Effects of stromal cell-derived factor-1 and survivin gene polymorphisms on gastric cancer risk

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Abstract. Stromal-cell derived factor-1 (SDF-1), a CXC chemokine, is important for growth, angiogenesis and metastasis of tumor cells. The SDF1-3'A polymorphism has been investigated in various types of cancer; however, no information is currently available on its role in gastric cancer. Survivin is a member of the inhibitor of apoptosis family of proteins and has a genetic polymorphism (-31G/C) located in the CDE/CHR repressor element of its promoter. In this study, 88 gastric cancer patients and 480 normal healthy control subjects were investigated for the genotype and allelic SDF1-3'A and survivin -31G/C frequencies using polymerase chain reaction-restriction fragment length polymorphism. The SDF1-3'A genotype frequencies for GG, GA and AA were 44.32, 48.86 and 6.92% in patients and 42.71, 47.71 and 9.58% in healthy subjects, respectively. GA+AA genotype frequency and A allele distribution were not identified as significantly different between gastric cancer cases and controls. The survivin frequencies for GG, GC and CC were 20.45, 50 and 29.54% in patients and 33.96, 45 and 21.04% in healthy subjects, respectively. The C carriers (GC+CC genotype) and the C allele were over-represented among the gastric cancer cases (P=0.013 and P=0.0083, respectively). Overall, no statistically significant association was identified for SDF-1 and survivin gene examined alleles and genotypes and any parameter investigated, (e.g., stage, differentiation status and survival). The survivin promoter -31G/C polymorphism may confer an increased susceptibility to gastric cancer, while the SDF1-3'A polymorphism may not be a candidate genetic variant to select individuals at higher risk of developing gastric cancer.

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Introduction

Gastric cancer is the fourth most common cancer and the second most frequent cause of mortality in the world. The prognosis is poor, with a five-year survival rate below 30% (1,2). Carcinogenesis of gastric cancer is a complex and multifactorial process, in which genetic and environmental factors are involved (3,4). Marked racial and geographic differences in gastric cancer incidence may be also due to polymorphisms of genes being involved in various steps of carcinogenesis and implicated in gastric cancer susceptibility (5,6).

Stromal cell-derived factor-1 (SDF-1), also known as CXCL12, is a chemokine acting as a growth factor for B-cell progenitors and a chemotactic factor for T cells and monocytes (7-9). SDF-1 binds to the CXCR4 receptor, resulting in a SDF-1/CXCR4 receptor-ligand system involving a one-on-one interaction (10,11). The cytokine possesses angiogenic properties and mediates the dissemination of CXCR4-positive tumor cells to distant organs (12). SDF-1 promotes angiogenesis directly by binding to its receptors CXCR4 and/or CXCR7 expressed on endothelial cells or indirectly by the induced secretion of matrix-metalloproteases or angiogenic factors, respectively (13,14). Furthermore, the SDF-1/CXCR4 axis is involved in tumor metastasis to sites which are characterized by high production of SDF-1, including liver, lung and bone marrow (13,15). SDF-1 has three isoforms. The beta variant has a single nucleotide polymorphism (G→A) at position 801 of the 3'-untranslated region of its gene (15-17). Allele A is a target for *cis*-acting factors, capable of upregulating expression of SDF-1 protein (15,17,18). It was previously suggested that homozygotes for SDF1-3'A (3'A/3'A) express higher levels of SDF-1 compared with wild-type individuals (3'G/3'G). However, this observation requires additional confirmation (15,19,20). The SDF-1 G→A polymorphism has been investigated in various types of cancer; however, limited information is currently available with respect to its role in gastric cancer (21-30). The first aim of our study was to explore the correlation between the SDF1-3'A polymorphism with the risk of gastric cancer development.

Survivin is a member of the inhibitors of apoptosis protein family, involved in regulation of apoptosis, cell cycle progres-

sion and microtubule stability (30-34). Survivin contains a single baculovirus IAP repeat domain (35) and blocks death receptor and mitochondrial apoptosis pathways by directly inhibiting caspase-3 and caspase-7 and by interfering with caspase-9 activity/processing (36,37). Additionally, survivin counteracts apoptotic stimuli induced by interleukin 3, Fas, Bax, tumor necrosis factor α, caspases, anticancer drugs and X-rays (38,39). It has been reported that survivin is associated with angiogenesis (40). Overexpression of survivin is frequently observed in various human malignancies, including colorectal cancer, lung cancer, hepatocellular carcinoma, pancreatic cancer and osteosarcoma (41-46). In addition, its overexpression is correlated with poor prognosis of those types of cancer.

Survivin is expressed in a cell-cycle-dependent manner. Expression levels peak in the G2/M phase of the cell cycle, when it is associated with microtubules of the mitotic spindle, followed by a rapid downregulation in the G1 phase (33). This is controlled at the transcriptional level and mediated by cell cycle-dependent elements (CDEs) and cell cycle homology regions (CHRs) located in the proximal region of the survivin promoter (47). Several single-nucleotide polymorphisms have been identified within the promoter region of the survivin gene, one of which is located at the CDE/CHR repressor binding site (-31G/C). This polymorphism has been associated with overexpression of survivin and aberrant cell cycle-dependent transcription, mediated through the functional disruption of binding at the CDE/CHR repressor motifs in a number of cancer cell lines (48). However, the -31G/C polymorphism within the CDE/CHR repressor element of the promoter has not been extensively studied in human malignancies (49-51). Currently, the role of this polymorphism in gastric cancer is not fully understood. Therefore, based on these data, the second aim of the present study was to investigate whether the genetic polymorphism -31G/C located in the CDE/CHR repressor element of the human survivin promoter is a risk factor for gastric cancer.

In the present study, we hypothesized that the aforementioned SDF-1 and survivin polymorphisms are associated with increased risk of gastric cancer and an aggressive tumor phenotype.

Materials and methods

Patients. The subjects in the hospital-based case-control were 88 unrelated gastric cancer patients (62 males and 26 females; mean age ± SEM, 63.33±12.13 years; median, 70; range, 27-82) with 480 ethnically, gender- and age-matched healthy control individuals (blood donors randomly selected from our DNA database) without evidence of malignancy or autoimmune disease. All patients and controls were born in and live in Greece. Informed consent was obtained from all patients and the hospital review board approved the study. According to the International Union Against Cancer classification and TNM staging system (54), 24 of the tumors (27.27%) were stage I, 16 (18.18%) stage II, 27 (30.68%) stage III and 21 (23.86%) stage IV (Table I). The patients were followed up until May 2012 or until mortality. The median time (± SD) of follow-up was 36.27 (±19.5) months (range, 6-60 months). Follow-up was not performed for 17 patients as they could not be located.

Table I. Patient and tumor characteristics.

Characteristics	Gastric cancer patients
Age at diagnosis (years), mean ± SD	63.33±12.13
Male/female, n	62/26
Lymph node metastasis, n	
Negative	26
Positive	62
Other metastasis, n	
Negative	66
Positive	22
TNM stage at diagnosis, n	
I	24
II	16
III	27
IV	21
Tumor size (cm), n	
≤5	40
>5	48
Lauren classification, n	
Intestinal	39
Diffuse	49

Determination of SDF-1 801G/A and survivin -31G/C polymorphism. Genomic DNA was isolated using a NucleoSpin Blood kit (Macherey-Nagel, GmbH & Co. KG, Dueren, Germany) according to the manufacturer's instructions. Genotyping analysis was performed using polymerase chain reaction-restriction fragment length polymorphism for the polymorphisms. Primers used for SDF-1 genotyping were forward, 5'-CAGTCAACCTGGGCAAAGCC-3' and reverse, 5'-CCTGAGAGTCCTTTTGCGGG-3' (GenBank accession number L36033). The amplification included an initial denaturation at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 sec, annealing at 57˚C for 90 sec and extension at 72˚C for 90 sec and ended with a final elongation at 72˚C for 5 min. Amplicons of 293 bp were subjected to restriction digestion by the *Hpa*II enzyme. SDF-1 wild-type alleles yielded 100- and 193-bp products, while SDF-1 3'A alleles (polymorphic) yielded a 293-bp product. Primers used for survivin genotyping were forward, 5'-GTTCTTTGAAAGCAGTCGAG-3' and reverse, 5'-GCCAGTTCTTGAATGTAGAG-3'. The amplification included an initial denaturation at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 sec, annealing at 57˚C for 90 sec and extension at 72˚C for 90 sec and ended with a final elongation at 72˚C for 5 min. Amplicons of 341 bp were then digested with the restriction enzyme *Eco*O109I (New England Biolabs Inc., Ipswich, MA, USA) at 37˚C for 18 h. The G allele was cleaved by the enzyme, generating two fragments (236 and 105 bp), whereas the C allele was not digested. Digestion patterns were analyzed by electrophoresis in a 2% agarose gel stained with ethidium bromide.

Statistical analysis. Statistical calculations were performed using the SPSS for Windows software package (SPSS Inc.,

Table II. Distribution of SDF1-3'A genotypes and alleles in patients and controls.

Genotype/allele	Cases, n (%, n=88)	Controls, n (%, n=480)	P-value; OR (95% CI)
GG	39 (44.32)	205 (42.71)	Reference
GA	43 (48.86)	229 (47.71)	1; 0.98 (0.61-1.58)
AA	6 (6.82)	46 (9.58)	0.52; 0.68 (0.27-1.72)
GA+AA	49 (55.68)	275 (57.29)	0.81; 0.94 (0.59-1.48)
G	121 (68.75)	639 (66.56)	Reference
A	55 (31.25)	321 (33.44)	0.6; 0.9 (0.64-1.28)

OR, odds ratio; CI, confidence interval.

Table III. Distribution of survivin -31G/C genotypes and alleles in patients and controls.

Genotype/allele	Cases, n (%, n=88)	Controls, n (%, n=480)	P-value; OR (95% CI)
GG	18 (20.45)	163 (33.96)	Reference
GC	44 (50)	216 (45)	0.05; 1.84 (1.03-3.31)
CC	26 (29.54)	101 (21.04)	0.013; 2.33 (1.22-4.47)
GC+CC	70 (79.54)	317 (66.04)	0.013; 2 (1.15-3.47)
G	80 (45.45)	542 (56.46)	Reference
C	96 (54.54)	418 (43.54)	0.0083; 1.55 (1.13-2.15)

OR, odds ratio; CI, confidence interval.

Chicago, IL, USA). Frequency and susceptibilities of mutations were compared using the χ^2 test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using the corresponding χ^2 distribution test. P-values obtained were two-tailed and $P<0.05$ was considered to indicate a statistically significant difference. Hardy-Weinberg equilibrium was verified by calculating the expected frequencies and numbers and was tested separately in patients and in controls using the goodness-of-fit χ^2 test.

Results

Tumor characteristics. Tumor characteristics are presented in Table I. The SDF-1 genotype and allele distribution among the 88 patients and 480 healthy controls is presented in Table II. The observed genotype frequencies were in accordance with Hardy-Weinberg equilibrium. Only 6 patients and 46 cases among the control group presented with the AA genotype. As a result, these were grouped together with the GA cases during the statistical analysis (Table II). The genotype frequencies for GG, GA and AA were 44.32, 48.86 and 6.82% in patients and 42.71, 47.71 and 9.58% in healthy subjects, respectively. GA+AA genotype frequency and A allele distribution were not identified as significantly different between gastric cancer cases and controls (Table II).

Survivin genotype and allele distribution. The survivin genotype and allele distribution among the 88 patients and 480 healthy controls is presented in Table III. The -31G/C genotype and allele distribution was identified as significantly different between gastric cancer cases and controls. The genotype frequencies for GG, GC and CC were 20.45, 50 and 29.54% in patients and 33.96, 45 and 21.04% in healthy subjects, respectively. GC+CC genotype frequency and C allele distribution were identified as significantly different between gastric cancer cases and controls. The C carriers (GC+CC genotype) and the C allele were over-represented among the gastric cancer cases ($P=0.013$ and $P=0.0083$, respectively; Table III). With respect to the tumor characteristics, no statistically significant association was identified for SDF-1 and survivin examined alleles and genotypes and any parameter investigated (e.g., stage and differentiation status). A total of 19 mortalities occurred during the follow-up period and none of the alleles and genotypes conferred a survival advantage in this group of gastric cancer patients.

Discussion

It is commonly accepted that the clinical behavior of tumors depends on the interaction between tumor cells and the 'host'. The diverse cellular origins of SDF-1 and its constitutive expression in various organs warrant the study of this chemokine as an organ microenvironment 'host' factor in malignant and non-malignant pathologies (15-30). The majority of molecular studies have focused on mutational analysis of cancer-associated genes or carcinogen metabolizing genes; however, screening genetic polymorphisms of malignant stroma or microenvironment-related (i.e. SDF-1) and apoptosis (i.e. survivin) genes with the aim to uncover their potential predictive role must still be performed. In the present study

we investigated the correlations between common genetic variants of SDF-1 and survivin promoter with gastric cancer risk and phenotypic aggressiveness in a cohort of patients of Greek descent. Although the polymorphisms of the two genes have been studied in several diseases and various other types of cancer, limited data are available with regard to the significance of the polymorphisms in gastric cancer.

Previous studies have demonstrated the oncogenic properties of the SDF-1 (CXCL12)/CXCR4 pathway (12,21,28,29). In addition, the SDF-1/CXCR4 axis is a good candidate for chemical intervention, with the aim to generate a new therapeutic approach for various tumors (55). SDF-1/CXCR4 expression levels have been studied in gastric cancer; however, current literature is limited with respect to evaluation of the main genetic polymorphism of this molecule (i.e., SDF1-3'A) in this type of malignancy (56-59). For this reason, the elucidation of the potential role of this polymorphism in tumor development and potentially its progression is of great interest. The present results indicate that the SDF1-3'A polymorphism may not be a good candidate genetic variant to select human subjects at higher risk of developing gastric cancer. A correlation between adverse clinicopathological variables was not identified; however, the number of gastric cancer patients in the study was limited. Previous studies on colorectal cancer have also failed to identify a significant link between the SDF-1 polymorphism and the risk of colorectal cancer development (28,29). However, the polymorphism has been previously associated with susceptibility to breast, lung, prostate and pancreatic cancer (21,23,25-27,30). Extensive studies in breast cancer have demonstrated that the combination of low plasma SDF-1 levels and the SDF-1-3'A polymorphism may identify a cohort of patients with an intrinsic susceptibility for poorer survival (21). Variations in molecular mechanisms and modifications associated with the pathogenesis of various types of cancer may account for these diverse associations. Specifically, based on tissue origin, the effect of the SDF-1 gene polymorphism may contribute differentially to tumor progression, angiogenesis, metastasis and leukocyte migration.

According to the present results, no correlation has been identified among the typical progression markers, including histological grade, tumor size or metastatic profile. Nevertheless, the absence of an association of SDF1-3'A with gastric cancer risk and phenotype does not exclude the possibility that SDF-1 itself may affect cancer progression and aggression. It was previously reported that SDF-1 and CXCR4 expression in intestinal-type cancer is correlated with a significant increase in tumor size, depth of invasion and lymphatic and liver metastasis (56,57). Serum SDF-1 levels were identified to be higher in patients with metastasis from gastric cancer, indicating that SDF-1 protein-expressing gastric cancer cells may be associated with tumor aggressiveness (58). In a previous study, CXCR4 was expressed in 50% of gastric cancer cases and was upregulated to a greater degree in gastric cancer than in normal gastric tissues. In addition a significant increase in SDF-1 mRNA in lymph nodes with cancer cell metastasis in comparison with normal lymph nodes was identified. The latter observation confirmed that cancer cells migrate towards an SDF-1 gradient established in specific target organs (59).

The mechanism behind the correlation between the SDF-1 polymorphism and clinical behavior of tumors, observed in a number of the single-nucleotide polymorphisms studied, remains under investigation. Alterations in SDF-1 gene functional levels, taking into consideration the biological role of this molecule in the tumor microenvironment and metastasis, may serve as a rational explanation. The mode of action of chemokines depends heavily on the local environment and the secreted SDF-1 produces a gradient for CXCRCR4-bearing cells (30,59).

Survivin, a unique antiapoptotic factor, is involved in cell cycle regulation. Numerous clinical studies have demonstrated that survivin is markedly overexpressed in the majority of common types of cancer, indicating that transcriptional deregulation is a major mechanism associated with aberrant expression of survivin in cancer (42-48). In the present study, we investigated whether the -31G/C polymorphism in the survivin gene contributes to the development of gastric cancer. Previous studies have identified an elevated frequency of the -31C allele in patients with lung, urothelial, renal, nasopharyngeal, thyroid, endometrial, esophageal and colorectal cancer. Consistent with these findings, the present results identified an association between the -31CC genotype and the -31C allele and a significantly increased risk of gastric cancer (50,60-69). However, Yang *et al* observed that the variant genotype (GG and GC) was associated with risk of distal gastric cancer or well-differentiated tumor. A statistically significant association was not identified between gastric cancer risk overall and variant genotype. However, the authors were in agreement with the hypothesis that the genotype is involved in distal gastric carcinogenesis and tumor differentiation in the Chinese population (52). By contrast, consistent with the present findings, Cheng *et al* demonstrated that the frequencies of survivin-31C allele and C/C genotype were identified as significantly higher in gastric carcinoma patients than in healthy subjects, concluding that the -31C genotype of the survivin promoter is a risk factor of gastric carcinoma (53). Li *et al* identified that G carriers of the survivin promoter gene may have an increased relative risk of developing gastric tumors of the diffuse type, localized in the antrum at a younger age. C carriers with a high D17S250 microsatellite instability (TP53 gene) demonstrated an overall higher risk of developing gastric tumors, indicating that the mutated TP53 gene is unable to inhibit survivin expression, promoting gastric carcinogenesis (70). However, this polymorphism has yet to be identified as a risk marker in cervical, pancreatic and hepatocellular carcinomas (49,51,71).

Although overexpression of survivin in human cancer types is a well accepted hypothesis, the differential role of its polymorphism remains to be understood. A rational explanation for this tumor-dependent difference in risk, conferred by the examined survivin polymorphism, may be attributed to differences in the carcinogenesis pathways among various types of human cancer. A number of studies have revealed that the survivin promoter -31G/C polymorphism may modulate the expression of survivin (50,61,63). Due to its location at the CDE/CHR repressor binding site, this polymorphism may affect the affinity of repressor binding to this region and the expression of survivin. Previously, an *in vitro* promoter assay revealed that the -31G allele exhibited significantly lower transcriptional activity than the -31C allele, indicating that the -31G/C polymorphism affects survivin expression and contributes to genetic susceptibility to lung cancer (50). In addition,

the presence of the -31G/C polymorphism has been identified at higher frequencies in cancer cell lines and correlated with increased survivin expression at mRNA and protein levels (61). Since survivin functions as an inhibitor of apoptosis, a process important for the elimination of mutated or transformed cells from the body, it is possible that individuals carrying the higher production genotype of survivin -31G/C polymorphism may possess a decreased capacity to eliminate cells with DNA damage which may contribute to malignancies. Therefore, it is biologically plausible that the survivin promoter -31G/C polymorphism confers susceptibility to various types of cancer.

In conclusion, the present study comprised a homogeneous Greek population, in which it was identified that the survivin promoter -31G/C polymorphism confers an increased susceptibility to gastric cancer. This is an additional example of the role of apoptosis-related molecules in human malignancies. Owing to its association with poor prognosis and a global epidemiological distribution, gastric cancer remains at the forefront of contemporary epidemiological, basic and clinical research. Due to the small sample size of the present study, expansion of the study and inclusion of a higher number of patients is required. Well-designed and carefully executed multi-center studies, with enrollment of a large number of subjects, are predicted to reach more convincing and generalizable conclusions. Since genetic polymorphisms often exhibit ethnic differences, additional studies should include populations with diverse backgrounds to further elucidate the association of the examined polymorphisms with gastric cancer.

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