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EPIDEMIOLOGY

Association of interleukin-10 gene variation with breast cancer prognosis

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Abstract Genetic polymorphisms are responsible for inter-individual variation and diversity and have been recently considered as the main genetic elements involved in the development and progression of cancer. We examined associations between common germline genetic variants in 7 genes involved in folate metabolism, cell proliferation and apoptosis, prostaglandin synthesis, detoxification of compounds and inflammation, and disease-free survival among women diagnosed with invasive breast cancer. DNA from up to 432 women was genotyped for 8 polymorphisms. The genotypes of each polymorphism were tested for association with disease-free survival using univariate and multivariate Cox regression analysis. The model was adjusted for known breast cancer prognostic factors. The rare allele of the IL-10 592C>A polymorphism was significantly associated with reduced disease-free survival ($P = 0.018$, risk ratio of recurrence (RR) = 1.45, 95% confidence interval (CI) = 1.06–1.98), which was not attenuated after adjusting for age at diagnosis, tumor size, lymph node status, clinical stage, histological grade, estrogen receptor status, progesterone receptor status, and treatment modalities ($P = 0.019$, RR = 1.48, 95% CI = 1.066–2.044). No association was found between MTHFR 677C>T, TGFB1 29T>C, FASLG 844C>T, FAS 1377G>A, FAS 670A>G, PTGS2 8473T>C and SULT1A1 638G>A polymorphisms and disease-free survival. Our data suggest that the rare allele of IL-10 592C>A may be a potential prognostic marker in breast cancer for disease-free survival.

Keywords Breast cancer · Prognosis · Polymorphism · Interleukin

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Introduction

Genetic polymorphisms are responsible for inter-individual variation and diversity and have been recently considered as the main genetic elements involved in the development and progression of common and complex diseases [1]. Metastasis accounts for ~90% of cancer-related death and its impact in developed nations will likely escalate, which primarily reflects an aging population [2, 3]. A number of models have been proposed for the mechanism of metastasis, and although each model explains certain aspects of this process, none has yet provided a comprehensive explanation. Somatic alterations have been shown to correlate with breast cancer prognosis and survival, but less is known about the effects of common inherited genetic

variations [4]. Recent results suggest that germline polymorphism is a significant, previously unrecognized factor in breast cancer progression and metastasis. Germline polymorphisms contribute to the overall metastasis risk by providing a more or less permissive environment on which the secondary mutational events occur, mediated by the combination of subtle changes in gene function due to single nucleotide polymorphisms (SNPs) in coding sequence, splice sites promoters and enhancers [5]. The existence of inherited susceptibility to metastasis may have profound implications in regard to cancer diagnosis and assessment of prognosis [6]. The existence of germline polymorphisms that influence metastasis may enable the clinicians to better identify those patients at risk, providing individual treatment with low toxicity but maximum benefit [7, 8]. The purpose of this study was to assess whether common germline polymorphisms in a variety of pathways are associated with disease-free survival (DFS) in breast cancer. Seven genes involved in folate metabolism, cell proliferation and apoptosis, prostaglandin synthesis, detoxification of compounds and inflammation have been investigated in this study. This study was conducted adhering to as many of the reporting recommendations for tumor marker prognostic studies as applicable (REMARK; [9, 10]).

Materials and methods

Subjects

Between January and July 2002, 432 blood samples from women patients with histologically confirmed, sporadic breast cancers without synchronous metastasis were collected at the Division of Oncology, Department of Internal Medicine, Medical University of Graz, Austria. Patients were followed until January 2008 for breast cancer recurrence/metastasis and death due to breast cancer. Complete clinical (age at diagnosis, tumor size, lymph node status and stage) and tumor biological factors (grade, estrogen and progesterone receptor), therapy data (hormone-/tamoxifen-therapy, anthracycline-/nonanthracycline-chemotherapy), genotyping results and breast cancer relapse information were available for 383 patients. The main reason for incomplete patients' data was missing clinical data (Table 1). Follow-up information was available for all patients. The study was performed according to the Austrian Gene Technology Act and has been approved by the Ethical Committee of the Medical University Graz. Written informed consent was obtained from all participating subjects. All subjects were Caucasian.

Table 1 Clinical, tumor biological and treatment characteristics

Follow-up time (month)	78.5 (median)	3.8–120 (range, censored)
Disease-free survival (month)	83.2 (median)	3.5–120 (range, censored)
Age (years)	55.9 (median)	28–83 (range)
Menopausal status		
Premenopausal	52	12%
Postmenopausal	380	88%
Tumor size		
T1	223	51.6%
T2	145	33.6%
T3	15	3.5%
T4	32	7.4%
Unknown	17	3.9%
Lymph node involvement		
N0	202	46.8%
N1	202	46.8%
N2	14	3.2%
Unknown	14	3.2%
Grade		
G1	20	4.6%
G2	188	43.5%
G3	205	47.5%
Unknown	19	4.4%
Stage		
I	129	29.9%
II	230	53.2%
III	56	13%
Missing	17	3.9%
Estrogen receptor		
Negative	103	23.8%
Positive	316	73.1%
Unknown	13	3%
Progesterone receptor		
Negative	141	32.6%
Positive	275	63.7%
Unknown	16	3.7%
Hormone therapy		
No	189	43.8%
Yes	243	56.2%
Tamoxifen therapy		
No	197	45.6%
Yes	235	54.4%
Anthracycline therapy		
No	340	78.8%
Yes	92	21.3%
Nonanthracycline therapy		
No	360	83.3%
Yes	72	16.7%

Selection of genetic polymorphisms

For the present analysis, SNPs previously tested for breast cancer risk assessment by our study group were evaluated. Eight SNPs involved in a variety of pathways, including folate metabolism (MTHFR 677C>T), inflammation (IL10 592C>A), cell proliferation and apoptosis (FAS 1377G>A, FAS 670A>G, FASL 844C>T, TGFB1 29T>C), prostaglandin synthesis (PTGS2 8473T>C) and detoxification of compounds (SULT1A1 638G>A) were analyzed [11–16].

Genotyping

Genotypes were determined by a 5V-nuclease assay (TaqMan) with primers and probes designed and manufactured using Applera's "Assayby-Design" custom service (Applied Biosystems, Vienna, Austria). PCR and evaluation of fluorescence data were done as described previously [17]. For each set of reactions, one negative control containing water instead of DNA was added to check for contamination. Genotyping failed in 4 cases for TGFB1, 6 cases for MTHFR and 11 cases for FASL.

Statistical analysis

The primary outcome was disease-free survival. The endpoints included cancer recurrence/metastasis or death due to breast cancer. Follow-up was censored at 10 years. The relationship between the genotype frequency of the SNPs and clinicopathological factors were assessed by χ^2 and Fisher's exact probability tests. Models were adjusted for known breast cancer prognostic factors by multivariate regression analysis. Disease-free survival curves were generated by the Kaplan–Meier method and verified by the log-rank test. Cox's proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values. SNPs were re-evaluated in a model adjusted for known breast cancer prognostic factors, which included age at diagnosis (<40, 40–49, 50–59, or ≥60 years), tumor size (T 1, 2, 3, 4), lymph node status (N 0, 1, 2), clinical stage (TNM 1, 2, 3), histological grade (grade 1, 2, 3), estrogen receptor status (ER), progesterone receptor status (PR) and treatment modalities (hormone-/tamoxifen therapy, anthracycline-/nonanthracycline-chemotherapy). Differences were considered significant when a P value < 0.05 was obtained. All analyses were performed using the SPSS 14.0 statistical software package (SPSS Inc., Sunnyvale, USA).

Results

Frequency data for clinical and tumor biological factors and therapy modalities are shown in Table 1. The patients were genotyped for a panel of eight SNPs in various pathways. We found an association between the SULT1A1 genotypes with lymph node status and tumor stage. The SULT1A1 638A allele was significantly associated with a higher prevalence of lymph node involvement ($P = 0.04$) and higher tumor stage ($P = 0.04$). No correlation was found between the other SNPs and clinicopathological features. When the models were adjusted for known breast cancer prognostic factors no association between genotypes and clinical variables were found (data not shown). We investigated whether the SNPs were associated with DFS. No association was found between MTHFR 677C>T, TGFB1 29T>C, FASLG 844C>T, FAS 1377G>A, FAS 670A>G, PTGS2 8473T>C and SULT1A1 638G>A and DFS. In contrast, the A-allele of the IL-10 592C>A polymorphism was significantly associated with reduced DFS by Kaplan–Meier analysis, as shown in Fig. 1 ($P = 0.017$), and univariate Cox's proportional hazards regression analysis ($P = 0.018$, risk ratio of recurrence [RR] = 1.45, 95% confidence interval [CI] = 1.06–1.98; Table 2; disease-free survival table: Table 3). In this analysis, we combined the C/A heterozygous and A/A homozygous genotypes of IL-10 592C>A, because of the small number of A/A genotypes. The risk associated with IL-10 was not significantly attenuated after adjusting for age at diagnosis, tumor size, lymph node status, clinical stage, histological grade, ER, PR and treatment modalities ($P = 0.019$, RR = 1.48, 95% CI = 1.066–2.044; Fig. 2). In addition, we performed independent cohort analyses of IL10 592C>A for each treatment group (anthracycline therapy,

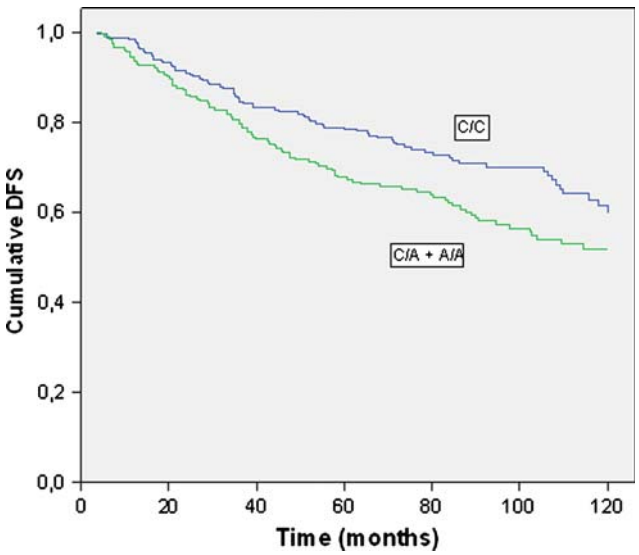

Fig. 1 Disease-free survival of patients with breast cancer after diagnosis stratified by the genotypes of IL-10 592C>A (C/A heterozygous and A/A homozygous genotypes are combined; $N = 432$ cases; C/C: 231 cases, 157 censored; C/A and A/A: 201 cases, 113 censored)

Table 2 Univariate Cox's model (disease-free survival) of analyzed polymorphisms

Variables	*n*	*P*	RR (95% CI)
MTHFR 677C>T	426	0.14	1.18 (0.95–1.48)
TGFB1 29T>C	428	0.13	1.18 (0.95–1.47)
FASLG 844C>T	421	0.84	0.98 (0.77–1.24)
FAS 1377G>A	432	0.16	0.78 (0.56–1.10)
FAS 670A>G	431	0.55	1.07 (0.85–1.35)
PTGS2 8473T>C	432	0.99	0.99 (0.80–1.25)
SULT1A1 638G>A	431	0.56	1.08 (0.84–1.37)
IL-10 592 592C>A	432	0.018	1.45 (1.07–1.98)

Table 3 Disease-free survival table for IL10 592C>A (C/C: 231 cases; C/A and A/A: 201 cases)

Time (month)	C/C		C/A and A/A	
	N of cumulative events	*N* of remaining cases	*N* of cumulative events	*N* of remaining cases
12	5	226	12	189
24	22	209	29	172
36	35	196	40	161
48	41	190	57	144
60	50	181	66	135
72	57	138	69	110
84	62	112	75	97
96	65	92	82	73
108	68	67	86	56
120	74	39	88	39

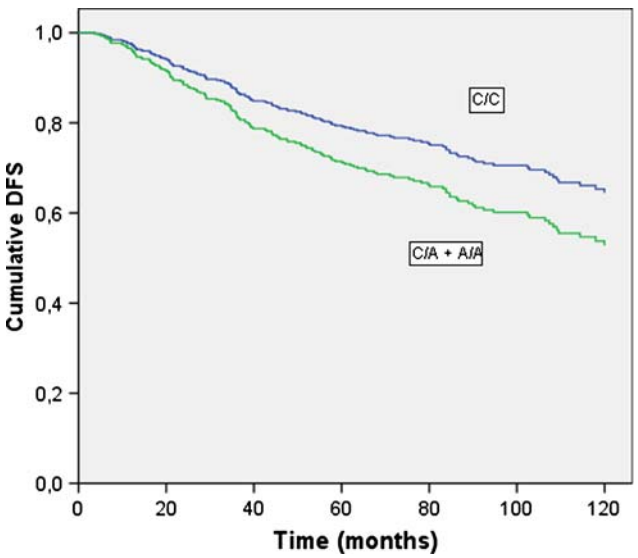

Fig. 2 Disease-free survival of patients with breast cancer after diagnosis stratified by the genotypes of IL-10 592C>A (C/A heterozygous and A/A homozygous genotypes are combined) after adjusting for known breast cancer prognostic factors ($N = 398$ cases)

nonanthracycline therapy, hormone-/tamoxifen therapy) and found no association with DFS (data not shown).

Discussion

In this study we assessed whether common germline polymorphisms in a variety of pathways are associated with DFS in breast cancer. The findings that the SULT1A1 genotypes are associated with lymph node status and tumor stage have been discussed by our research group in a previous study [15]. We demonstrate that the 592C>A polymorphism of the IL-10 gene may have an important role in breast cancer metastasis. The IL-10 592C>A polymorphism showed a significant association with DFS after the diagnosis of breast cancer (unadjusted *P* value = 0.018). This finding remains significant after adjusting for clinicopathological and therapy factors (adjusted *P* value = 0.019). There was no evidence of association with DFS for polymorphisms in the MTHFR, TGFB, FASL, FAS, PTGS2 and SULT1A1 genes. To evaluate the "predictive" value of IL10 592C>A we performed independent cohort analyses of the marker within the treatment groups and found no association with DFS.

As a multifunctional Th2-cytokine, IL-10 is an immunosuppressive cytokine with anti-angiogenic functions and participates in the development and progression of various tumors [18]. The IL-10 gene comprises 5 exons, spans ~5.2 kb, and is located on chromosome 1 at 1q31-1q32. Physiologically, interleukin-10 secreted by antigen-presenting cells promotes the development of immunologic tolerance and suppresses the production of inflammatory cytokines [19]. In the context of breast cancer risk, IL-10 may act as a two-edged sword: On the one hand, elevated IL-10 levels could facilitate development of cancer by supporting tumor escape from the immune response. On the other hand, anti-angiogenic effects of IL-10 are supposed to prevent or reduce tumor growth and metastazing. A [TCATA] haplotype formed by polymorphisms at positions −3,575, −2,763, −1,082, −819 and −592 in the promoter of the IL-10 gene has been associated with increased IL-10 expression [20]. Due to linkage disequilibrium the presence of this haplotype can be fully determined by analysis of the −592C>A polymorphism. The −592A allele indicates the presence of the [TCATA] haplotype, whereas the −592C allele indicates its absence. Although the genetic control of IL-10 expression is not fully understood yet, previous studies indicated that the [TCATA] haplotype is associated with high levels of IL-10 [21, 22]. Recently, homozygosity for the IL-10 [TCATA] haplotype has been associated with a reduced risk of acute graft-versus-host-disease after hematopoietic stem-cell transplantation [22]. Together with the reported biological

functions of interleukin-10 in vitro and in vivo [19, 23], these data suggest that the T-C-A-T-A haplotype is defined by high levels of interleukin-10. Results of IL polymorphism studies with respect to cancer prognosis are conflicting [24, 25]. The association found in our study has not been previously reported but is biologically possible. The mechanism for this remains to be determined, but may likely include tumor escape by IL-10 mediated immunosuppression. The strengths of the present study are: its relatively high number of participants as well as the clinically validated phenotypes. Some limitations of the present study should be taken into account: potential confounding factors, such as age at menarche, number of full-term pregnancies or dietary factors, were not available. Furthermore, due to its retrospective design, a survival bias cannot be excluded. The conflicting data in the literature indicate that larger and prospective studies are needed to clarify the role of IL gene polymorphisms in breast cancer. However, our findings hold promise for further investigations.

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