higher; however, the level of circulating adiponectin is lower in gastric cancer. Our study suggests that the expression of *AdipoR1* and *AdipoR2*, besides the low level of adiponectin, may play an important role in the development and/or progression of gastric cancer.

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Introduction

Gastric cancer (GC) is the fourth most common cancer in humans and is the second leading cause of death due to all forms of cancer worldwide [1]. Incidence of GC in Western countries has been increased since the 1940s and still remains as an important health issue [2]. It has been also reported that GC is rising dramatically in Iran with the highest incidence in Ardabil Province [3].

One of the proteins that are considered having anticancer effects is adiponectin. The receptors associated with adiponectin cause activation of caspases that can lead to programmed cell death and prevent the growth and proliferation of cells [4–6]. Adiponectin also increases the expression levels of *BCL2* (a protein involved in apoptosis) [7, 8]. Polymorphisms in adiponectin receptors and their lower expression correlate with insulin resistance [5, 6, 9]. In vitro studies have demonstrated that adiponectin is associated with the growth inhibition and apoptosis induction in cancer cell lines [9]. In vivo studies in mice have shown that the development of intestinal tumors is associated with disruptions in serum adiponectin [10]. Adiponectin receptor 1 (*AdipoR1*) gene is located on the large arm of chromosome 1 (1q32.1) [11], and adiponectin receptor 2 (*AdipoR2*) gene is located on chromosome 12 (12p13.31), also contains 8 exons [6].

This study was designed with the aim to evaluate the role of expression of adiponectin receptors (*AdipoR1* and *AdipoR2*) genes in patients with GC. According to the increasing rate of GC in Iran, considering the gene expression of receptors for anticancer proteins is an inevitable issue through improving the prognosis of patients.

Materials and Methods

Study Population

The study included 42 patients with GC who visited GMC Hospital, Guwahati, India, and 52 healthy volunteers without any signs of digestion problem. Informed consent was obtained from all individuals. The study protocol was approved by the ethics committee of Gauhati University (No. GUEC-03/2015).

Two milliliters of peripheral blood was collected in EDTA vials for whole blood conservation, and 3 mL was collected in serum clot activator vials for serum separation (red vials). Samples were immediately transferred to Biomedical Laboratory, Gauhati University, and stored at 4°C. Immediately after blood collection, serums were isolated at 3,000-rpm speed and kept in 2-mL Eppendorf Tubes stored at −20°C.

RT-PCR and Real-Time PCR Analysis

Total RNA was extracted by using an RNA extraction mini kit (Qiagen). The RNA was dissolved in 50-μL RNase-free water. The concentration of RNA was determined at 260 nm with a NanoDrop spectrophotometer. The cDNA (complementary) was synthesized from the total RNA (100 ng) by using the cDNA Kit (Thermo Fisher Scientific). The cDNA was then used as a template.

Real-time quantitative PCR was performed. The set of primers for *AdipoR1* and *AdipoR2* designed by NCBI information is as follows:

AdipoR1:

- Forward: 5′-TCTCGGACTTTTTCCAAACT-3′
- Reverse: 5′-GTCCCAGGAACACGCCTGCT-3′

AdipoR2:

- Forward: 5′-TGGAAGAATTTGTTTGTAAGGTATG-3′
- Reverse: 5′-ACAGGAAGAATACACAACCTAAGAG-3′

To control the variation for DNA available through PCR, *AdipoR1* and *AdipoR2* gene expression was normalized in relation to the expression of a housekeeping gene (the human β-actin). The PCR conditions were set as follows: 95°C for 10 min, followed by 38 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s.

Serum Level of Adiponectin

The total level of human serum adiponectin was measured by using a commercially available ELISA kit according to the manufacturer's instructions (Thermo Fisher Scientific; Fig. 2).

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Table 1. Clinical characteristics of patients with GC and healthy control subjects

Variants	GC patients (*n* = 42)	Control (*n* = 52)
Gender, *n* (%)		
Male	32 (76)	30 (58)
Female	10 (24)	22 (42)
Age, years		
Mean ± SD	45.1±7	35±5
BMI, kg/m^2		
Mean ± SD	34.2±7.0	29.0±4.5
FBS, mmol/L		
Mean ± SD	9.8±4.1	6.5±2.0
Stage of tumor, *n* (%)		
Stage II	23 (55)	–
Stage III	19 (45)	–

None of the patients had any prior history of alcohol consumption. GC, gastric cancer; *n*, number; BMI, body mass index; FBS, fasting blood sugar.

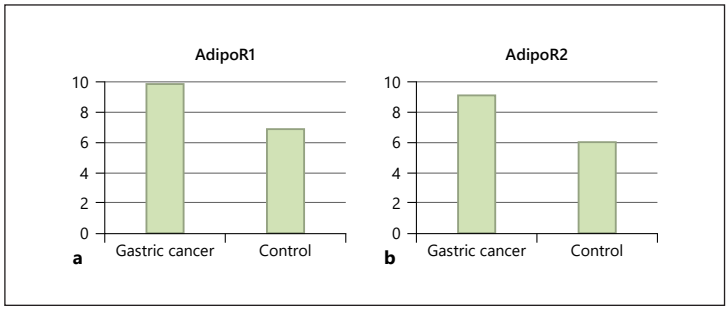

Fig. 1. Real-time quantitative PCR analyses of *AdipoR1* (**a**) and *AdipoR2* (**b**) in GC patients. *AdipoR1* expression levels in GC cases were higher than those in the control group. Expression levels were normalized to the housekeeping gene (β-actin). GC, gastric cancer.

Statistical Analysis

For statistical analysis, SPSS software version 16 was used, and the significant possibility <0.05 was considered. BMI was calculated as weight (kg) divided by height squared (m^2). For the determination of relative levels of *AdipoR1* and *AdipoR2* gene expression, the Pfaffl method of relative quantification, based on the comparison of the threshold cycle of a constitutive gene (mRNA of ACTB) with the test gene of each sample in duplicate, was carried out. The fold of *AdipoR1* and *AdipoR2* was normalized with regard to the reference genes expressed constitutively and was then compared with the nontreated controls as follows: $2^{-\Delta\Delta CT}$, where $\Delta\Delta CT$ = (CT-target – CT-reference) treated sample – (CT-target – CT-reference) calibrating sample. "Calibrating sample" refers to the level of expression (1×) of the target gene normalized for the constitutive gene.

Result

The clinical characteristics of GC and control participants of the present study are summarized in Table 1. The mean age of GC patients was 45.1 ± 7 years, and the mean BMI was 34.2 ± 7 kg/m^2. The mean age of the control group was 35 ± 5 years, and the mean BMI was 29.0 ± 4.5 kg/m^2. The GC group consisted of 32 male and 10 female patients (M/F ratio: 3.2), while the control group consisted of 30 males and 22 females (M/F ratio: 1.4).

Expression of AdipoR1 and AdipoR2 in GC

To identify how expression of *AdipoRs* is altered in patients with GC, a real-time PCR analysis was performed by using cDNA made of total RNA extracted from human peripheral

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Table 2. Difference in serum adiponectin concentration between patients with GC and the control group

	GC patients (n = 209)	Control (n = 211)	p value*
Adiponectin level, μg/mL, mean ± SD	4.4±3.2	6.6±3.7	0.03

Data are expressed as mean ± SD. * *T* test: p value is significant at 0.05 levels.

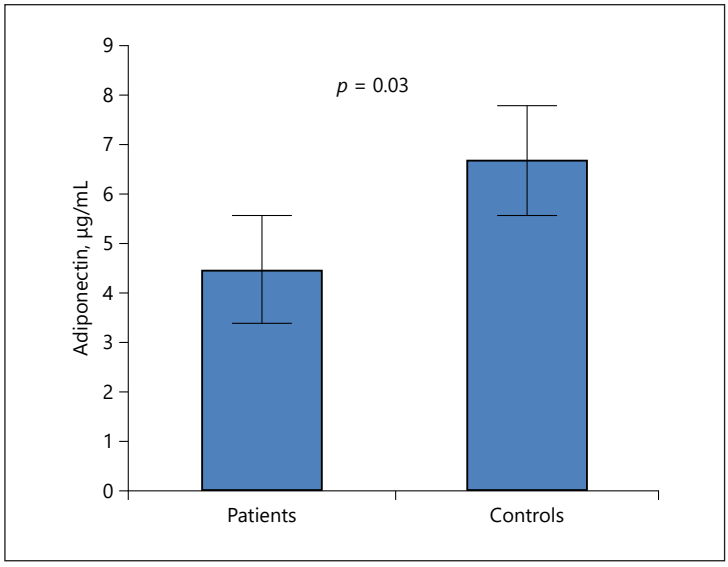

Fig. 2. Difference in serum adiponectin concentration between GC patients and controls. GC, gastric cancer.

blood samples. Expression of *AdipoR1* was significantly higher among GC groups (p = 0.02) (Fig. 1a) although expression of *AdipoR2* was significant in patients with GC (Fig. 1b).

Serum Adiponectin Level in GC Patients

The serum adiponectin levels were measured in 42 GC patients and 52 control subjects. In the GC patients, mean serum levels of adiponectin were significantly lower compared with the healthy control subjects (total adiponectin 4.4 ± 3.2 vs. 6.6 ± 3.7 μg/mL) (Table 2; Fig. 2).

The mean serum levels of adiponectin were significantly lower in male subjects with GC compared with the male healthy control subjects (total adiponectin 4.3 vs. 5.5 μg/mL). In addition, the mean serum levels of adiponectin were significantly lower in female subjects with GC compared with the control healthy subjects (total adiponectin 5.3 vs. 7.1 μg/mL).

Discussion

Adiponectin, which is also referred to as gelatin-binding protein 28 (GBP28) [12], AdipoQ [13], ACP30 (Acrp30) [14], or gene product of the adipose which is the most abundant gene transcript-1 (apM1) [15], is secreted predominantly by white adipose tissue. The recent studies reported that adiponectin may play a role in gastric [16] and renal [17] cancers, and also in leukemia [18, 19]. Plasma adiponectin concentrations have been found to be lower in patients with GC than in healthy control subjects and to be inversely correlated with tumor

size, depth of invasion, and tumor stage [16]. The present study provides evidence of lower adiponectin circulating levels in GC cases. Adiponectin has well-documented insulin-sensitizing effects [8, 20, 21], which may in part explain the reduction in cancer risk associated with higher circulating levels of this adipokine. However, still the role of adiponectin in cancer is not fully understood. However, adiponectin may provide indirect protection against carcinogenesis by affecting inflammatory states. The expression of adiponectin receptors is negatively regulated by insulin through activation of phosphoinositide 3-kinase and inactivation of FOXO1 [22]. Thus, the expression of *AdipoR1*/*R2* is inversely correlated with plasma insulin concentrations in vivo under physiologic (i.e., increase with fasting and decrease with feeding) and pathologic conditions [22]. Moreover, the expression of adiponectin receptors in GC cell lines has already been reported [23].

In this study, we found adiponectin circulating levels are lower in patients with GC and adiponectin receptors *AdipoR1* and *AdipoR2* are highly expressed in GC; thus, the expression of adiponectin receptors was significantly higher in GC. These data indicate the potential for adiponectin receptors to be involved in GC and their potential utility in the diagnosis and/or therapy for this carcinoma.

AdipoR1 and *AdipoR2* are present approximately in 1% of T cells, 93% of monocytes, 47% of B cells, and 21% of NK cells [24]. *AdipoR2* mRNA expression and plasma concentration of adiponectin are decreased in patients with obesity, type 2 diabetes, and coronary heart disease [25–27].

In our study, we found that the adiponectin plasma level is lower in GC cases compared with the healthy control group ($p = 0.03$). The expression of *AdipoR1* receptor was significantly higher among the GC cases compared with the healthy control group ($p = 0.03$), and the expression of *AdipoR2* was significantly high in GC ($p = 0.04$). These data suggest the role of adiponectin in GC and confirm a remarkable association between reduced level of adiponectin production in adipose tissue with GC and insulin resistance [28]. Relation between high expressions of *AdipoR1* and *AdipoR2* versus low level of adiponectin needs to be investigated. Adiponectin has also been found to inhibit prostate cancer cell growth [29]. More recently, adiponectin, acting through its specific membrane receptors *AdipoR1* and *AdipoR2*, was shown to inhibit the growth and peritoneal metastasis of GC in vivo [16]. Adiponectin has anti-inflammatory effects and also plays a role in suppressing neovascularization, which is required for tumor proliferation [30]. It remains uncertain whether adiponectin may act directly on the tumor cells to inhibit proliferation and/or induce apoptosis.

Conclusion

Expression of *AdipoR1* and *AdipoR2* are strongly higher in GC, and level of circulating adiponectin is lower. Our study suggests that the expression of *AdipoR1* and *AdipoR2*, besides the low level of adiponectin, plays an important role in the development and/or progression of GC. These data (*AdipoR1* and *AdipoR2* high expression) could be a novel anticancer therapeutic target in GC. However, further studies are necessary to confirm this potential mechanism of adiponectin to act directly on GC.

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Statement of Ethics

The study protocol was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. This study was approved by the ethics committee of Gauhati University (No. GUEC-03/2015). Also, written informed consent was obtained from the patients for publication of this study.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Morteza Kordafshari and Narjes Mehrvar: writing the manuscript draft; Mahyar Nourian and Aida Etemadi: data analysis and editing; Shahrokh Iravani: primary investigator in collaboration with Azim Mehrvar; Ali Reza Khoshde and Hasan Jalaeikhoo: critical reviewing and editing; Mohammad Ghaznavi Idris: performing the experimental tasks.

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