A common hereditary single-nucleotide polymorphism in the gene of FAS and colorectal cancer survival

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Abstract

Apoptosis plays an important role in embryogenesis, autoimmunity and tumourigenesis. Cell surface death receptors such as TNFRSF6 (FAS) confer a major apoptotic effect. A single-nucleotide polymorphism in the FAS promoter gene, -670A/G, modulates apoptotic signalling and has been related to susceptibility and progression of a variety of cancers. The present study aimed to evaluate the role of this polymorphism for survival of patients with colorectal cancer. We performed a retrospective analysis including 433 patients with histologically confirmed colorectal cancer. A Cox regression model including FAS -670 genotypes, age at diagnosis, tumour grading, primary tumour size, number of lymph nodes examined, number of metastatic lymph nodes, tumour stage and application of fluorouracil-based adjuvant chemotherapy was used to estimate the effect of the FAS genotype on survival. FAS -670A/G genotype frequencies were 24.2% (AA), 46.3% (AG) and 29.5% (GG). Forty-nine patients were excluded from the Cox regression analysis because of missing values. Out of the remaining 384 patients, 69 (18%) died during a follow-up of maximum 10 years. Mean follow-up time was 58 ± 34 months (median 55 months). Carriers of the homozygous FAS -670GG genotype had a significantly lower survival rate compared with AA/AG genotype carriers (relative risk 1.76, 95% confidence interval 1.08–2.87; $P = 0.023$). The FAS -670A/G polymorphism may be associated with overall survival time of patients with colorectal cancer.

Keywords: FAS • apoptosis • colorectal cancer • survival • polymorphism

Introduction

Half a million deaths and one million cases of new cancers in 2002 were caused by colorectal cancer (CRC) worldwide [1], and it was estimated that 148,710 men and women will be diagnosed with and 49,960 men and women will die of CRC in the United States in 2008 [2]. These data make CRC a major burden for health systems all over the world.

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FAS (also known as CD-95, APO-1 or TNFRSF6) is a death receptor that is a member of the tumour necrosis factor (TNF) receptor superfamily. Together with FAS-ligand (FASL), FAS initiates the extrinsic pathway of the death signalling cascade that leads to apoptotic cell death. Apoptosis is crucial for maintaining homeostasis for tissues, and abnormal regulation can lead to a variety of diseases, including cancer [3–5]. On the other hand, the FAS–FASL system is able to induce apoptosis in tumour-infiltrating lymphocytes (TILs) and therefore can help malignant cells escape from defence mechanisms. This immune-privilege phenomenon may lead to cancer formation and progression and even play an essential role in developing metastases [6–8].

The promoter region of the FAS-gene contains a functional single-nucleotide polymorphism (SNP), the -670A/G SNP.

Table 1 Cox regression analysis of survival time in colorectal cancer patients

Variable	RR	95% CI	P-value
FAS -670GG genotype	1.76	1.08–2.87	0.023
Age at diagnosis	1.04	1.01–1.07	0.006
pT	1.09	0.71–1.67	0.688
Evaluated N	0.95	0.92–0.98	0.001
Positive N	1.11	1.07–1.16	<0.001
Tumour grading	1.06	0.65–1.73	0.810
Stage (AJCC)	1.85	1.29–2.65	0.001
Adjuvant CTX	0.54	0.32–0.92	0.023

RR, relative risk; CI, confidence interval; pT, primary tumour size according to AJCC-TNM system; Evaluated N, count of lymph nodes evaluated after resection; Positive N, count of metastatic lymph nodes; Adjuvant CTX, application of fluorouracil (5-FU)-based adjuvant chemotherapy.

The -670A/G SNP lies within the interferon (IFN) gamma-activated sequence (GAS) motif, a STAT1-binding site. The different allelic expressions influence the gene expression of FAS [9, 10]. This functionality may influence FAS-mediated apoptosis and therefore lead to different individual susceptibility of cancer. It was demonstrated that these polymorphisms are associated with a higher risk of developing breast cancer, squamous cell carcinoma of the head and neck, bladder cancer, T-cell leukaemia, lung cancer, cutaneous malignant melanoma, prostate cancer, cervical cancer, esophageal squamous cell carcinoma and acute myeloid leukaemia [10–25]. The FAS -670A/G polymorphism showed to have a correlation with survival in adult T-cell leukaemia as well [26].

Therefore, we hypothesized that this -670A/G SNP could be associated with survival in CRC patients, and to test our hypothesis, the following retrospective study was performed.

Materials and methods

A total of 433 consecutive patients with histologically confirmed sporadic colorectal cancer without synchronous and/or metachronous secondary malignancy were recruited at the Division of Oncology, Department of Internal Medicine, of the Medical University of Graz, Austria, from January 1993 until June 2004. The study was performed according to the Austrian Gene Technology Act and has been approved by the Ethics Committee of the Medical University of Graz. Written informed consent was obtained from all participating subjects and all of the probands were of Caucasian ethnicity.

Genomic DNA for genetic analyses was isolated from venous blood using a GeneMole automated DNA extraction system (Mole AS, Lysaker, Norway) and stored at 4°C in the facilities of the Medical University of Graz. FAS genotypes were determined by 5′-nuclease assays (TaqMan™). Applied Biosystems 'Assay-by-Design' custom service (Applera, Austria) was used for designing and manufacturing Primer and probe sets. For each set of reactions, DNA of cases was taken, and a negative control containing H_2O instead of DNA to check for contaminations was added. Fifty samples were re-analyzed and results were identical for all samples. The data were exported into Excel format and analyzed as scatter plot.

Statistical analysis was done using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Survival was defined as the time from diagnosis to death from any cause. Numeric values were analyzed by Student's t-test, and proportions of groups were compared by chi-square test. A Cox regression analysis including age at diagnosis, tumour stage (according to the AJCC TNM-classification), size of primary tumour (as pT-parameter), tumour grading, count of lymph nodes evaluated after resection, count of metastatic lymph nodes, application of fluorouracil (5-FU)-based adjuvant chemotherapy and the FAS -670A/G genotypes was used to identify independent prognostic variables influencing survival. Threshold for significance was $P < 0.05$.

Results

Demographic data of study subjects are described elsewhere [27, 28]. The genotype frequencies among patients were AA (wild-type) 24.2, AG 46.3 and GG 29.5%, respectively. These frequencies did not deviate from the Hardy–Weinberg equilibrium.

Forty-nine patients were excluded from the Cox regression analysis because of missing values. Out of the remaining 384 patients, 69 (18.0%) died during a follow-up of maximum 10 years. Mean follow-up time was 58 ± 34 months (median 55 months). In this study population, we observed a statistically significant association between survival and age at diagnosis, count of lymph nodes evaluated after resection, count of metastatic lymph nodes, tumour stage according to the American Joint Committee on Cancer (AJCC), application of 5-FU-based adjuvant chemotherapy and the GG genotype (see Table 1). Carriers of the homozygous FAS -670GG genotype had a significantly increased mortality rate compared with AA/AG genotype carriers (relative risk 1.76, 95% confidence interval 1.08–2.87; $P = 0.023$, see Fig. 1).

Cause of death was not cancer-related in one patient. In an analysis investigating cancer-related mortality (68 deaths), the adjusted relative risk for the FAS -670GG genotype was 1.86 (95% confidence interval 1.14–3.03; $P = 0.013$).

Discussion

This investigation is the first one to our knowledge that has tried to search for a correlation between a FAS SNP and survival in CRC patients. There exist some established risk factors that modify survival for CRC from whom the pathologic stage represents the
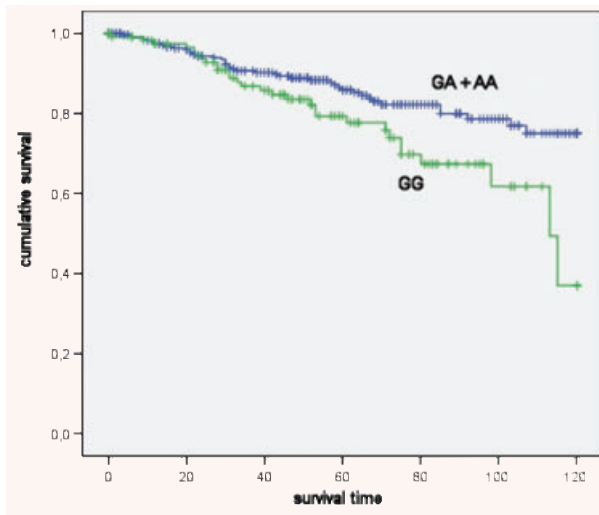
Fig. 1 Kaplan–Meier survival graph. The *x* axis shows survival time in months, the *y* axis cumulative survival. The green line marks the GG genotype, the blue line the GA and AA genotypes. As there was no significant discrepancy detectable in survival between patients with AA and AG genotype, they were subsumed and compared to patients with GG genotype.

most important prognostic factor [29, 30]. The most commonly used staging system is the tumour–node–metastasis (TNM) system defined by the American Joint Committee on Cancer, which consists of primary tumour size, count of metastatic lymph nodes and existence of distant metastases [30]. Tumour grading and number of lymph nodes evaluated after surgery are important independent prognostic factors as well [31, 32]. It was demonstrated in the 1990s that 5-FU-containing adjuvant chemotherapy is able to prolong survival in CRC patients [33, 34]. We included these proven risk factors together with the FAS -670 genotypes in our statistical model. Using this model, we were able to detect a possible influence of this polymorphism in survival in addition to the established risk factors.

It was demonstrated that modified mechanisms in the FAS-mediated apoptosis pathway play an essential role in early colorectal carcinogenesis [35, 36]. The different allelic expressions of the

-670G/A SNP, which is located in the consensus sequence of GAS, leads to different STAT1-binding affinity and therefore to varying FAS gene expression. The GG variant was found to be correlated with lower STAT1-binding capacity, and this led to decreased transcriptional activity of the FAS promoter [9, 37]. Farre and co-workers demonstrated that IFN-gamma stimulation leads to a gradual increase of FAS expression rising from nearly no expression in the GG genotype to a significant expression in the AA genotype [26]. These different levels of FAS expression could influence FAS-mediated apoptosis of cancer and immune cells and hence tumourigenesis, early tumour dissemination and survival. In addition, the activation of non-apoptotic signalling pathways of FAS in tumour microenvironment that might be influenced by different FAS-gene expressions could play a role in cancer development or protection of tumour [4, 38, 39]. Interestingly, the GG genotype, which was associated with higher mortality in colorectal cancer patients in the present study, led to decreased mortality in adult T-cell leukaemia patients [26]. The reason for this apparent contrast could be differential FAS-mediated apoptosis pathways as well as non-apoptotic functions in solid tumour cells and immune cells.

To date, little is known about these non-apoptotic functions of FAS, and further basic research is necessary to clarify the involvement of SNPs and their functionality in these matters.

The FAS -670G/A polymorphism and its potential association with susceptibility was investigated for different types of cancer and resulted in various results even for the same cancer entity, for example, cervical cancer [14, 23, 40–42]. These controversial findings could have their origin in the geography and ethnicity of the investigated populations, but also alternating non-apoptotic signalling in different tissues could have led to these results.

Limitations of our study are its retrospective design and the relatively small sample of patients. In addition, the recruitment of subjects started in the early 1990, and hence the patients that were included at the beginning did not receive any or insufficient adjuvant therapy. Regrettably, K-RAS mutation analysis and microsatellite instability (MSI) testing of tumour tissue, which are interesting risk factors for colorectal cancer at the genetic level, could not be performed for the present study.

Therefore, we conclude that prospective trials with larger sample size and state-of-the-art treatment as well as MSI and K-RAS mutation analysis are warranted to confirm our findings and clarify the role of these factors for colorectal cancer survival.

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