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BRIEF ARTICLE

Association of the *GNAS1* T393C polymorphism with tumor stage and survival in gastric cancer

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Abstract

AIM: To analyze the impact of the *GNAS1* T393C polymorphism on prognosis and histopathology of gastric cancer.

METHODS: Genomic DNA was extracted from paraffin-embedded tissues of 122 patients with primary gastric carcinoma and from the blood of 820 healthy white individuals. Allelic discrimination was performed by quantitative real-time polymerase chain reaction. Genotyping was correlated with histopathologic parameters and with overall survival according to the Kaplan-Meier approach and with multivariate analysis by multiple stepwise regression.

RESULTS: Thirty-nine (32%) patients displayed a CC genotype, 57 (46.7%) a CT genotype and 26 (21.3%) a TT genotype. The frequency of the C allele (fC) in the patient group was 0.55, which was not significantly different from that of healthy blood donors. The distribution was compatible with the Hardy-Weinberg equilibrium. Analysis of clinicopathological parameters did not show any significant correlation of the T393C genotype with gender (P = 0.50), differentiation (P = 0.29), pT-category (P = 0.19), pN-category (P = 0.30), pM-category (P = 0.25), R-category (P = 0.95), the classifications according to WHO (P = 0.34), Laurén (P = 0.16), Goseki (P = 1.00) and Ming (P = 0.74). Dichotomization between C+ (CC+CT) and C-genotypes (TT), however, revealed significantly more advanced tumor stages (P = 0.023) and lower survival rates (P = 0.043) for C allele carriers.

CONCLUSION: The present study provides strong evidence to suggest that the *GNAS1* T393C allele carrier status influences tumor progression and survival in gastric cancer with higher tumor stages and a worse outcome for C allele carriers.

Key words: Gastric cancer; G Protein; Polymorphism; Prognosis; Tumor stage

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INTRODUCTION

Gastric cancer has substantially decreased in incidence over the past decades, but it still remains one of the most common cancers in the world and the second most frequent cause of cancer-related death after lung cancer[1]. Most patients are diagnosed with advanced gastric cancer, and overall survival remains poor[2,3]. The 5-year survival rate for gastric cancer is still only at 40%[4,5].

Of particular interest are prognostic factors, as they give the basis to identify gastric cancer patients with high-risk and poor prognosis. The identification of patients with poor outcome can help to set up novel treatment strategies at the beginning of treatment and may lead to better and more individualized therapy strategies with better survival[2]. Current efforts in research are therefore focused on the detection and validation of biomarkers and genetic markers that give additional information about prognosis to classical prognostic factors such as the TNM classification. The majority of new detected markers are related to properties of the tumor itself, e.g. somatic mutations or differential expression of genes or proteins. However, difficulties in standardization of such markers often prevent their routine application in clinical practice[6].

In recent years, studies have focused on the detection of single nucleotide polymorphisms (SNPs) that have a prognostic impact in cancer. One major advantage of SNPs as prognostic markers is that they can be determined independently from the availability and quality of tumor material as they can be easily evaluated from a blood sample from individual patients.

The T393C polymorphism of the gene *GNAS1* is one such polymorphism. This SNP is located in exon 5 of the gene *GNAS1*, which encodes the ubiquitously expressed Gαs subunit of heterotrimeric G proteins. Previous studies indicate that increased expression of Gαs enhances apoptosis[7,8] and that Gαs mRNA expression is different between T393C genotypes[9]. For various solid tumors, previous studies demonstrated that patient survival and tumor progression depended on T393C genotype[10-17].

Until now, nothing has been published about the impact of the *GNAS1* T393C polymorphism on gastric cancer. Thus, the aim of the present study was to determine the influence of this polymorphism on prognosis in gastric cancer. Furthermore, we looked for possible correlations between the *GNAS1* T393C polymorphism and clinicopathological parameters.

MATERIALS AND METHODS

Patients

Of 159 patients, who were treated surgically between May 1996 and January 2005 for primary gastric carcinoma at the Department of General, Visceral and Cancer Surgery of the University of Cologne, 13 (8.2%) patients with a second tumor, a previous operation of the upper digestive tract or missing paraffin-embedded tissue from normal cells, and 24 (15.1%) patients with neoadjuvant treatment received before surgery were excluded. Excluded patients did not differ in age and gender from the remaining patients.

All of the included 122 patients [median age 67.6 years, range 33-87 years; 78 (63.9%) male, 44 (36.1%) female] were initially treated by operation with curative intention. Gastroscopic examination, endoscopic ultrasound and computed tomography (CT) of the chest and abdomen were performed before surgery on all patients for clinical staging.

One hundred and six (86.9%) of the 122 patients underwent a gastrectomy with D2-lymphadenectomy (compartment I and II) and in 16 (13.1%) cases, a subtotal gastrectomy with D2-lymphadenectomy was performed. The median number of resected lymph nodes was 36.0 (range 15-80).

The present study was performed according to the guidelines of the local Research Ethics Commission.

Histopathology

The specimens were removed *en bloc* and the lymph nodes of the specimens were dissected with the cooperation of surgeons and pathologists according to a standardized protocol. The resected specimens were routinely fixed in 5% phosphate-buffered formalin and embedded in paraffin. Histopathologic examination of all resected specimens consisted of a thorough and standardized evaluation of the tumor stage, residual tumour (R) category, grading and the number of resected and infiltrated lymph nodes. The gastric lymph nodes were documented according to the classification of the Japanese Research Society of Gastric Cancer (JRSGC) with lymph node groups 1 to 13[18]. The tumor localization was defined according to the International Classification of Diseases for Oncology. The lesions were further classified and graded in accordance with WHO recommendations, the Laurén-classification and tumor differentiation. Postoperative staging was performed according to the 6th edition of the TNM-classification of malignant tumors[19].

Genotyping

DNA was extracted from paraffin-embedded tissues from resection boundaries containing exclusively normal cells using a DNA extraction kit (QIAamp, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genotyping was performed in 96-well plates by 5'nuclease assay (TaqMan) using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Darmstadt, Germany).

The pre-developed TaqMan assay ID C_9901536_10 (Applied Biosystems, Darmstadt, Germany) was used for genotyping of *GNAS1* T393C polymorphism (dbSNP rs7121). Polymerase chain reaction (PCR) reactions contained 10 ng DNA, 200 μmol/L dNTPs and 900 nmol/L primers (Figure 1).

PCR conditions were: 95℃ for 10 min followed by 40 cycles of 15 s at 92℃ and 60 s at 60℃.

Reference group

The Caucasian control sample consisted of 820 healthy

Table 1 Allele frequencies and genotype distribution of *GNAS1* T393C polymorphism in 122 gastric cancer patients and in the reference group (n = 820) n (%)

T393C	Patients (n = 122)			Reference group (n = 820)			χ^2	P	Odds ratio	95% CI
Allele	C 135 (55.3)		T 109 (44.7)	C 873 (53.2)		T 767 (46.7)	0.38	0.54	0.92	0.70-1.20
Genotype	CC 39 (32.0)	CT 57 (46.7)	TT 26 (21.3)	CC 235 (28.7)	CT 403 (49.1)	TT 182 (22.2)	0.37	0.54	0.92	

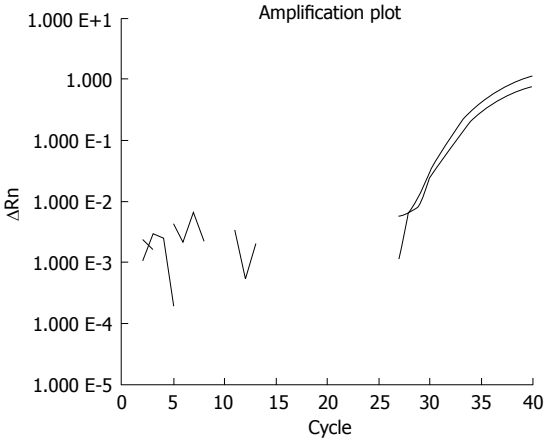

Figure 1 Amplification plot of one heterozygous *GNAS1* T393C (CT) by two allele specific TaqMan probes.

white individuals who were recruited at the local Department for Transfusion Medicine, University Hospital, Essen. All samples were collected at random from subjects donating blood. The details of this sample have been published previously[12].

Statistical analysis

Associations between T393C genotype and clinicopathological parameters were evaluated using the χ^2 test. Pearson's χ^2 was used for Hardy-Weinberg analysis and to examine differences in allele frequencies between our patient group and the reference group. Relations to overall survival were evaluated with univariate analysis according to the Kaplan-Meier approach using the log-rank test to assess statistical differences between groups. Prognostic factors were determined by multiple stepwise regression analysis using the Cox model. Only potential prognostic factors were included in the multivariate analysis. The level of significance was set at $P < 0.05$ and P values were for 2-sided testing. All statistical tests were performed using the Software Package SPSS for Windows, Version 17.0 (Chicago, IL, USA).

RESULTS

Genotype distribution and reference group

Thirty-nine (32.0%) patients displayed a CC genotype, 57 (46.7%) a CT genotype and 26 (21.3%) a TT genotype. The frequency of the C allele (fC) in the entire patient group was 0.55, which is not significantly different from that of healthy blood donors (Table 1). The distribution was compatible with the Hardy-Weinberg equilibrium.

Clinicopathological characteristics

Clinicopathological characteristics of the whole patient group with genotype distribution are displayed in Table 2. Thirty (24.6%) patients showed an early gastric carcinoma (pT1). In 73 (59.8%) cases, lymph node metastasis (pN+) was detected. An M1 category was found in 23 (18.9%) patients with localized peritoneal carcinosis, distant lymph node metastasis (M1 lymph) or single liver metastasis (M1 Hep). Patients with diffuse peritoneal or multiple liver metastasis had been treated non-surgically and were excluded from the study.

Analysis of clinicopathological parameters did not show any significant correlation of the T393C genotype with gender (P = 0.50), differentiation (P = 0.29), pT-category (P = 0.19), pN-category (P = 0.30), pM-category (P = 0.25), R-category (P = 0.95), or the classifications according to WHO (P = 0.34), Laurén (P = 0.16), Goseki (P = 1.0), Ming (P = 0.74) and UICC (P = 0.15).

When genotypes were dichotomized in C+ (CC+CT) and C-genotypes (TT), a significantly higher rate of advanced tumor stages (stage Ⅲ and Ⅳ), according to the UICC classification, was seen for C allele carriers (P = 0.023). Only 6 (23.1%) of 26 patients with TT genotype were diagnosed with a tumor stage of Ⅲ or Ⅳ. In contrast, an advanced tumor stage was detected in 50 (52.1%) of 96 C allele carriers.

Univariate survival analysis

Overall survival dependent on T393C genotypes is displayed in Figure 2. The 5-year survival rate for patients with a TT genotype was 56.9% (SE ± 10.4%), followed by patients with CC genotype with a 5-year survival rate of 42.6% (SE ± 8.3%). Heterozygous CT patients showed a 5-year survival rate of 32.7% (SE ± 6.3%). Survival was not significantly associated with the T393C genotype when the three genotypes were compared (P = 0.082). However, dichotomization between C+ (CC+CT) and TT demonstrated a significantly (P = 0.043) lower survival rate for C allele carriers (Figure 3) with a 5-year survival rate for the C+ group of only 36.7% (SE ± 5.1%) *vs* 56.9% (SE ± 10.4%) for the TT group.

Multivariate survival analysis

In the multivariate Cox regression analysis, known prognostic factors for gastric cancer (pT, pN, pM and R-category) and T393C genotype with dichotomization between C+ (CC+CT) and TT were included. pT-category (P < 0.001), R-category (P = 0.022) and pM-category (P = 0.027) maintained their prognostic independence (Table 3). pN-category (P = 0.55), and the T393C genotype (P = 0.33) lost their prognostic independence.

DISCUSSION

Gastric cancer is the fourth most common cancer with

Table 2 Clinicopathological characteristics of 122 patients with gastric cancer *n* (%)

	All	T393C genotypes			*P*
		CC	CT	TT	
n (%)	122 (100)	39 (32)	57 (46.7)	26 (21.3)	
Gender					
Male	78 (63.9)	25 (32.1)	34 (43.6)	19 (24.4)	
Female	44 (36.1)	14 (31.8)	23 (52.3)	7 (15.9)	0.274
WHO					
Papillary/Tubular/Mucinous	76 (62.3)	23 (30.3)	34 (44.7)	19 (25)	
Signet-ring cancer	38 (31.1)	12 (31.6)	19 (50)	7 (18.4)	
Other	8 (6.6)	4 (50)	4 (50)	0	0.340
Differentiation					
Well/Moderate (G1-G2)	42 (34.4)	12 (28.6)	22 (52.4)	8 (19)	
Poor (G3-G4)	80 (65.6)	27 (33.8)	35 (43.8)	18 (22.5)	0.805
Laurén					
Intestinal	52 (42.6)	16 (30.8)	25 (48.1)	11 (21.2)	
Diffuse	55 (45.1)	17 (30.9)	29 (52.7)	9 (16.4)	
Mixed	15 (12.3)	6 (40)	3 (20)	6 (40)	0.171
Ming					
Expanding	47 (38.5)	14 (29.8)	24 (51.1)	9 (19.1)	
Infiltrative	75 (61.5)	25 (33.3)	33 (44)	17 (22.7)	0.620
pT-category					
T1	30 (24.6)	7 (23.3)	13 (43.3)	10 (33.3)	
T2	44 (36.1)	12 (27.3)	22 (50)	10 (22.7)	
T3	38 (31.1)	14 (36.8)	18 (47.4)	6 (15.8)	
T4	10 (8.2)	6 (60)	4 (40)	0	0.110
pN-category					
N0	49 (40.2)	11 (22.4)	24 (49)	14 (28.6)	
N1	34 (27.9)	13 (38.2)	13 (38.2)	8 (23.5)	
N2	14 (11.5)	6 (42.9)	6 (42.9)	2 (14.3)	
N3	25 (20.5)	9 (36)	14 (56)	2 (8)	0.196
pM-category					
M0	99 (81.1)	30 (30.3)	45 (45.5)	24 (24.2)	
M1	23 (18.9)	9 (39.1)	12 (52.2)	2 (8.7)	0.101
R-category					
R0	118 (96.7)	38 (32.5)	54 (46.2)	25 (21.4)	
R1/R2	4 (3.3)	1 (25)	2 (50)	1 (25)	0.950
UICC stage					
Ⅰa	26 (21.3)	5 (19.2)	11 (42.3)	10 (38.5)	
Ⅰb	22 (18)	7 (31.8)	12 (54.5)	3 (13.6)	
Ⅱ	18 (14.8)	4 (22.2)	7 (38.9)	7 (38.9)	
Ⅲa	11 (9)	4 (36.4)	5 (45.5)	2 (18.2)	
Ⅲb	4 (3.3)	2 (50)	2 (50)	0	
Ⅳ	41 (33.6)	17 (41.5)	20 (48.8)	4 (9.8)	0.023

P values are given for dichotomization between C+ (CC+CT) and C- (TT) genotypes.

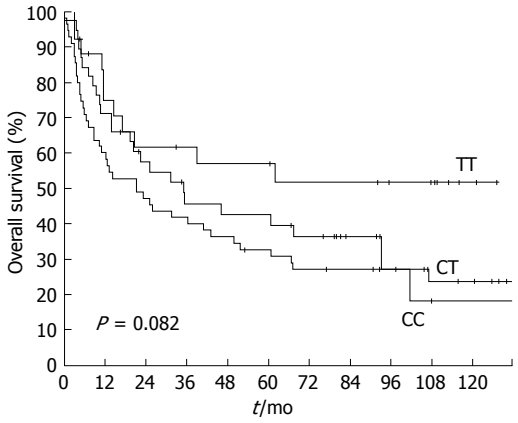

Figure 2 Overall survival of 122 resected gastric cancer patients based on *GNAS1* T393C genotype (Kaplan-Meier analysis), *P* = 0.082 (Mantel-Cox log-rank test).

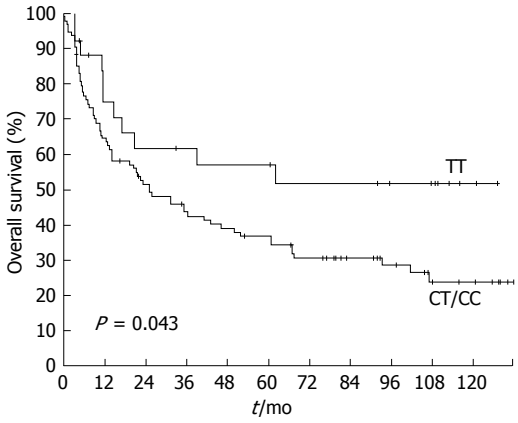

Figure 3 Overall survival of 122 resected gastric cancer patients based on *GNAS1* T393C genotype with dichotomization between C+ and C- genotypes, *P* = 0.043.

Table 3 Univariate and multivariate survival analysis of 122 gastric cancer patients

Covariate	*n*	Univariate analysis			Multivariate analysis		
		P value	5-yr-SR (%)	SE (±%)	*P* value	HR	95% CI
pT-category		< 0.001			< 0.001		
pT1	30		85.4	6.8		1	
pT2	44		44.5	7.8	< 0.001	6.212	2.31-16.70
pT3	38		5.4	3.7	< 0.001	13.026	4.44-38.23
pT4	10		33.3	15.7	0.001	7.838	2.24-27.46
pN-category		< 0.001			0.549		
pN0	49		61.6	7.4		1	
pN1	34		47.1	8.6	0.226	0.663	0.34-1.29
pN2	14		16.9	10.9	0.986	0.993	0.43-2.27
pN3	25		8.0	5.4	0.814	0.905	0.40-2.07
T393C SNP		0.043			0.333		
CC/CT	96		36.7	5.1		1	
TT	26		56.9	10.4		0.712	0.36-1.42
pM-category		< 0.001			0.027		
M0	99		48.1	5.2		1	
M1	23		9.2	6.2		2.087	1.09-4.01
R-category		< 0.001			0.022		
R0	118		42.3	4.7		1	
R+	4		0	0		3.128	1.18-8.27

SNP: Single nucleotide polymorphism; 5-yr-SR: 5-yr-survival; HR: Hazard ratio.

Table 4 Summary of the effect of the *GNAS1* T393C polymorphism on various carcinomas

Cancer type	Yr	*n*	Effect	Benefit (survival)
Gastric cancer	2009	122	The present study demonstrates a significant survival benefit for the TT genotype with a 5-yr-survival rate of 56.9% *vs* the CC/CT group with a 5-yr-survival rate of only 36.7% ($P = 0.043$)	TT-genotype
Squamous cell cancer of larynx[15]	2008	157	Survival was significantly dependent on the T393C genotype in advanced American Joint Committee on Cancer (AJCC) stages (Ⅲ-Ⅳ) with higher 5-yr survival rates for TT, followed by TC and CC ($P = 0.0437$)	TT-genotype
Oro- and hypo-pharyngeal squamous cell carcinoma[16]	2008	202	C homozygous patients displayed a higher risk for disease progression than T homozygous patients ($P = 0.019$) and a higher risk for death ($P = 0.015$). In multivariate analysis, besides cancer stage and tumor localization, the T393C polymorphism was an independent prognostic factor for disease progression and death	TT-genotype
Clear cell renal cell carcinoma[11]	2006	150	Tumor progression, development of metastasis and tumor-related death was significantly associated with the T393C polymorphism. In multivariate analysis CC patients were at highest risk for progression or tumor-related death compared with T-allele carriers ($P = 0.018$)	TT-genotype
Chronic lymphocytic leukemia[17]	2006	144	Median progression-free survival was significantly higher for T-allele carriers ($P = 0.007$). In multivariate analysis, the T393C polymorphism kept its prognostic independence ($P = 0.01$) besides of ZAP-70 ($P = 0.005$) and Binet stage ($P < 0.001$). Regarding overall survival, CC genotypes were significantly at highest risk for death compared to T-alleles both in univariate ($P < 0.001$) and multivariate analysis ($P = 0.002$)	TT-genotype
Bladder cancer[10]	2005	254	Progression-free survival ($P = 0.011$), metastasis-free survival ($P = 0.001$) and cancer-specific survival ($P = 0.014$) were significantly increased in TT genotypes compared with CC genotypes. In multivariate analysis, the T393C polymorphism kept its prognostic independence	TT-genotype
Sporadic colorectal cancer[12]	2005	151	In UICC stages Ⅰ to Ⅱ, the 5-yr survival rate was significantly ($P = 0.009$) higher in TT genotypes (88%) compared with TC (71%) and CC genotypes (50%). In multivariate analysis, the T393C polymorphism was also an independent prognostic factor. No significant effect could be seen for UICC stages Ⅲ to Ⅳ	TT-genotype
Cholangio-carcinoma[14]	2007	87	Disease-specific overall survival was significantly dependent on the T393C genotype ($P = 0.02$), with TT genotypes showing reduced survival compared to patients carrying at least one C allele. In multivariate analysis (TT/C+) the T393C genotype kept its prognostic independence ($P = 0.04$)	CC-genotype
Breast carcinoma[13]	2007	279	Overall survival was significantly ($P = 0.033$) associated with the T393C polymorphism with lowest survival rates for the TT-genotype and highest survival rate for the CC-genotype. In multivariate analysis, the TT-genotype still had a significant survival benefit compared to the CC genotype ($P = 0.045$)	CC-genotype
Esophageal cancer[28]	2009	51	T393C polymorphism was significantly associated with tumor response to Cisplatin/5-FU-based radiochemotherapy. 63% of the T allele carriers had a minor histopathologic response (MiHR) with more than 10% residual vital tumor cells in resection specimens. For the CC genotype MiHR was seen only in 20%. In binary logistic regression analysis, the T393C genotype kept its independence ($P < 0.05$)	CC-genotype

approximately 800 000 new cases per year and the second leading cause of cancer-related death worldwide[20]. Many patients have advanced disease at the time of diagnosis, resulting in poor prognosis and high mortality[2,21,22]. Pretreatment staging of the disease is of high importance as it provides the basis for selecting the most appropriate therapeutic strategy[23]. Based on the preoperative staging, patients with early stage tumors are treated by endoscopic mucosal resection, while patients with advanced tumors are treated by partial or total gastrectomy[5]. Accurate staging is also the basis for selecting patients for neoadjuvant, adjuvant or palliative treatment[24]. By identifying patients with poor outcome, novel treatment strategies could be set up at the beginning of treatment which can lead to better and more individualized therapy strategies with superior survival[2].

The present study demonstrated that besides the known prognostic factors pT, pM, pN and R-category, the T393C polymorphism was also a significant prognostic factor in the univariate analysis with a survival benefit for homozygous TT patients. In addition, it demonstrated that compared to C allele carriers, homozygous TT patients were diagnosed with significantly less advanced tumor stages according to UICC, which is possibly the main reason why the T393C genotype lost its independence in the multivariate analysis.

The gene *GNAS1* is mapped to chromosome 20q13 and consists of 13 exons. Somatic activating mutations of *GNAS1* have been implicated in the etiology of McCune Albright Syndrome[25] and sporadic, isolated endocrine tumors[26,27] which supports a role of *GNAS1* in tumor initiation and progression.

Recent studies have shown that genotypes of the T393C polymorphism are significantly associated with survival of patients suffering from colorectal cancer, bladder cancer, clear cell renal carcinoma, intrahepatic cholangiocarcinoma, invasive breast carcinoma and squamous cell carcinoma of the larynx, oropharynx and hypopharynx (Table 4)[10-14].

Comparable to previous results in bladder cancer, clear cell renal carcinoma and colorectal cancer, the present study also demonstrated significantly higher survival rates for TT genotypes in gastric cancer (Figure 3). Patients with the TT genotype showed a 5-year survival rate of 57%, whereas the 5-year survival rate for C allele carriers was only at 37%.

In contrast to our findings in gastric cancer and previous findings in the above-mentioned tumor types, an unfavourable clinical course for T allele carriers has been described in studies of invasive breast cancer and intrahepatic cholangiocarcinoma, suggesting that the biological effect of the T393C polymorphism may be different in different tumor types. In a recent study, we demonstrated that this polymorphism is a predictive molecular marker for tumor response to cisplatin/5-FU-based radiochemotherapy in esophageal cancer, with CC genotypes mostly showing a major response[28].

In vitro experiments suggest that expression of Gαs is associated with enhanced apoptosis[7,8]. The second messenger, cyclic AMP, which is generated by activated Gαs, seems to play a major role in this proapoptotic process. An increased concentration of the intracellular second messenger, cyclic AMP promotes apoptosis in several cell types including leukemic cells[29], ovarian cancer cells[30], and lymphoma cells[25]. Increased Gαs expression in tissues of patients with TT genotypes may therefore confer enhanced apoptosis in 393T allele carriers. Hypothetically, this mechanism may contribute to the described more favorable clinical course and the less advanced tumor stages of homozygous TT patients. This hypothesis remains to be supported by additional functional studies which were beyond the scope of the present study. The T393C polymorphism as a risk factor for gastric cancer could not be established in the present study.

In conclusion, this study demonstrated for the first time that in primary gastric cancer, homozygous *GNAS1* 393T patients have less advanced tumor stages and higher survival rates than C allele carriers. These findings further support the concept of a general role for the *GNAS1* T393C polymorphism in tumor progression.

COMMENTS

Background

Identification of gastric cancer patients with poor outcome can help to set up novel treatment strategies at the beginning of treatment and may lead to better and more individualized therapy strategies with better survival. In recent years, studies have focused on the detection of single nucleotide polymorphisms (SNPs) as prognostic molecular markers in cancer.

Research frontiers

The *GNAS1* T393C polymorphism is located in exon 5 of the gene *GNAS1*. In this study the authors describe, for the first time, the impact of this SNP in gastric cancer. The study demonstrates that the *GNAS1* T393C polymorphism affects tumor stage and prognosis in gastric cancer.

Innovations and breakthroughs

For various solid tumors, previous studies have demonstrated that patient survival and tumor progression depend on the *GNAS1* T393C genotype. In the present study, the authors have described for the first time that the *GNAS1* T393C polymorphism affects tumor stage and prognosis in gastric cancer.

Applications

The *GNAS1* T393C polymorphism will contribute to identifying high-risk patients with gastric cancer and might help to establish a more individualized treatment strategy for gastric cancer.

Terminology

A single-nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide - A, T, C, or G - in the genome differs between members of a species. The *GNAS1* T393C is located in exon 5 of the gene *GNAS1*. For several cancer types, studies have demonstrated that patient survival is affected by this SNP.

Peer review

Overall, this paper provides information on *GNAS1* T393C allele carrier status which influences tumor progression and survival in gastric cancer, with higher tumor stages and worse outcome for C allele carriers.

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