Upregulation of SIRT1 gene in gastric adenocarcinoma

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ABSTRACT

Background/Aims: SIRT1 gene overexpression is reportedly associated with cancer development, via the triggering of DNA repair impairment, and cell proliferation. The study aimed to investigate SIRT1 expression in patients with gastric cancer and its correlations with the clinical and pathological characteristics of the disease.

Materials and Methods: All patients (64 patients) who underwent gastric biopsy and were diagnosed with gastric adenocarcinoma and signet ring cell carcinoma between January 2011 and December 2013 were enrolled in the study, and patients with benign gastric biopsy were enrolled in the control group (34 patients). The previously prepared gastric tissues were collected from the pathology department, and SIRT1 gene expressions were evaluated in the gastric tissues of all study patients. Patients were subclassified according to their demographic, clinical, and pathologic features, and the patient and control groups were compared.

Results: Sixty-four patients were included in the study (25 females and 39 males). The mean age of the patients was 66±1 (range: 33-88) years. The SIRT1 gene 2' Average delta cycle threshold (CT) value was 0.102 in the control group, whereas it was 0.292 in the patients with gastric cancer (relative risk: 2.86; p=0.014). The SIRT1 gene was upregulated in all tumor stage subgroups except stage I, female patients, young patients (≤45 years), and corpus and cardia tumor subgroups compared to the control group.

Conclusion: SIRT1 gene overexpression is associated with gastric adenocarcinoma, and it can be argued that SIRT1 gene upregulation is associated with unfavorable gastric adenocarcinoma prognosis.

Keywords: SIRT1 gene, gastric adenocarcinoma, prognosis

INTRODUCTION

Gastric cancer accounts for approximately 10% of cancers worldwide and its frequency is rare in individuals before the age of 30 years; the frequency increases after the age of 50 years. Gastric cancer occurs more frequently (almost twice) in males compared to females. There are several risk factors associated with gastric cancer; however, no single reason is responsible for the disease development (1).

Sirtuins are a class of proteins with nicotinamide adenine dinucleotidedependent deacetylase and adenosine diphosphate (ADP) ribosyl transferase activity. Seven sirtuins, Sirt (silent information regulator) 1-7, have been identified in mammals. SIRT1 gene overexpression is reportedly associated with cancer development through triggering of DNA repair impairment and cell proliferation (2). Moreover, SIRT1 overexpression has been identified in epithelial cancers, such as cancer of the breast, colon, gastric system, prostate, and ovaries; lymphoma; leukemia; central nervous system tumors; and soft tissue sarcomas (2-10).

Cancer pathogenesis is a multifactorial process, and the etiologic factors may differ according to geographic areas. There is no information about SIRT1 expression levels in gastric cancer in our region. The aim of this study was to investigate SIRT1 expression in patients with gastric cancer in our region and to determine the association between SIRT1 expression and clinicopathological features.

MATERIALS AND METHODS

Patient selection

Overall, 64 patients who underwent gastric biopsy and were diagnosed with gastric adenocarcinoma and signet ring cell carcinoma between January 2011 and December 2013 were enrolled in the study along with 34 patients with benign gastric biopsy as the control group. An ethical approval was obtained from the Ethical Com-

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Table 1. Preparation of primers SIRT1 primers (Delta Gene Assays, Fluidigm, South San Francisco, CA, USA)

Target	Assay ID	Assay Name	FP	RP	Design RefSeq	Blast Hits	Gene Full Name
SIRT1	GEA00013147	SIRT1_13147_i5	ACAAAGTTGACT-GTGAAGCTGTAC	GTTCATCAGCTGG-GCACCTA	NM_012238.4	NM_001142498.1| NM_012238.4	sirtuin 1

Table 2. SIRT-1 gene expression in gastric cancer patients and control group

	Control Group 2^(-Avg. (Delta(Ct))	Gastric Cancer 2^(-Avg. (Delta(Ct))	Fold Change	p
SIRT-1	0.102	0.292	2.858^	0.014

mittee of Muğla Sıtkı Koçman University (30265539-622.01.00.00.13/196668). Patient records from the pathology department were searched to identify patients who underwent gastric biopsy or surgery between 2011 and 2013. The age of the patients in the control group ranged from 40 to 70 [(mean: 60±1) years; 17 males (mean age: 64±1 years) and 17 females (mean age: 57±1 years)].

The previously obtained gastric tissues (from surgery or biopsy) were collected from the pathology department, and SIRT1 gene expressions were evaluated in the gastric tissues of all patients.

Tissue analysis

Tissue samples were prepared using a microtome from paraffin blocks, with a size of 5-9 µ each. RNA easy Mini Kit (QIAGEN Sample & Assay Technologies, Germany) was used to obtain total RNA; after all tissue samples were deparaffinized, cDNA was synthesized from RNA, using RT2 qPCR primary assays (SABiosciences, Frederick, MD). SYBR Green Master Mix (Qiagen) was used to determine SIRT1 gene expression and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used for internal controls. Gene expressions were analyzed using a realtime polymerase chain reaction.

Preparation of primers

SIRT1 primers (Delta Gene Assays, Fluidigm, South San Francisco, Calif., USA) supplied in a lyophilized from were diluted with the required amount of Tris-ethylenediaminetetraacetic acid buffer to 100 µM for each primer pair according to the manufacturer's instructions, vortexed, briefly centrifuged, and stored at -20°C (Gene 6000 Real-Time PCR; table 1).

Regarding pathologic analysis, tumor staging, and histologic type of the tumor were evaluated. Tumor stages were determined according to the tumor, node, metastasis (TNM) classification.

Groups and statistical analysis

Patients were classified according to the age (≤45 years and >45 years), gender (female, male), tumor stage, tumor localization, and histologic type. Firstly, statistical significance of SIRT1 gene expression was investigated between all patients with gastric cancer and the control group (table 2). Secondly, statistical analysis was conducted between gastric cancer subgroups according to the clinicopathological features and the control group (table 3).

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 12.0 software program (SPSS Inc.; Chicago, IL, USA). The Wilcoxon signed rank test was performed for statistical analysis using the SPSS software. The results were showed as mean±standard deviation. A p value <0.05 was accepted as statistically significant. To understand homogeneity in the distribution of the groups, a variance analysis was used. Data analysis was performed using RT^2 Profiler Data Analysis Software-Qiagen and 2′ Average delta CT values, and standard deviation was used. For relative gene expression, 2′ Average delta CT method and standard deviation was used.

RESULTS

Sixty-four patients were included in the study. Twenty-five patients were females (39.06%), whereas 39 were males (60.94%). Nine patients were under the age of 45 (14.06%) years. Regarding tumor stages, 12 patients were in stage II, 11 in stage IIIa, 13 in stage IIIb, and 28 in stage IV. Eleven patients had signet ring cell carcinoma. The mean age of the patients was 62±1 (range: 33-88) years. The mean age of the female and male patients was 65±1 (range: 33-88) years and 66±1 (range: 31-88) years, respectively. SIRT1 gene 2′ Average delta CT values were 0.102 in the control group and 0.292 in the patients with gastric cancer (relative risk: 2.86; p=0.014; fold change value 2.858 was calculated; p<0.05; table 2).

Table 3. SIRT gene expression according to the clinicopathologic features

Variables	2^(-Avg. (Delta(Ct))	Fold Change	p
Stage			
Stage-I	-	-	-
Stage-II	0.546	5.338^	0.008*
Stage-IIIa	0.631	6.166^	0.0001*
Stage-IIIb	0.404	3.954^	0.004*
Stage-IV	0.344	3.3623^	0.004
Tumor Localization			
Antrum	0.234	23.920^	0.062
Corpus	0.401	3.920^	0.001*
Cardia	0.227	2.221^	0.043*
Histologic type			
Adeno Ca	0.307	3.000^	0.009*
Signet Ring			
Cell Carcinoma	0.225	2.200^	0.115
Gender			
Female	0.307	3.000^	0.009*
Male	0.225	2.300^	0.115
Age			
<45	0.321	3.145^	0.008*
>45	0.206	2.018^	0.071
Control g.	0.102		

^up-regulation; *significantly p value

SIRT1 gene 2′ Average delta CT values were statistically higher in the adenocarcinoma group ($p<0.05$) for all stages except stage 1 ($p<0.05$), tumor localization in the corpus group ($p<0.01$), tumor localization in the cardia group ($p<0.05$), female group ($p<0.05$), and under 45 years age group ($p<0.05$; table 3).

SIRT1 gene 2′ Average values were not statistically significant in the antrum group, signet ring group, male group, and over 45 years age group ($p>0.05$; table 3).

DISCUSSION

With the aim to investigate the expression of SIRT1 genes in patients with gastric cancer and its relation to the clinicopathological features, we found that SIRT1 gene is significantly over expressed in gastric carcinoma compared to control patients. We found that SIRT1 gene was upregulated in all tumor stages (Stage II-IV, except stage I) compared to the control group. Overexpression of SIRT1 was also detected in antral and cardiac tumors compared to corpus, in adenocarcinoma compared to signet ring cell carcinoma, in females compared to males, and in patients who were aged ≤45 years compared to those aged >45 years.

Gastric cancer is one of the most challenging cancers in the world. According to National Cancer Institute of the United States of America, almost 0.9% of population have a lifetime risk for gastric carcinoma. Although it is treated with surgery, chemotherapy, and radiotherapy together, the 5-year survival is around 29.3%. Gastric cancer comprises 1.8% of all cancer deaths (11). A majority of the gastric cancers are sporadic; however, familial and hereditary factors have also been determined. Different mutations and impaired DNA repair mechanisms have been determined in hereditary syndromes, such as CDH1 heterozygous germ line mutation, MSH-2 or MLH-1, and mutations in TP53, STK11, and APC. Moreover, environmental factors also influence pathogenesis of gastric carcinoma (12).

SIRT1 and deleted in breast cancer 1 (DBC-1) genes play an antagonistic role on p53 tumor suppressor gene. SIRT1 deacetylates p53 gene, decreases the activity of p53, and increases carcinogenesis. However, the activity of SIRT1 is controlled by DBC-1 (13,14). In contrast, some studies have reported that SIRT1 prevents β catenin pathway and acts as a tumor suppressor gene (15,16). However, our study results suggest that SIRT1 acts as a tumor promoter gene. Moreover, Feng et al. (17) evaluated the tissue microarray technique and immunohistochemical expression of SIRT1, p53, and Ki-67 in 176 gastric cardiac carcinoma tissues and 32 normal gastric cardiac region tissues and found that SIRT1 overexpression was also significantly associated with lymphatic metastasis, TNM stage, proliferative status, and prognosis, consistent with our results.

Kang et al. (13) also investigated the association between immunohistochemical expression of SIRT1, DBC-1, p53, and β-catenin and various clinicopathological characteristics in 452 gastric carcinoma patients who underwent gastrectomy and found that SIRT1 and DBC-1 can be considered good prognostic factors in gastric adenocarcinoma. The correlation between SIRT1 and DBC-1 expres-

sion was investigated in 177 patients with gastric cancer. Immunohistochemical expression of SIRT1, DBC-1, and p53 was evaluated, and SIRT1 overexpression was found to be associated with shorter overall and relapse-free survival in univariate analyses. Zhang et al. (18) also suggested that SIRT1 and STAT3 mRNA expression indicate poor prognosis in gastric cancer, suggesting the use of SIRT1 in diagnosis and follow-up (18). However, Yang et al. (19) reported that SIRT1 is downregulated in gastric cancer, and SIRT1 activators can be used therapeutically. However, in this study, we found that the SIRT1 gene is upregulated in gastric cancer. The study of Qui et al. (20) showed that the expression of Beclin-1 and SIRT1 were shared independently or together with poor prognosis in gastric cancer. Yan et al. (21), in the case of Yes-associated protein and gastric cancer, found an inverse correlation between SIRT1 and wap and showed that the reduction in WAP concentration resulted in poor prognostic outcome in stomach cancer with activation in SIRT1 expression and inhibitory effect on Mfn2-mediated mitophagy. Jiang et al. (22) reported that SIRT1 expression is associated with poor prognosis in stomach cancer patients over a short period of time (3 years) and that patient age, tumor stage, and differentiation are associated with SIRT1 expression in the systematic metaanalysis.

It has recently been published that there is a relationship between SIRT1 and treatment resistance. Chen et al. (23) reported an inverse correlation between tNOX and SIRT1 and oxalplatin-induced apoptosis in their study of gastric cancer cell line (AGS cell line) cultures. Zhank et al. (24) showed that upregulation of miR-132 in Lgr5+ gastric cancer stem cell-like cells leads to cisplatin resistance through the SIRT1/CREB/ABCG2 signaling pathway.

In conclusion, we think that most of the gastric adenocarcinomas are with overexpression of SIRT1, and this overexpression may be associated with unfavorable gastric adenocarcinoma characteristics and poor prognosis. As a conclusion, SIRT1 gene expression is associated with clinicopathological features in gastric adenocarcinoma. The significant SIRT1 upregulation in females, corpus localized tumors, and in patients who are under the age of 45 years suggest that it is a poor prognostic factor. Although the role of SIRT1 in gastric tumorigenesis is still unclear, our results suggest that their expression is a clinically significant prognostic indicator for gastric carcinoma. In addition to being a prognostic marker, SIRT1 may also serve as a therapeutic target in gastric cancer.

Several limitations can be considered in our study. This study was conducted as a cross-sectional study for not including stage 1 gastric cancer tumor samples due to the disease rarity. Moreover, a survival analysis was not performed to determine gene's prognostic profile because no follow-up data were available.

Ethics Committee Approval: This study was conducted in accordance with the Helsinki Declaration of 1975 and approved by the Ethics Committee of the Muğla Sıtkı Koçman University (30265539-622.01.00.00.13/196668).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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