The *IL-8* and *IL-13* Gene Polymorphisms in Inflammatory Bowel Disease and Colorectal Cancer

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Inflammatory bowel diseases (IBD) and colorectal cancer (CRC) are disorders that originate from immune disturbances. In our study, we evaluated the association between the –251 T/A interleukin (*IL*)-8 and the –1112 C/T *IL*-13 polymorphisms, the risk of IBD, and CRC development. Genotypes were determined by PCR-restriction fragment length polymorphism in 191 patients with CRC, 150 subjects with IBD, and 205 healthy controls. We found an association between CRC and the presence of the –251 TA genotype and A allele of the *IL*-8 gene (odds ratios [ORs] 2.28 and 1.65). A similar relationship was observed between these polymorphic variants and ulcerative colitis (OR 2.05 for the –251 TA genotype and OR 1.47 for the –251 A allele) as well as Crohn's disease (ORs 3.11 and 1.56, respectively). Our research also revealed that the CT and TT genotypes of the *IL*-13 –1112 C/T polymorphism may be connected with a higher risk of CRC (ORs 2.28 and 1.65). The same genotypes affected the susceptibility of IBD (ORs 2.26 and 3.72). Our data showed that the *IL*-8 –251 T/A and *IL*-13 –1112 C/T polymorphisms might be associated with the IBD and CRC occurrence and might be used as predictive factors of these diseases in a Polish population.

Introduction

COLORRECTAL CANCER (CRC) is one of the leading causes of death among people with cancer. Most cases of CRC originate from inflammatory bowel disease (IBD), of which the most common are ulcerative colitis (UC) and Crohn's disease (CD).

According to reports based on the data of the National Cancer Registry (2010), there were 14,441 new cases of CRC (ICD18-20) in Poland in 2008. About 80% of CRC cases have an unclear etiology, and they are classified as sporadic. Approximately 25% of new CRC cases occur in individuals who have a first-degree relative with the disease (Rawl *et al.*, 2008). The colon cancer instances classified as hereditary originate from genetic mutations and instabilities. Tumors may also arise at sites of chronic inflammation. Chronic inflammation probably promotes carcinogenesis by inducing gene mutations, inhibiting apoptosis, or stimulating apoptosis and cell proliferation, but to date, no genetic basis has been identified that explains the CRC predisposition in these IBD (Kraus and Arber, 2009).

The transition of the normal epithelium to inflammation and carcinoma is associated with acquired molecular events. Reactive oxygen species (ROS) and reactive nitrogen species produced by inflammatory cells can interact with the key genes involved in carcinogenic pathways such as *TP53*, DNA mismatch repair genes, and even DNA base excision-repair

genes (Slupphaug *et al.*, 2003; Achanta and Huang, 2004). In the wide group of factors inducing IBDs, there are also immune disturbances causing improper stimulation of lymphocytes, macrophages, or other kinds of immunocompetent cells (Haarmann-Stemmann *et al.*, 2009). It conducts changes in cytokine expression, which may also play a role in carcinogenesis.

In the colon epithelium in both IBD and CRC, an increased level of proinflammatory mediators was observed. One of them—interleukin (IL)-8—is the cytokine that plays a crucial role in chemotaxis and a migration of neutrophils, monocytes, lymphocytes, and fibroblasts (Miller and Krangel, 1992). IL-8 also regulates angiogenesis, cancer cell proliferation, and is involved in the pathogenesis of cancers, such as melanoma (Singh *et al.*, 2010), lung cancer (Zhu *et al.*, 2004), gastric cancer (Liu *et al.*, 2010), pancreatic cancer (Le *et al.*, 2000), liver cancer (Miyamoto *et al.*, 1998), bladder cancer (Inoue *et al.*, 2000), and prostate cancer (Seaton *et al.*, 2008). IL-8 has elevated expression in colon carcinoma compared with normal colon tissue (Doll *et al.*, 2010). Moreover, IL-8 acts as an autocrine growth factor for human colon carcinoma cells (Brew *et al.*, 2000). It is also an angiogenic factor and data supports the existence of a pathway by which IL-8 is involved in the expression of vascular endothelial growth factor (VEGF) in endothelial cells (Martin *et al.*, 2009). The trials conducted on CRC cells have shown an increased expression level of CXCR1 and CXCR2-specific receptors for

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IL-8. The enhanced expression of these receptors may affect the sensitivity of cancer cells on IL-8 (Waugh and Wilson, 2008). IL-8 may also regulate the ROS metabolism and, in that way, influences the colon motor activity and absorption. The decrease in the ROS metabolism level results in cell damage caused by free radicals, exacerbation of inflammatory processes, and immune cells recruitment. What is more, ROS are well-known factors that contribute to carcinogenesis and angiogenesis (Ono, 2008).

IL-13 plays an opposite role to IL-8. In monocytes and macrophages, this anti-inflammatory cytokine inhibits the secretion of pro-inflammatory mediators such as prostaglandins, ROS and nitrogen species, tumor necrosis factor (TNF) alpha, IL-1, -6, -8, and -12 (Minty *et al.*, 1997). IL-13 also stimulates B-lymphocytes and induces the synthesis of immunoglobulin-E (Hajoui *et al.*, 2004). It may also affect the level of activation-induced cytidine deaminase (AID) and induce the colon inflammation as well as transformation into cancer cells (Endo *et al.*, 2008). Moreover, IL-13 influences eosinophils and cause their prolonged survival, activation, and migration to inflammatory lesions (Rothenberg, 2009). Data show that IL-13 receptors are highly expressed in colonic epithelial cells. The increased level of this Th2 cytokine by natural-killer T cells may extend the colitis development by affecting epithelial apoptosis, tight junctions, and restitution velocity (Heller *et al.*, 2005; Fuss and Strober, 2008). A higher level of IL-13 expression is also observed in colon cancer, bladder cancer, prostate cancer, and pancreatic cancer. Trials have shown that polymorphic variants of the *IL-13* gene may increase the risk of breast cancer and mastocytosis occurrence. It may also promote tumor growth and metastasis (Formentini *et al.*, 2009).

It is supposed that in the molecular background of these disturbances (IBD and CRC) may lay the polymorphism or mutations in genes that encode cytokines and other proinflammatory mediators. In our study, we examined an association between the -251T/*Ail-8*(rs4073) and the -1112C/T *IL-13*(also known as -1055 C/T; rs1800925) polymorphisms in a promoter region that may facilitate the gene expression level with IBD and CRC.

Materials and Methods

Patients

Peripheral blood samples were obtained from 150 IBD patients (80 men and 70 women; median age 44, quartiles: 29, 56 years). Among them, 92 subjects had UC (53 men and 40 women; median age 46, quartiles: 32, 57 years), 50 subjects were diagnosed with CD (23 men and 27 women; median age 40, quartiles: 27, 51 years), and 8 patients were unable to be diagnosed unequivocally, so they were classified as the UC/CD group (5 men and 3 women; median age 45, quartiles: 33, 55 years). In the UC/CD group, all features are shown to be highly suggestive of a diagnosis of UC and CD, and they cannot be detected with high reliability. The second group of patients consists of 191 subjects diagnosed with CRC (124 men and 67 women; median age 64, quartiles: 54, 73 years). The control group consists of age- (± 5 years), sex-, and ethnicity-matched 205 cancer-free blood donors.

The diagnosis of cancer was made after direct colonoscopy and a histopathological examination of the patients' biopsies. To assess the malignancy grade of CRC, the American Joint

Committee on Cancer staging classification of histological changes was used (Table 1). Patients with IBDs were selected during direct colonoscopy, histological examination, and C-reactive protein level assay (Table 2). Before the examination, the patients and control subjects did not receive any drugs such as antibiotics or steroids. All patients and control subjects were recruited from the Department of General and Colorectal Surgery, Medical University of Lodz, Poland, and the Department of Gastroenterology and Internal Diseases, Medical University of Lodz, Poland. All subjects involved in the study were unrelated Caucasians and resided in the Lodz district, Poland. The study was approved by the local ethics committee, and written consent from each patient or healthy blood donor was obtained before their participation in the study.

Genotyping

Genomic DNA was prepared using the QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA (Qiagen, Chatsworth, CA). PCR-restriction fragments length polymorphism was employed to determine the genotypes of the *IL-8* -251 T/A and *IL-13* -1112 C/T polymorphisms. Each 20 μ L of the PCR reaction mix was prepared according to the manufacturer's instructions (Qiagen) and also contained 10 ng genomic DNA and 250 nM of each primer. The -251 T/A polymorphism of the *IL-8* gene was determined using the following primers (Sigma-Aldrich, St. Louis, MO): sense, 5'- TTC TAA CAC CTG CCA CTC TAG-3'; antisense, 5'-CTG AAG CTC CAC AAT TTG GTG-3'. The 108 bp PCR product was digested overnight at 37°C with 5 U of the restriction enzyme *Mfe*I. The fragment of the *IL-8* gene containing A variant was digested into 76 and 32 bp fragments, whereas the fragment containing the T allele remained intact. The -1112 C/T polymorphism of the *IL-13* gene was determined using the following primers: sense, 5'- GGA ATC CAG CAT GCC TGG TGA GG-3'; antisense, 5'- GTC GCC TTT TCC TGC TCT TCC CGC-3'. The 246 bp product was digested for 6 h at 60°C with 5 U of the restriction enzyme *Bst*UI. The fragment of the *IL-13* gene containing the C variant was digested into 223 and 23 bp fragments, and the fragment containing C remained intact. More than 10% of the samples were repeated, and the results were 100% concordant.

Statistical analysis

Statistical power was calculated by the two-sample one-tail test using percentage values. To compare the distributions of demographic variables and selected risk factors between patients and controls, chi-square tests were used. The Hardy-Weinberg equilibrium (HWE; $p^2 + 2pq + q^2 = 1$), where p is the frequency of the variant allele ($q = 1 - p$), was tested by a goodness-of-fit chi-square test to compare the observed genotype frequencies with the expected genotype frequencies in cancer-free controls. The potential linkage between genotype and cancer was assessed by the logistic regression. Analyses were performed using STATISTICA 6.0 package (Statsoft, Tulsa, OK).

Results

Distributions of *IL-8* and *IL-13* genotypes in Polish population

The studied population consisted of 150 IBD and 191 CRC patients as well as 205 controls. The characteristics of the

CRC and IBD groups can be found in Tables 1 and 2. The statistical power of our experiment was 100%. Patients and controls were subdivided according to their genotype for the -251 T/A polymorphism of the *IL-8* gene and the -1112 C/T polymorphism of the *IL-13* gene. The genotype frequencies of the investigated polymorphisms are presented in Tables 3–5.

The observed genotype frequencies of *IL-13* -1112 C/T SNP in the control subjects were consistent with HWE ($p=0.546$; $\chi^2=0.36$), but the observed genotype frequencies of the *IL-8* -251 T/A SNP were not in agreement with HWE ($p<0.001$; $\chi^2=11.083$). It is caused by the low abundance of the *IL-8* TA genotype in the examined Polish population. The similar trials conducted on much larger populations living in Central Europe (including Poland) have shown that the genotype frequencies in control groups were also not consistent with HWE ($p=0.391$; $\chi^2=0.73$ and $p=0.206$; $\chi^2=1.62$) (Campa *et al.*, 2005; Savage *et al.*, 2006).

The association of *IL-8* and *IL-13* polymorphisms and cancer risk

The association was found between the TA genotype and A allele of the -251 T/A *IL-8* polymorphism and an increased risk for the development of CRC (odds ratio [OR] 2.28; 95% CI 1.52–3.42 and OR 1.65; 95% CI 1.24–2.19, respectively; Table 3). We also found a strong association between the CT and TT genotypes of the -1112 C/T *IL-13* polymorphism and the risk for CRC (OR 2.42; 95% CI 1.58–3.69 and OR 3.48; 95% CI 1.04–11.64, respectively; Table 3). A similar association was found between CRC occurrence and the T allele of the -1112 C/T *IL-13* polymorphism (OR 2.12; 95% CI 1.5–2.99; Table 3).

In Table 4, the correlation between the investigated gene polymorphisms and the stage of CRC was performed. We did not observe a statistically significant association between the tumor size or the level of spread and the -251 T/A *IL-8* or -1112 C/T *IL-13* polymorphic variants.

The association of *IL-8* and *IL-13* polymorphisms and the risk of IBD

A significant association was found between the genotype frequencies for the heterozygote of the -251 T/A *IL-8* polymorphism and the IBD (OR 2.08; 95% CI 1.3–3.12; Table 5). This association was also observed when the group of IBD patients was subdivided in two groups—UC and CD subjects (OR 2.05; 95% CI 1.17–3.56 and OR 3.11; 95% CI 1.51–

6.4, respectively; Table 5). In addition, a significant difference was shown between the A allele and the UC and CD cases separately (OR 1.47; 95% CI 1.03–2.09 and OR 1.56; 95% CI 1.002–2.43, respectively; Table 5), but not in both these summarized groups (IBD).

The presence of the *IL-13* -1112 CT genotypes in the investigated groups seems to be connected with a higher risk of IBD as well as UC occurrence (OR 2.52; 95% CI 1.51–4.22 and OR 1.98; 95% CI 1.37–2.86, respectively; Table 5). The statistically significant differences in the T-allele distribution were observed in all the investigated groups compared with the controls (ORs 1.72–1.98; Table 5).

Discussion

The purpose of our study was to assess the role of the *IL-8* and *IL-13* polymorphisms as the risk factors for CRC and IBD in a case-control setting. There is very little research considering the association between the *IL-8* -251 T/A polymorphism and CRC. Hull *et al.* (2000) reported that the presence of the -251 A allele affects the expression of the *IL-8* gene and is associated with increased plasma levels of *IL-8*. Further studies, including promoter assays, confirmed the functional role of the *IL-8* -251 T/A polymorphism (Lee *et al.*, 2005; Ohyauchi *et al.*, 2005).

The presence of the *IL-8* -251 T/A polymorphism in the transcription start site has been associated with various diseases; however, results of different studies are often contradictory. In our trial, we have found an association between the TA genotype and A allele and the risk of CRC. Our results are consistent with the outcomes of the study performed in the American population (90% of recruited participants were Caucasian) (Gunter *et al.*, 2006). Gunter *et al.* (2006) trial showed that carriers of the *IL-8* -251 A allele were at a 1.5-fold increased risk of colon adenoma (OR 1.5; 95% CI 1.0–2.4; $p_{\text{trend}}=0.002$). This research also indicates that the AA homozygote may increase the risk of colon adenoma occurrence (OR 2.7; 95% CI 1.5–4.9). This has been confirmed by Lurje *et al.* (2008), who revealed that that stage III CRC American patients carrying the AA genotype were at a higher risk of developing tumor recurrence (OR 2.14; 95% CI 1.01–4.53; $p=0.048$). We suggest this disagreement is likely due to a different genotype distribution in the American population.

Wilkening *et al.* (2008) investigated the association between the CRC tumor stage (according to Dukes staging system) and the *IL-8* -251 T/A polymorphism in a Swedish

TABLE 1. THE CHARACTERISTICS OF PATIENT GROUPS WITH COLORECTAL CANCER

No. of patients	Gender		Cancer staging according to TNM classification									Cancer staging according to AJCC classification		
			T				N			M				
	Female	Male	1	2	3	4	0	1	2	0	1	I° (T1-2N0)	II° (T3-4N0)	III° (T1-4N1-2)
191	67	124	4	45	133	9	101	59	31	191	0	36	65	90

T (1-4) the size of the tumor.

N (0-2) spread of cancer to regional lymph nodes.

M (0-1) presence of distant metastasis.

TABLE 2. THE CHARACTERISTICS OF PATIENT GROUPS WITH INFLAMMATORY BOWEL DISEASE

No. of patients	Gender		Type of IBD		
	Female	Male	UC	CD	UC/CD
150	70	80	92	50	8

CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

population. The results obtained by Wilkening *et al.* were in agreement with our outcomes. The connection between *IL-8* polymorphic variants and C+D stage in comparison with A+B stage occurred to be statistically insignificant. Contrary to our results, they also did not find a relation between overall CRC risk and *IL-8* polymorphic variants.

Landi *et al.* (2003) found no statistically significant association between the *IL-8* -251 TA and AA genotypes in the Spanish population considered, as the CRC cancer was divided into the colon cancer and rectal cancer groups. What is more, they observed a reduced risk of tumor development in the CRC group (OR 0.7; 95% CI 0.5–0.99; $p=0.43$) as well as in the colon cancer group (OR 0.65; 95% CI 0.44–0.95; $p=0.025$). The discrepancy between our results may have two causes: population differences (Spanish vs. Polish) and Landi *et al.*'s misgiving that controls recruited by them from a hospital were not ideal, even though they tried to minimize the potential bias. No correlation was also found in Greek patients. Theodoropoulos *et al.* (2006) and Cacev *et al.* (2008) did not find any association between the *IL-8* SNP and sporadic CRC in that population.

The *IL-8* -251 T/A polymorphism is also taken into consideration as a factor deciding the clinical outcomes in patients treated with a variety of drugs. The survival of metastatic CRC patients who were given CPT-11 (according to Stoecklmacher *et al.* research, 2002) is prolonged when subjects were the *IL-8* TT genotype carriers with the concomitant presence of the Ile105Ile homozygote of the GSTP1 gene ($p=0.005$). Tsilidis *et al.* (2009) performed a research concerning an association between CRC and polymorphisms in the genes related to inflammatory response and obesity. Among the nonsteroidal anti-inflammatory drugs (NSAIDs)

users, the *IL-8* -251 SNP was positively associated with CRC (AT/AA vs. TT, OR 2.25; 95% CI 0.98–5.16; $p_{\text{interaction}}=0.03$). No such association was observed in NSAID nonusers.

In our work, we were also concerned with a role of the *IL-8* -251 T/A in IBDs susceptibility. To our knowledge, there are no literature data on the connection of the *IL-8* polymorphic variants in IBD as well as CD. We have found only one publication that investigates an association of this polymorphism with a higher risk of UC occurrence. The trials performed on a Chinese population by Li *et al.* (2009) did not reveal a connection between the *IL-8* -251 T/A polymorphism individual and UC. They also investigated a role of other polymorphisms of the *IL-8* gene and its impact on the level of IL-8 in serum. The frequency of the -353A/-251A/+678T haplotype was significantly higher in UC patients than in healthy controls (OR=1.454, $p=0.036$). By subgroup analyses, this haplotype tended to be more common in severe UC patients than in those with mild-to-moderate disease (OR=2.281, $p=0.027$). Furthermore, patients with AAT diplotype showed significantly increased serum IL-8 concentrations than those with other diplotypes ($p<0.001$).

Entertaining the role of the *IL-8* -251 T/A SNP in CRC and IBDs, we observed a lot of conflicting results. We suggest these disagreements are likely due to different allele distributions in different investigated populations. Our analysis indicates that the frequency of the *IL-8* -251 T/A polymorphic variants in healthy subjects in the Polish population varies from other nations (it is not with agreement with HWE). Finally, these data show a possible ethnic variability in the allelic distribution of the -251 T/A polymorphism, which strongly suggests that more examinations of the *IL-8* genotypes should be performed on a well-characterized and clinically specified population.

In our research, we also examined the association between the *IL-13* -1112 C/T polymorphism and colon diseases. Data showed that the CT genotype of the investigated *IL-13* polymorphism increased the risk of IBD and colon cancer, either individually or grouped in one studied population (Tables 5, 3 and 4, respectively). To the best of our knowledge, to date, there are a lack of similar literature data on the connection of the *IL-13* -1112 C/T polymorphism and CRC or IBD occurrence. The only *IL-13* gene polymorphism investigated in association with IBD was the *IL-13* +2044 G/A

TABLE 3. THE GENOTYPE FREQUENCIES, ALLELE FREQUENCIES, AND ODDS RATIOS OF THE *IL-8* -251 T/A AND *IL-13* -1112 C/T POLYMORPHISMS IN COLORECTAL CANCER PATIENTS AND HEALTHY CONTROLS

Polymorphism	Genotype	No. of Patients (n=191)	Patient frequency	Control no. (n=205)	Control frequency	OR (95% CI)	p
<i>IL-8</i> -251 T/A	TT	50	0.26	99	0.48	Ref.	
	TA	104	0.55	71	0.35	2.90 (1.84–4.57)	<0.0001
	AA	37	0.19	35	0.17	2.09 (1.18–3.72)	0.011
	Allele T	204	0.54	269	0.66	Ref.	
	Allele A	178	0.46	141	0.34	1.65 (1.24–2.19)	0.0006
<i>IL-13</i> -1112 C/T	CC	93	0.49	144	0.7	Ref.	
	CT	89	0.47	57	0.28	2.42 (1.58–3.69)	<0.0001
	TT	9	0.05	4	0.02	3.48 (1.04–11.64)	0.03
	Allele C	275	0.72	354	0.84	Ref.	
	Allele T	107	0.28	65	0.16	2.12 (1.5–2.99)	<0.0001

IL, interleukin; OR, odds ratio.

TABLE 4. THE GENOTYPES FREQUENCIES, ALLELE FREQUENCIES AND ODDS RATIOS OF THE *IL-8* - 251 T/A AND *IL-13* - 1112 C/T POLYMORPHISMS IN COLORECTAL CANCER PATIENTS WITH DIFFERENT CANCER STAGES ACCORDING TO AMERICAN JOINT COMMITTEE ON CANCER CLASSIFICATION

Polymorphism	Genotype	<i>I</i> ^o (<i>T1-2N0</i>)			<i>II</i> ^o (<i>T3-4N0</i>)			<i>III</i> ^o (<i>T1-4N1-2</i>)			<i>II</i> ^o vs. <i>I</i> ^o			<i>III</i> ^o vs. <i>II</i> ^o		
		No.	Frequency	No.	Frequency	No.	Frequency	No.	Frequency	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	p
<i>IL-8</i> - 251 T/A	TT	9	0.25	21	0.32	19	0.21	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	0.07
	TA	19	0.53	32	0.49	58	0.64	0.72 (0.27-1.9)	0.5	1.45 (0.56-3.73)	0.44	2 (0.94-4.27)	0.07	2 (0.94-4.27)	0.07	0.73
	AA	8	0.22	12	0.18	13	0.14	0.64 (0.2-2.11)	0.47	0.77 (0.24-2.52)	0.66	1.2 (0.44-3.26)	0.73	1.2 (0.44-3.26)	0.73	0.73
	Allele A	37	0.51	74	0.57	96	0.53	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	0.53
	Allele T	35	0.49	56	0.43	84	0.47	0.8 (0.45-1.43)	0.45	0.93 (0.54-1.6)	0.78	1.16 (0.73-1.82)	0.53	1.16 (0.73-1.82)	0.53	0.53
<i>IL-13</i> -1112 C/T	CC	20	0.56	27	0.42	46	0.51	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	0.2
	CT	13	0.36	36	0.55	40	0.44	2.05 (0.87-4.84)	0.09	1.34 (0.59-3.03)	0.48	0.65 (0.34-1.26)	0.2	0.65 (0.34-1.26)	0.2	0.61
	TT	3	0.08	2	0.03	4	0.04	0.49 (0.08-3.24)	0.39	0.58 (0.12-2.83)	0.39	1.17 (0.2-6.84)	0.61	1.17 (0.2-6.84)	0.61	0.61
	Allele C	53	0.74	90	0.69	132	0.73	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	0.43
	Allele T	19	0.26	40	0.31	48	0.27	1.24 (0.65-2.36)	0.51	1.01 (0.55-1.88)	1	0.82 (0.5-1.35)	0.43	0.82 (0.5-1.35)	0.43	0.43

TABLE 5. THE GENOTYPE FREQUENCIES, ALLELE FREQUENCIES, AND ODDS RATIOS OF THE *IL-8* - 251 T/A AND *IL-13* - 1112 C/T POLYMORPHISMS IN INFLAMMATORY BOWEL DISEASE, ULCERATIVE COLITIS, CROHN'S DISEASE, AND HEALTHY CONTROLS

Polymorphism		IBD no. (n=150)			IBD frequency (n=92)			UC no. (n=92)			UC frequency (n=50)			CD no. (n=50)			CD frequency (n=8)			UC/CD no. (n=8)			UC/CD frequency (n=205)			Control no. (n=205)			Control frequency (n=205)			IBD OR (95% CI)			UC OR (95% CI)			CD OR (95% CI)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
		Genotype	IBD no.	IBD frequency	UC no.	UC frequency	CD no.	CD frequency	UC no.	UC frequency	CD no.	CD frequency	UC/CD no.	UC/CD frequency	Control no.	Control frequency	OR	p	OR	p	OR	p	OR	p	OR	p	OR	p	OR	p	OR	p	OR	p																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
IL-8 -251 T/A	TT	51	0.34	30	0.33	13	0.26	5	0.63	99	0.48	Ref.	0.002	Ref.	0.002	0.002	0.011	Ref.	0.011	Ref.	0.002	0.011	Ref.	0.002	0.011	Ref.	0.002	0.011	Ref.	0.002	0.011	Ref.	0.002	0.011	Ref.	0.002	0.011																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
	TA	76	0.51	44	0.48	29	0.58	3	0.38	71	0.35	2.08 (1.3-3.12)		0.35	0.35			2.05 (1.17-3.56)		3.11 (1.51-6.4)						3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)			3.11 (1.51-6.4)		

(Dotan, 2006). It appeared to have no significant role in the susceptibility to IBD.

Trials concerning the role of the *IL-13* –1112 C/T polymorphism in cancer development were performed in an Iranian population, but neither in lung cancer nor breast cancer, there was no association observed (Faghih *et al.*, 2009; Sameni *et al.*, 2009). The similar trials were performed on a mixed population in patients with glioma, but there was also no connection indicated between the *IL-13* –1112 C/T polymorphic variants and cancer occurrence (Schwartzbaum *et al.*, 2007).

The obtained results confirm the outcomes of other trials that investigate the function and role of IL-13 in colon cancer cells. Research also shows the elevated level of both IL-13 and its receptors (IL-13R α 1 and IL-13R α 2) in colon mucosa and colon cancer cells (Mandal and Levine, 2010). IL-13 also appeared to be an activator of the STAT6 pathway (Mandal and Levine, 2010). Moreover, IL-13 also activates other intracellular signal transducers in cancer cells, for example, JAK2 kinase (Murata *et al.*, 1996). IL-13 increases the expression level of activation-induced cytidine deaminase AID, which causes hypersomatic mutations and may conduct CRC development (Endo *et al.*, 2008). IL-13 also regulates the expression level of adhesive molecules, for example, the CD44 protein influencing cell-cell adhesions in tumors (Trejdosiewicz *et al.*, 1998; Kanai *et al.*, 2000). It suggests that IL-13 may participate in cancer metastasis.

Our results show an association between the *IL-13* –1112 C/T polymorphism and CRC prevalence. The lack of correlation between this polymorphism and other types of cancer suggest that *IL-13* –1112 C/T polymorphic variants may be considered the specific prognostic markers of CRC, but further trials are needed to confirm this statement.

Conclusion

We observed an association between the TA genotype and A allele of the *IL-8* T/A polymorphism as well as all polymorphic variants of the *IL-13* –1112 C/T SNP and CRC, but we did not find any such correlation with tumor stage. We postulate that the extension of the tumor or the tumor spread is not associated with the genetic polymorphism of the investigated cytokines and may be caused by other factors. Further research is needed to confirm this hypothesis. Our work also revealed that the same polymorphisms of the *IL-8* and *IL-13* genes affect the susceptibility of IBD as well as CRC. It suggests that these variants may have an impact on carcinogenesis and the transformation of epithelial as well as subepithelial cells of the inflamed colon into cancer cells, but this point should be confirmed by the correlation of our outcomes with histopathological trials.

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Disclosure Statement

The authors declare no conflict of interest exists.

References

- Achanta, G., and Huang, P. (2004). Role of p53 in sensing oxidative DNA damage in response to reactive oxygen species-generating agents. *Cancer Res* **64**, 6233–6239.
- Brew, R., Erikson, J.S., West, D.C., Kinsella, A.R., Slavin, J., and Christmas, S.E. (2000). Interleukin-8 as an autocrine growth factor for human colon carcinoma cells *in vitro*. *Cytokine* **12**, 78–85.
- Cacev, T., Radosevic, S., Krizanac, S., and Kapitanovic, S. (2008). Influence of interleukin-8 and interleukin-10 on sporadic colon cancer development and progression. *Carcinogenesis* **29**, 1572–1580.
- Campa, D., Hung, R.J., Mates, D., Zaridze, D., Szeszenia-Dabrowska, N., Rudnai, P., Lissowska, J., Fabianova, E., Bencko, V., Foretova, L., Janout, V., Boffetta, P., Brennan, P., and Canzian, F. (2005). Lack of association between –251 T>A polymorphism of IL8 and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* **14**, 2457–2458.
- Doll, D., Keller, L., Maak, M., Boulesteix A-L, Siewert, J.R., Holzmann, B., and Janssen, K.P. (2010). Differential expression of the chemokines GRO-2, GRO-3 and interleukin-8 in colon cancer and their impact on metastasis disease and survival. *Int J Colorectal Dis* **25**, 573–581.
- Dotan, I. (2006). [Inflammatory bowel diseases: in search of novel cytokines and genes]. *Harefuah* **145**, 817–819, 861.
- Endo, Y., Marusawa, H., Kou, T., Nakase, H., Fujii, S., Fujimori, T., Kinoshita, K., Honjo, T., and Chiba, T. (2008). Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. *Gastroenterology* **135**, 889–898, 898.
- Faghih, Z., Erfani, N., Razmkhah, M., Sameni, S., Talei, A., and Ghaderi, A. (2009). Interleukin13 haplotypes and susceptibility of Iranian women to breast cancer. *Mol Biol Rep* **36**, 1923–1928.
- Formentini, A., Prokopchuk, O., Strater, J., Kleeff, J., Grochola, L.F., Leder, G., Henne-Bruns, D., Korc, M., and Kornmann, M. (2009). Interleukin-13 exerts autocrine growth-promoting effects on human pancreatic cancer, and its expression correlates with a propensity for lymph node metastases. *Int J Colorectal Dis* **24**, 57–67.
- Fuss, I.J., and Strober, W. (2008). The role of IL-13 and NK T cells in experimental and human ulcerative colitis. *Mucosal Immunol* **1**(Suppl. 1), S31–S33.
- Gunter, M.J., Canzian, F., Landi, S., Chanock, S.J., Sinha, R., and Rothman, N. (2006). Inflammation-related gene polymorphisms and colorectal adenoma. *Cancer Epidemiol Biomarkers Prev* **15**, 1126–1131.
- Haarmann-Stemmann, T., Bothe, H., and Abel, J. (2009). Growth factors, cytokines and their receptors as downstream targets of arylhydrocarbon receptor (AhR) signaling pathways. *Biochem Pharmacol* **77**, 508–520.
- Hajoui, O., Janani, R., Tulic, M., Joubert, P., Ronis, T., Hamid, Q., Zheng, H., and Mazer, B.D. (2004). Synthesis of IL-13 by human B lymphocytes: regulation and role in IgE production. *J Allergy Clin Immunol* **114**, 657–663.
- Heller, F., Florian, P., Bojarski, C., Richter, J., Christ, M., Hillenbrand, B., Mankertz, J., Gitter, A.H., Burgel, N., Fromm, M., Zeitz, M., Fuss, I., Strober, W., and Schulzke, J.D. (2005). Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology* **129**, 550–564.
- Hull, J., Thomson, A., and Kwiatkowski, D. (2000). Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax* **55**, 1023–1027.

- Inoue, K., Slaton, J.W., Kim, S.J., Perrotte, P., Eve, B.Y., Bar-Eli, M., Radinsky, R., and Dinney, C.P. (2000). Interleukin 8 expression regulates tumorigenicity and metastasis in human bladder cancer. *Cancer Res* **60**, 2290–2299.
- Kanai, T., Watanabe, M., Hayashi, A., Nakazawa, A., Yajima, T., Okazawa, A., Yamazaki, M., Ishii, H., and Hibi, T. (2000). Regulatory effect of interleukin-4 and interleukin-13 on colon cancer cell adhesion. *Br J Cancer* **82**, 1717–1723.
- Kraus, S., and Arber, N. (2009). Inflammation and colorectal cancer. *Curr Opin Pharmacol* **9**, 405–410.
- Landi, S., Moreno, V., Gioia-Patricola, L., Guino, E., Navarro, M., de OJ, Capella, G., and Canzian, F. (2003). Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. *Cancer Res* **63**, 3560–3566.
- Le, X., Shi, Q., Wang, B., Xiong, Q., Qian, C., Peng, Z., Li, X.C., Tang, H., Abbruzzese, J.L., and Xie, K. (2000). Molecular regulation of constitutive expression of interleukin-8 in human pancreatic adenocarcinoma. *J Interferon Cytokine Res* **20**, 935–946.
- Lee, W.P., Tai, D.I., Lan, K.H., Li, A.F., Hsu, H.C., Lin, E.J., Lin, Y.P., Sheu, M.L., Li, C.P., Chang, F.Y., Chao, Y., Yen, S.H., and Lee, S.D. (2005). The -251T allele of the interleukin-8 promoter is associated with increased risk of gastric carcinoma featuring diffuse-type histopathology in Chinese population. *Clin Cancer Res* **11**, 6431–6441.
- Li, K., Yao, S., Liu, S., Wang, B., and Mao, D. (2009). Genetic polymorphisms of interleukin 8 and risk of ulcerative colitis in the Chinese population. *Clin Chim Acta* **405**, 30–34.
- Liu, L., Zhuang, W., Wang, C., Chen, Z., Wu, X.T., and Zhou, Y. (2010). Interleukin-8 -251 A/T gene polymorphism and gastric cancer susceptibility: a meta-analysis of epidemiological studies. *Cytokine* **50**, 328–334.
- Lurje, G., Zhang, W., Schultheis, A.M., Yang, D., Groshen, S., Hendifar, A.E., Husain, H., Gordon, M.A., Nagashima, F., Chang, H.M., and Lenz, H.J. (2008). Polymorphisms in VEGF and IL-8 predict tumor recurrence in stage III colon cancer. *Ann Oncol* **19**, 1734–1741.
- Mandal, D., and Levine, A.D. (2010). Elevated IL-13Ralpha2 in intestinal epithelial cells from ulcerative colitis or colorectal cancer initiates MAPK pathway. *Inflamm Bowel Dis* **16**, 753–764.
- Martin, D., Galisteo, R., and Gutkind, J.S. (2009). CXCL8/IL8 stimulates vascular endothelial growth factor (VEGF) expression and the autocrine activation of VEGFR2 in endothelial cells by activating NFkB through the CBM (Carma3/Bcl10/Malt1) complex. *J Biol Chem* **284**, 6038–6042.
- Miller, M.D., and Krangel, M.S. (1992). Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines. *Crit Rev Immunol* **12**, 17–46.
- Minty, A., Ferrara, P., and Caput, D. (1997). Interleukin-13 effects on activated monocytes lead to novel cytokine secretion profiles intermediate between those induced by interleukin-10 and by interferon-gamma. *Eur Cytokine Netw* **8**, 189–201.
- Miyamoto, M., Shimizu, Y., Okada, K., Kashii, Y., Higuchi, K., and Watanabe, A. (1998). Effect of interleukin-8 on production of tumor-associated substances and autocrine growth of human liver and pancreatic cancer cells. *Cancer Immunol Immunother* **47**, 47–57.
- Murata, T., Noguchi, P.D., and Puri, R.K. (1996). IL-13 induces phosphorylation and activation of JAK2 Janus kinase in human colon carcinoma cell lines: similarities between IL-4 and IL-13 signaling. *J Immunol* **156**, 2972–2978.
- Ohyauchi, M., Imatani, A., Yonechi, M., Asano, N., Miura, A., Iijima, K., Koike, T., Sekine, H., Ohara, S., and Shimosegawa, T. (2005). The polymorphism interleukin 8 -251 A/T influences the susceptibility of *Helicobacter pylori* related gastric diseases in the Japanese population. *Gut* **54**, 330–335.
- Ono, M. (2008). Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. *Cancer Sci* **99**, 1501–1506.
- Rawl, S.M., Champion, V.L., Scott, L.L., Zhou, H., Monahan, P., Ding, Y., Loehrer, P., and Skinner, C.S. (2008). A randomized trial of two print interventions to increase colon cancer screening among first-degree relatives. *Patient Educ Couns* **71**, 215–227.
- Reports Based on Data of National Cancer Registry. (2010). Available at <http://85.128.14.124/krn/english/index.asp> (last accessed February 24, 2012).
- Rothenberg, M.E. (2009). Biology and treatment of eosinophilic esophagitis. *Gastroenterology* **137**, 1238–1249.
- Sameni, S., Ghayumi, M.A., Mortazavi, G., Faghih, Z., Kashef, M.A., and Ghaderi, A. (2009). Lack of association between interleukin-13 gene polymorphisms (-1055 C/T and +2044 G/A) in Iranian patients with lung cancer. *Mol Biol Rep* **36**, 1001–1005.
- Savage, S.A., Hou, L., Lissowska, J., Chow, W.H., Zatonski, W., Chanock, S.J., and Yeager, M. (2006). Interleukin-8 polymorphisms are not associated with gastric cancer risk in a Polish population. *Cancer Epidemiol Biomarkers Prev* **15**, 589–591.
- Schwartzbaum, J.A., Ahlbom, A., Lonn, S., Malmer, B., Wigertz, A., Auvinen, A., Brookes, A.J., Collatz, C.H., Henriksson, R., Johansen, C., Salminen, T., Schoemaker, M.J., Swerdlow, A.J., Debinski, W., and Feychting, M. (2007). An international case-control study of interleukin-4Ralpha, interleukin-13, and cyclooxygenase-2 polymorphisms and glioblastoma risk. *Cancer Epidemiol Biomarkers Prev* **16**, 2448–2454.
- Seaton, A., Scullin, P., Maxwell, P.J., Wilson, C., Pettigrew, J., Gallagher, R., O'Sullivan, J.M., Johnston, P.G., and Waugh, D.J. (2008). Interleukin-8 signaling promotes androgen-independent proliferation of prostate cancer cells via induction of androgen receptor expression and activation. *Carcinogenesis* **29**, 1148–1156.
- Singh, S., Singh, A.P., Sharma, B., Owen, L.B., and Singh, R.K. (2010). CXCL8 and its cognate receptors in melanoma progression and metastasis. *Future Oncol* **6**, 111–116.
- Slupphaug, G., Kavli, B., and Krokan, H.E. (2003). The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat Res* **531**, 231–251.
- Stoehlmacher, J., Park, D.J., Zhang, W., Groshen, S., Tsao-Wei, D.D., Yu, M.C., and Lenz, H.J. (2002). Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. *J Natl Cancer Inst* **94**, 936–942.
- Theodoropoulos, G., Papaconstantinou, I., Felekouras, E., Niki-teas, N., Karakitsos, P., Panoussopoulos, D., Lazaris, A.C., Patsouris, E., Bramis, J., and Gazouli, M. (2006). Relation between common polymorphisms in genes related to inflammatory response and colorectal cancer. *World J Gastroenterol* **12**, 5037–5043.

- Trejdosiewicz, L.K., Morton, R., Yang, Y., Banks, R.E., Selby, P.J., and Southgate, J. (1998). Interleukins 4 and 13 upregulate expression of cd44 in human colonic epithelial cell lines. *Cytokine* **10**, 756–765.
- Tsilidis, K.K., Helzlsouer, K.J., Smith, M.W., Grinberg, V., Hoffman-Bolton, J., Clipp, S.L., Visvanathan, K., and Platz, E.A. (2009). Association of common polymorphisms in IL10, and in other genes related to inflammatory response and obesity with colorectal cancer. *Cancer Causes Control* **20**, 1739–1751.
- Waugh, D.J., and Wilson, C. (2008). The interleukin-8 pathway in cancer. *Clin Cancer Res* **14**, 6735–6741.
- Wilkening, S., Tavelin, B., Canzian, F., Enquist, K., Palmqvist, R., Altieri, A., Hallmans, G., Hemminki, K., Lenner, P., and Forsti, A. (2008). Interleukin promoter polymorphisms and prognosis in colorectal cancer. *Carcinogenesis* **29**, 1202–1206.
- Zhu, Y.M., Webster, S.J., Flower, D., and Woll, P.J. (2004). Interleukin-8/CXCL8 is a growth factor for human lung cancer cells. *Br J Cancer* **91**, 1970–1976.

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