Single Nucleotide Polymorphisms of Integrin Alpha-2 and Beta-3 Genes Are not Associated with Relapse-free and Overall Survival in Colorectal Cancer Patients

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Abstract. *Background: Integrins influence tumourigenesis, tumor progression and development of metastases. The impact of polymorphisms in integrin genes on relapse-free survival (RFS) and overall survival (OS) for 433 Caucasian patients with colorectal cancer was analysed in this retrospective study. Patients and Methods: A Cox regression model including integrin genotype, age, grading, tumour size, number of lymph nodes examined, number of metastatic lymph nodes, stage and application of fluorouracil-based adjuvant chemotherapy was used to estimate their effect. Results: After a median follow-up of 41 months for RFS and 55 months for OS, no significant correlation between the ITGA2 1648A allele (RFS p=0.618, OS p=0.604), the ITGA2 807T allele (RFS p=0.603, OS p=0.807) and the ITGB3 176C allele (RFS p=0.719, OS p=0.261) and survival was detectable. Conclusion: The investigated integrin polymorphisms are not associated with RFS or OS in colorectal cancer patients.*

Colorectal cancer (CRC) is the most common neoplasm of the gastrointestinal tract in humans and the third cause of cancer worldwide (1). It was estimated that in 2010 about 148,000 people would be diagnosed with and 52,000 people would die of CRC in the U.S.A. alone (2). Since CRC is a significant burden for health care systems all over the world, the identification of new cellular and genetic markers which can help clinicians estimating relapse-free survival (RFS) and overall survival (OS) and, subsequently, tailoring adjuvant or palliative treatment for every individual is badly needed.

Integrins are transmembrane glycoprotein heterodimers which consist of α- and β-subunits. These 18 α-subunits and 8 β-subunits combine to form at least 25 distinct pairings that are specific for a unique set of ligands. They function as key surface adhesion and cell-signalling receptors by mediating cell-extracellular matrix and cell–cell interactions. Hence, integrins regulate cell proliferation and migration, as well as cell survival and cell shape (3-5). It was demonstrated recently that, in addition to their ligation-dependent effects, unligated integrins are able to influence tumour growth and metastasis (6). Moreover, integrins and integrin-induced signalling contribute to tumour progression by mediating angiogenesis, lymphangiogenesis and inflammation in the tumour microenvironment (7, 8).

The functional 176T>C single nucleotide polymorphism (SNP) (NCBI SNP ID: rs5918) in the *ITGB3* gene, which codes for the integrin β3 subunit, causes increased activation of mitogen-activated protein kinase and increased ability of platelet aggregation (9, 10). *ITGA2* is the gene coding for the α2 component and contains two functional polymorphisms which are known to be in genetic linkage (11). The 1648G>A polymorphism (NCBI SNP ID: rs1801106) and the 807C>T polymorphism (NCBI SNP ID: rs1126643) have been associated with different integrin α2β1 density on cell surfaces (11-13). Correlations of these genetic variants in integrins with the risk for developing melanoma, breast, ovarian, prostate and colorectal cancer have been described

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(14-18). The ITGB3 176T>C polymorphism could be linked with a higher risk of developing metastases in breast cancer patients, as well (19, 20).

This retrospective study was performed with the aim of testing the hypothesis that these integrin polymorphisms may be associated with RFS and, possibly, OS in CRC patients.

Patients and Methods

From January 1993 until June 2004, 433 consecutive patients with sporadic CRC, without synchronous and/or metachronous secondary malignancy, attending the Division of Oncology, Department of Internal Medicine of the Medical University of Graz, Austria were recruited to this study. The study was approved by the Ethics Committee of the Medical University of Graz and was performed according to the Austrian Gene Technology Act. Written informed consent was obtained from all participants and all probands were Caucasian.

All patients were included in the aftercare measures programme of the Division of Clinical Oncology, providing follow-up at regular intervals for adjuvant setting (3-month intervals in years 1-3, 6-months intervals in years 4-5 and 12-month interval in years 6-15 after diagnosis). Follow-up investigations included clinical check-up, laboratory and radiological (computed tomography, abdominal ultrasound and chest X-ray) examinations. If patients failed to show up to their follow-up appointment, their general practitioner was contacted to retrieve information from the patient's record. The last update in this procedure was performed in May 2010.

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. Integrin genotypes were determined by 5'-nuclease assays (TaqMan®; Applied Biosystems, Carlsbad, CA, USA). Applied Biosystems 'Assay-by-Design' custom service (Applied Biosystems, Carlsbad, CA, USA) was used for designing and manufacturing primer and probe sets. The data were exported into Excel (Microsoft, Inc., Redmont, WA, USA) format and analysed as scatter plot. For each set of reactions, DNA was taken and a negative control containing H_2O instead of DNA was added to check for contaminations. Fifty samples were reanalysed and the results were identical for all samples. Additionally, in a subset of 24 samples, genotypes were verified by a commercial laboratory for molecular genetics (http://www.labor-renner.at) and the results were consistent for all samples.

Statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). RFS was defined as the time period from diagnosis to the detection of metastases or first relapse or death from any cause (21). OS was defined as the time from diagnosis to death from any cause. Numeric values were analysed by Student's *t*-test, proportions of groups were compared by chi-squared test. A Cox regression analysis including age at diagnosis, tumour stage (according to the AJCC TNM classification (22); see Table I), 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 integrin genotypes was used to identify independent prognostic variables influencing RFS and OS. The threshold for statistical significance was $p<0.05$.

Table I. *Tumour staging according to the AJCC modified by Wolpin BM and Mayer RJ, 2008 (22).*

Stage (AJCC)	TNM classification
I	T_{1-2} N_0 M_0
IIa	T_3 N_0 M_0
IIb	T_4 N_0 M_0
III	any T N_{1-2} M_0
IV	any T any N M_1

Table II. *Demographic and clinical data of CRC patients.*

CRC patients, n	433
Gender	
Female	175 (40.4%)
Male	258 (59.6%)
Age, years	61.0±10.9
TNM staging	
T_1	11 (2.6%)
T_2	41 (9.5%)
T_3	281 (64.9%)
T_4	80 (18.6%)
N_0	156 (36.1%)
N_1	158 (36.5%)
N_2	102 (23.6%)
M_1	87 (20.1%)
Tumour grading	
G1	8 (1.8%)
G2	274 (63.3%)
G3	151 (34.9%)
Adjuvant CTX received#	273 (63.1%)

#Application of fluorouracil (5-FU)-based adjuvant chemotherapy.

Results

Demographic data of study subjects are described in Table II and elsewhere (15, 23). Genotype frequencies among patients did not deviate from the Hardy-Weinberg equilibrium and are shown in Table III.

Fifty-two patients had to be excluded from the Cox regression analysis for RFS because of missing data. Of the remaining 381 patients, 149 (39.1%) developed metastases, relapsed or died during a median follow-up time period of 41 months (range 0-198 months; Table IV).

Forty-nine patients were excluded from the Cox regression analysis for OS because of missing data. Of the remaining 384 patients, 69 (18.0%) died during a follow-up of maximum 10 years. Mean follow-up time period was 58 ± 34 months (median 55 months; Table V).

In both analyses for RFS as well as OS, no influence of the investigated SNPs on these endpoints was observed. The relative risk for the ITGA2 1648A, ITGA2 807T and ITGB3 176C alleles and RFS are shown in Table IV. The relative

Table III. *Genotype frequencies of CRC patients.*

	Number of patients (%) or genotype frequency
Total number of patients	433
ITGA2 807C>T	
CC	171 (40.6%)
CT	192 (45.6%)
TT	58 (13.8%)
ITGA2 807T frequency	0.366
ITGA2 1648G>A	
GG	350 (82.7%)
GA	71 (16.8%)
AA	2 (0.5%)
ITGA2 1648A frequency	0.089
ITGB3 176T>C	
TT	302 (69.9%)
TC	116 (26.9%)
CC	14 (3.2%)
ITGB3 176C frequency	0.167

Table IV. *Cox regression analysis of relapse-free survival in CRC patients.*

Variable	RR	95% CI	*p*-Value
Age at diagnosis, years	1.000	0.985-1.016	0.957
Gender, male *versus* female	1.093	0.788-1.518	0.593
Stage (AJCC-TNM)	3.924	2.734-5.630	<0.001
pT^	1.340	0.983-1.828	0.064
Tumour grading	1.096	0.788-1.524	0.586
Positive N†, count	1.043	1.008-1.079	0.015
Evaluated N‡, count	0.976	0.958-0.994	0.008
Adjuvant CTX#, yes/no	0.155	0.101-0.239	<0.001
ITGA2 1648A allele	1.115	0.728-1.709	0.618
ITGA2 807T allele	0.939	0.742-1.190	0.603
ITGB3 176C allele	0.944	0.689-1.293	0.719

RR, Relative risk; CI, confidence interval; ^primary tumour size according to AJCC-TNM system; †count of metastatic lymph nodes; ‡count of lymph nodes evaluated after resection; #application of fluorouracil (5-FU)-based adjuvant chemotherapy.

Table V. *Cox regression analysis of overall survival in CRC patients.*

Variable	RR	95% CI	*p*-Value
Age at diagnosis, years	1.039	1.011-1.068	0.006
Gender, male *versus* female	1.516	0.934-2.460	0.092
Stage (AJCC-TNM)	1.979	1.356-2.889	<0.001
pT^	1.106	0.715-1.710	0.652
Tumour grading	1.236	0.755-2.023	0.401
Positive N†, count	1.105	1.054-1.157	<0.001
Evaluated N‡, count	0.940	0.909-0.972	<0.001
Adjuvant CTX#, yes/no	0.604	0.356-1.023	0.061
ITGA2 1648A allele	1.182	0.628-2.223	0.604
ITGA2 807T allele	1.045	0.736-1.483	0.807
ITGB3 176C allele	0.764	0.477-1.222	0.261

RR, Relative risk; CI, confidence interval; ^primary tumour size according to AJCC-TNM system; †count of metastatic lymph nodes; ‡count of lymph nodes evaluated after resection; #application of fluorouracil (5-FU)-based adjuvant chemotherapy.

risk for *ITGA2* 1648A, *ITGA2* 807T and *ITGB3* 176C alleles and OS are shown in Table V.

A significant correlation was detected between RFS and count of lymph nodes evaluated after resection, count of metastatic lymph nodes, tumour stage according to the AJCC TNM classification and administration of adjuvant 5-FU-based chemotherapy in this study population. For OS, a statistically significant association with age at diagnosis, count of lymph nodes evaluated after resection, count of metastatic lymph nodes and tumour stage according to the AJCC TNM classification was demonstrated.

Discussion

To the Authors' best knowledge, this is the first study to investigate a potential correlation between specific integrin SNPs and RFS as well as OS in CRC patients. No association between both RFS and OS with the different genetic variants for integrin subunits was detected.

In both statistical models for RFS and OS, a clear effect for the well-established prognostic risk factors, tumour stage, count of operated and pathological lymph nodes was observed, concordant with previous studies (24-27). These findings emphasize the importance of these parameters, especially tumour staging (according to AJCC TNM classification), which was the only factor that emerged as being highly significant in both models. Administration of 5-FU-containing adjuvant chemotherapy had a statistical significant effect only on RFS in this study population, whereas for OS only, a trend with a *p*-value of 0.061 was observed. The reason for these results may be that patients that were recruited at the beginning of the study did not receive adequate (or, even, any at all) adjuvant chemotherapy. Although no correlation was found between RFS and OS and the investigated genetic variants, no conclusions can be drawn about the possible role of these SNPs with CRC in general or the potential for other functional integrin α-2 and β-3 SNPs not examined in this study to be associated with survival.

The importance of integrins and integrin-dependent signalling for development of neoplasms and metastases is evident (4-6). In preclinical and early clinical trials, various integrin antagonists have been tested against different tumour cell lines and entities and seem to be

effective as anticancer therapies (28-31). Perhaps these genetic variants of integrins influence these promising new therapy options in a similar way as described for other anticancer therapies (32, 33).

Limitations of this study are its retrospective design and the relatively small sample size of patients. In addition, the recruitment of subjects started in the early 1990 and hence the patients that were included at the beginning of this investigation received no or insufficient adjuvant therapy due to the standard of knowledge at that time. Furthermore, it is acknowledged that the size of the present study was too small to discover small effects of integrin gene variants on the clinical outcome of CRC patients. Nevertheless, the study was sufficiently powered to detect stronger effects with potential clinical relevance. For the SNP with the lowest minor allelic frequency (*ITGA2* 1648G>A), the present study had a statistical power of more than 0.8 to detect a relative risk of 1.6 for carriers of an *ITGA2* 1648A variant. For the two other SNPs (*ITGA2* 807C>T and *ITGB3* 176T>C), the statistical power was even higher.

In conclusion, the investigated polymorphisms are not associated with RFS and OS in Caucasian CRC patients.

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