Carcinogenesis vol.28 no.9 pp.2008–2012, 2007
doi:10.1093/carcin/bgm172
Advance Access publication August 11, 2007

Single-nucleotide polymorphisms at the TP53-binding or responsive promoter regions of *BAX* and *BCL2* genes and risk of squamous cell carcinoma of the head and neck

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Tumor protein 53 (TP53), a transcriptional factor, induces expression of the B-cell lymphoma 2-associated X protein (*BAX*) gene by directly binding to the TP53-binding element in the *BAX* promoter but inhibits B-cell lymphoma 2 (*BCL2*) promoter-driven transcription through a responsive region in the *BCL2* promoter. Therefore, we hypothesized that single-nucleotide polymorphisms (SNPs) of *BAX* and *BCL2* promoters and the *TP53* codon 72 SNP may jointly contribute to cancer risk. We tested this hypothesis in a hospital-based case–control study of 814 patients with squamous cell carcinoma of the head and neck (SCCHN) and 934 cancer-free controls in a US non-Hispanic white population. While there was no evidence of associations between *BAX* (−248 G>A), *BCL2* (−938 C>A) or *TP53* codon 72 SNPs and SCCHN risk in single-locus analyses, further analyses showed that, among *TP53* heterozygotes after adjustment for age, sex and smoking and alcohol status, the *BAX* AA genotype was associated with an elevated risk of SCCHN [odds ratio (OR) = 6.60, 95% confidence interval (CI) = 1.38–31.50 compared with the *BAX* GG genotype or OR = 6.58, 95% CI = 1.38–31.49 compared with the combined genotypes (GG + AG)], whereas *BCL2* A variant genotypes were associated with a decreased risk of SCCHN (adjusted OR = 0.68, 95% CI = 0.47–0.98 for CA vs CC and OR = 0.67, 95% CI = 0.48–0.95 for AA vs CA+CC). These altered risks appeared to be consistent with the roles of the anti-apoptotic *BCL2* and the pro-apoptotic *BAX*. Our data suggest that the risk of SCCHN may be associated with these two SNPs of *BAX* and *BCL2* promoter regions, particularly among *TP53* heterozygotes. Larger studies are needed to validate these findings.

Introduction

Head and neck cancer is the eighth leading cause of cancer death worldwide (1). In 2007, ∼40 566 new cases of squamous cell carcinoma of the head and neck (SCCHN) were diagnosed, and the poor survival of patients with SCCHN has not been improved for several decades (2). Whereas tobacco and alcohol are the primary risk factors for SCCHN development (3–5), epidemiological studies suggest an association of SCCHN with genetic susceptibility in the general population (6–8), and recent studies suggest that genetic polymorphisms in cell-cycle control and apoptosis genes may attribute partly to this predisposition (9,10).

Apoptosis is a process of programmed cell death under both normal physiological and pathological conditions. Deficiency in cell apoptosis alters cell homeostasis and leads to carcinogenesis and tumor progression (11). Apoptosis is regulated by different pathways involving a number of genes that either promote or inhibit apoptosis. The best-characterized apoptosis regulators include the anti-apoptotic B-cell lymphoma 2 (*BCL2*) gene and the pro-apoptotic B-cell lymphoma 2-associated X protein (*BAX*) gene. The protein products of these two genes physically interact with each other and the relative levels of the two proteins are important determinants of the apoptosis rate (12–14). Therefore, the relative expression of these two genes plays a key role in cellular homeostasis and cancer development. Another master regulator of cellular apoptosis is the tumor suppressor tumor protein 53 (*TP53*) gene. Research from the last decade has shown that TP53, which is a transcriptional factor, functions to regulate expression of many apoptosis and cell-cycle regulatory genes. The TP53 protein directly binds to the TP53-binding element in the promoter of *BAX* gene and induces *BAX* expression (15,16) and TP53 inhibits *BCL2* promoter-driven transcription (17). Recently, it was shown that it interacts with Brn-3a (18) that binds to the *BCL2* promoter and blocks the Brn-3a-mediated *BCL2* gene expression. Therefore, the interaction between TP53 and the promoters of this pair of apoptosis-regulatory genes is probably important for cellular apoptosis.

However, what factors affect this interaction are less clear except that TP53 mutations that abolish the DNA-binding ability may render *TP53* incapable of regulating apoptosis. *TP53* mutations have been observed in >50% of all human cancers (19) and there is a high frequency of *TP53* mutations in many cancer types including SCCHN (19,20). It has been recognized that during tumor development, one copy of chromosome 17p (the second allele), where TP53 resides, is frequently lost [loss of heterozygosity (LOH)], and the other allele is mutated. Thus, in tumor cells, where two copies of the *TP53* gene are present, the gene is probably a wild-type, which provides the opportunity to study the role of variants of the *TP53* gene in interaction with other genes involved in the carcinogenesis.

The *BCL2* gene is located on chromosome 18q21.3. There is only one reported single-nucleotide polymorphism (SNP) (a C-to-A change) in the promoter region, which has been validated by the published allele frequency and genotype data, that locates at nucleotide position −938 (*BCL2* −838 C>A, rs2279115) in the promoter region; only one study with a relatively small sample size investigated the role of this SNP in the etiology of prostate cancer and reported a decrease in prostate cancer risk (21). The *BAX* gene has been mapped to chromosome 19q13.3. Recently, a SNP located within the 5′-untranslated region of the *BAX* promoter, G −248A (rs4645878), was reported to be associated with both reduced expression of *BAX* and altered susceptibility to chronic lymphocytic leukemia (22–27). However, there is no report on the association between the risk of SCCHN and this SNP of the *BAX* gene. These two promoter SNPs in the *BAX* and *BCL2* genes are particularly interesting because they are located within 100 bases from the TP53-binding element in the *BAX* promoter region and TP53 responsive element in the *BCL2* promoter region, respectively. Thus, these SNPs may affect the interaction between the TP53 protein and the TP53-regulated sequences in the promoters.

Another potentially important factor is a well-recognized non-synonymous SNP in the coding region of *TP53* that produces two isoforms of TP53 variants that differ at codon 72, TP53Asp72 or TP53Pro72 (rs1042522). This SNP has been shown to influence the risk of a number of cancer types including cervical cancers (28). However, our previous work has showed that this Arg72Pro polymorphism alone was not associated with an increased risk of SCCHN (29).

In this study, we tested two hypotheses: first, that the SNPs of *BAX* and *BCL2* promoters are associated with risk of SCCHN, and second, that the codon 72 SNP of *TP53* may modulate the risk of SCCHN associated with the SNPs of *BAX* and *BCL2* promoters. We tested our hypotheses by genotyping two polymorphisms of *BAX* (−248 G>A)

Abbreviations: BAX, B-cell lymphoma 2-associated X protein; BCL2, B-cell lymphoma 2; CI, confidence interval; LOH, loss of heterozygosity; OR, odds ratio; PCR, polymerase chain reaction; SCCHN, squamous cell carcinoma of the head and neck; SNP, single-nucleotide polymorphism; TP53, tumor protein 53.

and *BCL2* (−838 C>A) promoter regions and one functional polymorphism of *TP53* (Arg72Pro) and evaluating their associations with the risk of SCCHN in a case–control study of 814 patients with SCCHN and 934 cancer-free controls in a US non-Hispanic white population.

Materials and methods

Study subjects

This study included 814 patients with histologically confirmed incident SCCHN between October 1999 and February 2006, including cancers of the oral cavity, oropharynx, hypopharynx and larynx, identified at the University of Texas M. D. Anderson Cancer Center. The detailed methods of the case–control study have been described elsewhere (30). The patients with second SCCHN primaries, primaries of the nasopharynx or sinonasal tract, primaries outside the upper aerodigestive tract, cervical metastases of unknown origin or histopathologic diagnoses other than squamous cell carcinoma were excluded. All cases were non-Hispanic whites and had not received any treatment at the time of recruitment. The response rate of the eligible cases whom we approached for recruitment was ∼95%. The 934 cancer-free subjects we recruited in the same time period were genetically unrelated visitors or companions of patients seen at the University of Texas M. D Anderson Cancer Center clinics and were frequency matched to the cases by age (±5 years), sex and ethnicity. The response rate of the eligible controls whom we approached for recruitment was ∼90%. After being asked to sign an informed consent form, all subjects enrolled in the study were interviewed to gather demographic data and history of smoking and alcohol use. Each eligible subject donated 30 ml of blood collected in heparinized tubes to be used for biomarker assays including DNA extraction and genotyping. The research protocol was approved by the University of Texas M. D Anderson Cancer Center Institutional Review Board.

Genotyping

From each blood sample, a leukocyte cell pellet obtained from the buffy coat by centrifugation of 1 ml of whole blood was used for DNA extraction. Genomic DNA was isolated with the QIAGEN DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Restriction fragment length polymorphism–polymerase chain reaction (PCR) was used to identify *BAX* (−248 G>A) and *BCL2* (−838 C>A) polymorphisms in the promoter regions. Each PCR was performed in a 25 μl reaction mixture containing ∼50 ng of genomic DNA templates, 12.5 pmol of each primer, 0.1 mM of each deoxynucleoside triphosphate, 1× PCR buffer (50 mM KCl, 10 mM Tris–HCl and 0.1% Triton X-100), 1.5 mM $MgCl_2$ and 1.5 U *Taq* polymerase (Promega Corporation, Madison, WI). The PCR profile consisted of an initial melting step of 96°C for 5 min, 35 cycles of 96°C for 45 s, 56°C for 40 s and 72°C for 30 s and a final extension step of 72°C for 10 min.

For the *BAX* (−248 G>A) polymorphism in the promoter, the primers (5′-CATTAGAGCTGCGATTGGAC*C*G-3′, in which the -2 C was introduced to create a *MspI* restriction site, and $\overline{5}$′-GCTCCCTCGGGAGGTTTGGT-3′) amplified a 109 bp DNA fragment. Then, the PCR product was digested by *Msp*I (New England BioLabs, Beverly, MA) overnight at 37°C. The digested product was separated on a 2.5% NuSieve 3:1 agarose (FMC BioProducts, Rockland, ME) gel with ethidium bromide and photographed with Polaroid film. The wild-type allele (GG) produced two bands (89 and 20 bp); wild-type/variant allele (GA) produced 20, 89 and 109 bp and the variant allele (AA) lacks the *Msp*I restriction site and therefore produces a single 109 bp band (Table I).

For the *BCL2* (−838 C>A) polymorphism in the promoter, the primers (5′-CTGCCTTCATTTATCCAGCA-3′ and 5′-GGCGGCAGATGAATTACAA-3′) amplified a 300 bp DNA fragment. Then, the PCR product was digested by *Bcc*I (New England BioLabs, Beverly, MA) overnight at 37°C. The wild-type allele (CC) produced two bands of 189 and 111 bp; wild-type/variant allele (CA) produced three bands of 111, 189 and 300 bp and the variant allele (AA) produced a single 300 bp band (Table I).

For the *TP53* (Arg72Pro) polymorphism in the promoter, the primers (5′-CTGCCTTCATTTATCCAGCA-3′ and 5′-GGCGGCAGATGAATTACAA-3′) amplified a 296 bp DNA fragment. Then, the PCR product was digested by *BstU*I (New England BioLabs, Beverly, MA) overnight at 60°C. The wild-type allele (GG) produced two bands of 169 and 127 bp); wild-type/variant allele (GC) produced three bands of 127, 169 and 296 bp and the variant allele (CC) produced a single 296 bp band (Table I).

PCR was conducted and the results were evaluated without knowledge of the subjects' case–control status. More than 10% of the samples were randomly selected for repeated assays, and the results were 100% concordant.

Statistical analysis

We used the χ^2 test to compare the differences in frequency distributions of demographic variables, smoking status, alcohol use, each allele and genotype of the *BAX*, *BCL2* and *TP53* polymorphisms between the cases and controls. We also tested the Hardy–Weinberg equilibrium of genotype distributions separately for both cases and controls. Additionally, we used unconditional univariate and multivariate logistic regression analyses to examine the associations between the selected SNPs and SCCHN risk by estimating odds ratios (ORs) and 95% confidence intervals (CIs) with and without adjustment for age, ethnicity, smoking status and alcohol use.

We further stratified the genotype data by subgroups of age, sex, smoking status, alcohol use and the selected SNPs and assessed the risk of SCCHN using multivariate logistic regression models. To evaluate interactions between *BCL2*, *BAX* and *TP53* genotypes in the risk of SCCHN, logistic regression analysis was also used to assess potential interactions by evaluating departures from additive and multiplicative interaction models. All statistical tests were two-sided, a *P* value of 0.05 was considered significant, and all analyses were performed using the SAS software (version 9.1; SAS Institute, Cary, NC).

Results

Characteristics of the study population

We recruited a total of 821 cases and 934 controls for genotyping, but seven cases failed in the genotyping assays. Thus, the final data analysis included 814 cases and 934 controls. The frequency distribution of selected characteristics of the cases and controls is presented in Table II. Because of frequency matching, there was no statistically significant difference in the distributions of age and sex between cases and controls: 57.0 ± 11.0 (mean ± standard deviation) years and 76.2% males for cases and 55.9 ± 11.2 (mean ± standard deviation) years and 75.1% males for controls ($P = 0.548$ for age and $P = 0.589$ for sex). Of the 814 cases, 240 (29.5%) had cancers of the oral cavity, 398 (48.9%) of oropharynx, 41 (5.1%) of hypopharynx and 135 (16.6%) of larynx. However, the cases were more likely than the controls to be smokers and drinkers ($P < 0.001$ for both smoking and alcohol use) (Table II). Therefore, these variables were further adjusted for in the logistic regression analyses.

Distribution of genotypes and risk estimates

The genotype and allele distributions of the *BAX* (−248 G>A), *BCL2* (−938 C>A) and *TP53* Arg72Pro SNPs in the cases and controls are

Table I. Genotyping assays for the selected polymorphisms

Gene	Position and base change	Genotyping method	Primer	PCR product	Enzyme	Gel band pattern
BAX	−248 G>A (rs4645878)	PIRA–PCR[a]: mismatch, forward primer −2 G-to-C	5′-CATTAGAGCTGCGATTGGAC*C*G-3′ (forward) 5′-GCTCCCTCGGGAGGTTTGGT-3′ (reverse)	109 bp	*Msp*I	A allele: 89 and 20 bp; G allele: 109 bp
BCL2	–938 C>A (rs2279115)	PCR–RFLP[b]	5′-CTGCCTTCATTTATCCAGCA-3′ (forward) 5′-GGCGGCAGATGAATTACAA-3′ (reverse)	300 bp	*Bcc*I	A allele: 189 and 111 bp; C allele: 300 bp
TP53	R72P G>C (rs1042522)	PCR–RFLP[b]	5′-ATCTACAGTCCCCCTTGCCG-3′ (forward) 5′-GCAACTGACCGTGCAAGTCA-3′ (reverse)	296 bp	*BstU*I	G allele: 169 and 127 bp; C allele: 296 bp

[a]PIRA, primer-introduced restriction analysis; [b]RFLP, restriction fragment length polymorphism.

summarized in Table III. The observed genotype frequencies of these three SNPs in the controls were all in agreement with Hardy–Weinberg equilibrium ($P = 0.493$ for *BAX*, $P = 0.176$ for *BCL2* and $P = 0.458$ for *TP53*). As shown in Table III, we did not find any statistically significant difference in the frequency distributions of the three SNPs between cases and controls [$P = 0.625$ for *BAX* (−248 G>A), $P = 0.933$ for *BCL2* (−938 C>A) and $P = 0.454$ for *TP53* Arg72Pro]. In the single-locus analysis, when the *BAX* GG genotype was used as the reference group, no association was found between the AG and AA variant genotypes and risk of SCCHN (Table III); similarly, when the *BCL2* CC and *TP53* Arg/Arg genotypes were used as the references, no *BCL2* and *TP53* variant genotypes were associated with a significantly increased risk of SCCHN (Table III). In further stratification analysis, we combined the variant genotypes [i.e. *BAX* (AG + AA), *BCL2* (AC + AA) and *TP53* (Arg/Pro + Pro/Pro) genotypes] to avoid small numbers in the subgroups. Overall, there was no association between these three combined variant genotypes and risk of SCCHN (Table III) or was any association between these genotypes and SCCHN risk in the subgroups stratified by age, sex, smoking and alcohol use (data not shown).

Table II. Frequency distributions of selected variables in SCCHN cases and cancer-free controls

Variables	No. of subjects (%)			*P* value[a]
	Cases ($n = 814$)	Controls ($n = 934$)		
Age (years)				
≤56	404 (49.6)	477	(51.1)	0.548
>56	410 (50.4)	457	(48.9)	
Sex				
Female	194 (23.8)	233	(25.0)	0.589
Male	620 (76.2)	701	(75.0)	
Smoking status				
Never	215 (26.4)	454	(48.6)	<0.001
Former	300 (36.9)	341	(36.5)	
Current	299 (36.7)	139	(14.9)	
Alcohol use				
Never	214 (26.3)	410	(43.9)	<0.001
Former	179 (22.0)	147	(15.7)	
Current	421 (51.7)	377	(40.4)	
Tumor site				
Oral cavity	240 (29.5)			
Oropharynx	398 (48.9)			
Hypopharynx	41 (5.0)			
Larynx	135 (16.6)			

[a]Two-sided χ^2 test.

Gene–gene interaction between BAX, BCL2 and TP53 genotypes

Because we did not find any association in the single-locus analysis, we then tested the hypothesis that *BAX*, *BCL2* and *TP53* variant genotypes might jointly alter SCCHN risk. We first stratified one SNP distribution by the other SNP and then evaluated the association between any of the polymorphisms of *BAX* (−248 G>A), *BCL2* (−938 C>A) and *P53* (Arg72Pro) and cancer risk among each SNP subgroup (Table IV). The results showed that, among *TP53* heterozygotes, there was a significant increase in risk of SCCHN associated with the *BAX* homozygous variant genotype (AA), either compared with *BAX* homozygous common genotype (GG) or with combined genotypes (GG + AG) (adjusted OR = 6.58, 95% CI = 1.38–31.49 and OR = 6.60, 95% CI = 1.38–31.50, respectively). In contrast, among the same *TP53* heterozygotes, there was a significant decrease in risk of SCCHN associated with both *BCL2* heterozygous (AC) and combined variants genotypes (AC + AA) (adjusted OR = 0.68, 95% CI = 0.47–0.98 and OR = 0.67, 95% CI = 0.48–0.95, respectively) compared with the *BCL2* homozygous common genotype (CC). These associations were not observed among carriers of other *TP53* genotypes (Table IV) nor was an association between cancer risk and any of subgroups of the *BCL2* genotypes stratified by the *BAX* genotypes (Table V). However, further analyses did not find

Table III. Logistic regression analysis of associations between *BAX*, *BCL2* and *TP53* polymorphisms and risk of SCCHN

Polymorphism	No. of subjects (%)		*P* value	OR (95% CI)	Adjusted OR (95% CI)[a]
	Cases ($n = 814$)	Controls ($n = 934$)			
BAX (G > A; rs4645878)					
GG	627 (77.0)	723 (77.4)	0.314[b]	1.00	1.00
AG	170 (20.9)	200 (21.4)		0.98 (0.78–1.24)	0.99 (0.78–1.26)
AA	17 (2.1)	11 (1.2)		1.78 (0.83–3.83)	2.01 (0.91–4.47)
AG + AA	187 (23.0)	211 (22.6)	0.849[c]	1.02 (0.82–1.28)	1.04 (0.82–1.32)
A allele frequency	0.125	0.119	0.625[d]		
GG + AG	797 (97.9)	923 (98.8)	0.130[c]	1.00	1.00
AA	17 (2.1)	11 (1.2)		1.79 (0.83–3.84)	2.01 (0.91–4.48)
BCL2 (C > A; rs2279115)					
CC	226 (27.8)	257 (27.5)	0.937[b]	1.00	1.00
AC	382 (46.9)	446 (47.8)		0.97 (0.78–1.22)	0.97 (0.76–1.22)
AA	206 (25.3)	231 (24.7)		1.01 (0.78–1.31)	1.03 (0.78–1.35)
AC + AA	588 (72.2)	677 (72.5)	0.908	0.99 (0.80–1.22)	0.99 (0.79–1.23)
A allele frequency	0.488	0.486	0.933[d]		
CC + AC	608 (74.7)	703 (75.3)	0.782[c]	1.00	1.00
AA	206 (25.3)	231 (24.7)		1.03 (0.83–1.28)	1.05 (0.84–1.32)
TP53 Arg72Pro (G > C; rs1042522)					
GG	417 (51.2)	484 (51.8)	0.380[b]	1.00	1.00
GC	324 (39.8)	383 (41.0)		0.98 (0.81–1.20)	1.02 (0.83–1.26)
CC	73 (9.0)	67 (7.2)		1.27 (0.89–1.81)	1.21 (0.83–1.76)
GC + CC	397 (48.8)	450 (48.2)	0.805[c]	1.02 (0.85–1.24)	1.05 (0.86–1.28)
C allele frequency	0.289	0.277	0.454[d]		
GG + GC	741 (91.0)	867 (92.8)	0.168[c]	1.00	1.00
CC	73 (9.0)	67 (7.2)		1.28 (0.90–1.80)	1.20 (0.83–1.72)

[a]Adjusted for age, sex, smoking status and alcohol consumption status.
[b]Two-sided χ^2 test for difference in frequency distribution of genotypes between cases and controls.
[c]Two-sided χ^2 test for difference in frequency distribution of combined genotypes between cases and controls.
[d]Two-sided χ^2 test for difference in frequency distribution of alleles between cases and controls.

Table IV. Stratified analysis of the association between *BAX* and *BCL2* polymorphisms and SCCHN risk by *TP53* genotypes

All	*TP53* Arg72Arg			*TP53* Arg72Pro			*TP53* Pro72Pro		
	Cases No. (%) 417 (100)	Controls No. (%) 484 (100)	OR (95% CI)[a]	Cases No. (%) 324 (100)	Controls No. (%) 383 (100)	OR (95% CI)[a]	Cases No. (%) 73 (100)	Controls No. (%) 67 (100)	OR (95% CI)[a]
BAX (G > A; rs4645878)									
GG	333 (79.9)	383 (79.1)	1.00	239 (73.8)	292 (76.3)	1.00	55 (75.3)	48 (71.6)	1.00
AG	78 (18.7)	92 (19.0)	0.99 (070–1.41)	74 (22.8)	89 (232)	0.98 (0.68–1.43)	18 (24.7)	19 (28.4)	0.88 (0.38–2.06)
AA	6 (1.4)	9 (1.9)	0.92 (0.32–2.68)	11 (3.4)	2 (0.5)	6.58 (1.38–31.49)	0.00	0.00	—
AG + AA	84 (20.1)	101 (20.9)	0.99 (0.70–1.39)	85 (26.2)	91 (23.8)	1.11 (0.77–1.59)	18 (24.7)	19 (28.4)	0.88 (0.38–2.06)
GG + AG	411 (98.6)	475 (98.1)	1.00	313 (96.6)	381 (99.5)	1.00	73 (100)	67 (100)	1.00
AA	6 (1.4)	9 (1.9)	0.92 (0.32–2.68)	11 (3.4)	2 (0.5)	6.60 (1.38–31.50)	0.00	0.00	—
BCL2 (C > A; rs2279115)									
CC	96 (23.0)	132 (27.3)	1.00	107 (33.0)	101 (26.4)	1.00	23 (31.5)	24 (35.8)	1.00
AC	207 (49.6)	232 (47.9)	1.26 (0.90–1.77)	142 (43.8)	180 (47.2)	0.68 (0.47–0.98)	33 (45.2)	33 (49.3)	1.09 (0.47–2.54)
AA	114 (27.4)	120 (24.8)	1.00 (0.90–1.00)	75 (23.2)	101 (26.4)	0.66 (0.43–1.01)	17 (23.3)	10 (14.9)	1.99 (0.65–6.10)
AC + AA	321 (77.0)	352 (72.7)	1.28 (0.94–1.76)	217 (67.0)	282 (73.6)	0.67 (0.48–0.95)	50 (68.5)	43 (64.2)	1.27 (0.57–2.84)
CC + AC	303 (72.7)	364 (75.2)	1.00	249 (76.5)	282 (73.6)	1.00	56 (76.7)	57 (85.1)	1.00
AA	114 (27.3)	120 (24.8)	1.14 (0.83–1.55)	75 (23.2)	101 (26.4)	0.83 (0.58–1.20)	17 (23.3)	10 (14.9)	1.89 (0.70–5.07)

[a]Adjusted for age, sex, smoking status and alcohol consumption.

Table V. Stratified analysis of the association between *BCL2* polymorphisms and SCCHN risk by *BAX* genotypes

All	*BAX* GG			*BAX* AG			*BAX* AA		
	Cases No. (%) 627 (100)	Controls No. (%) 723 (100)	OR (95% CI)[a]	Cases No. (%) 170 (100)	Controls No. (%) 200 (100)	OR (95% CI)[a]	Cases No. (%) 17 (100)	Controls No. (%) 11 (100)	OR (95% CI)[a]
BCL2 (C > A; rs2279115)									
CC	169 (27.0)	206 (28.5)	1.00	53 (31.2)	49 (24.5)	1.00	4 (23.5)	2 (18.2)	1.00
AC	291 (46.4)	343 (47.4)	1.02 (0.78–1.33)	84 (49.4)	95 (47.5)	0.84 (0.50–1.40)	7 (41.2)	8 (72.7)	0.20 (0.02–2.50)
AA	167 (26.6)	174 (24.1)	1.16 (0.86–1.59)	33 (19.4)	56 (28.0)	0.57 (0.31–1.05)	6 (35.3)	1 (9.1)	4.23 (0.20–91.3)
AC + AA	458 (73.0)	517 (71.5)	1.07 (0.83–1.37)	117 (68.8)	151 (75.5)	0.74 (0.46–1.20)	13 (76.5)	9 (81.8)	0.54 (0.06–4.62)
CC + AC	460 (73.4)	549 (75.9)	1.00	137 (80.6)	144 (72.0)	1.00	11 (64.7)	10 (90.9)	1.00
AA	167 (26.6)	174 (24.1)	1.15 (0.89–1.49)	33 (19.4)	56 (28.0)	0.63 (0.38–1.07)	6 (35.3)	1 (9.1)	11.6 (0.89–152)

[a]Adjusted for age, sex, smoking status and alcohol consumption.

any evidence of potential multiplicative and additive gene–gene and gene–environment interactions among *BAX*, *BCL2* and *TP53* variant genotypes, age, sex, smoking status and alcohol use (data not shown).

Discussion

In this hospital-based case–control study, we assessed jointly the effects of functional SNPs of *BAX* (−248 G>A), *BCL2* (−938 C>A) and *TP53* Arg72Pro on the risk of SCCHN in a US non-Hispanic white population, because the two functional SNPs of the *BAX* and *BCL2* promoters are in the proximal region or sequences that are regulated by the TP53 transcriptional factor. Although our single-locus analysis did not reveal any significant associations between these functional SNPs and SCCHN risk after adjustment for age, sex and smoking and alcohol status, further analyses showed that among *TP53* heterozygotes, the *BCL2* A variant genotypes appeared to be protective against SCCHN risk, whereas the *BAX* AA variant genotype exhibited an elevated risk of SCCHN; however, no joint effect was observed between *BCL2* and *BAX* SNPs.

Although these subgroup findings may have been due to chance, as a result of multiple tests, they may reveal some biological interaction between TP53 and its two key effectors, BAX and BCL2. TP53, as a guardian of the genome, regulates cellular apoptosis by directly binding to the promoter elements and activating transcription of a number of apoptosis-regulatory genes, including *BAX* (31–33). TP53 has also been shown to indirectly inhibit the *BCL2* transcription activation through a direct interaction between TP53 with an apoptosis-blocking protein, Brn-3a (18,34).

It was not immediately apparent why our data showed a specific association between the SNP at either of the *BAX* or *BCL2* promoter regions and risk of SCCHN only among *TP53* codon 72 heterozygotes. Although we did not have sequencing data on tumor mutations and LOH to unravel their relationship with the codon 72 SNP, a study of the correlation among mutations, LOH and the codon 72 SNP of *TP53* in SCCHN has been reported, in which the overall *TP53*-LOH rate was 45.2% among 42 tumors, but the *TP53*-LOH rate was low (14.3%) among the Arg72Pro heterozygotes, compared with 33.3% and 64% among the Arg72Arg and Pro72Pro homozygotes, respectively (35). Pertinent to our current study, it is likely that only when the wild-type *TP53* was present, the effect of the regulation on *BCL2* or *BAX* by the *TP53* codon 72 SNP can be adequately manifested. We believe this regulatory hierarchy accounts for our observation that the association of SNPs in the *BAX* and *BCL2* promoters with risk of SCCHN is only observed in *TP53* codon 72 heterozygotes whose tumors are most likely in the absence of *TP53*-LOH. Therefore, it is not surprising that our analyses did not reveal an association between *BCL2* (−938 C>A) or *BAX* (−248 G>A) polymorphisms and the risk of SCCHN in *TP53* homozygotes for codon 72, because any association is likely be abolished by the *TP53*-LOH and mutations in the tumors of such individuals.

It can be speculated that the A variant genotypes at the position −938 of the *BCL2* promoter may render a better interaction with TP53, leading to a decrease in the *BCL2* expression, an up-regulated programmed cell death or reduced longevity of transformed cells, and thus a subsequent decrease in the risk of SCCHN. Likewise, our data also showed that there was an increased risk of SCCHN associated

with the *BAX* (−248 G>A) polymorphism among the *TP53* heterozygotes. This may be due to the weakening of *TP53* interaction with the −248AA genotype, leading to a decrease in the expression of pro-apoptotic *BAX* gene in the cells and a high risk of SCCHN. Such hypotheses may help explain the absence of LOH in the tumors of *TP53* codon 72 heterozygotes (35) and provide a support for an unknown molecular mechanism of carcinogenesis of cells heterozygous for the *TP53* codon 72.

Until now, there are only a few reports on the association between *BCL2* (−938 C>A) or *BAX* (−248 G>A) polymorphisms and risk of cancer, mostly chronic lymphocytic leukemia (22–27) but not solid tumors. Several studies have reported that the SNP located within the 5′-untranslated region of the *BAX* promoter (-248 G>A), was associated with reduced expression of *BAX* and increased susceptibility to chronic lymphocytic leukemia (25). Recently, a study has examined the effect of the promoter SNP of the *BCL2* gene on prostate cancer susceptibility in three ethnic populations, African-Americans, Jamaicans and European Americans. This study showed that the heterogynous *BCL2* genotype was associated with a decreased prostate cancer risk among the European Americans, although the study size ($n = 218$) was relatively small (21). To the best of our knowledge, our study is the first report showing an association between *BAX* and *BCL2* promoter SNPs and the risk of SCCHN stratified by the *TP53* codon 72 genotypes. We further demonstrated that in solid tumors where *TP53* mutations are frequent, stratification by the *TP53* codon 72 SNP as a surrogate for LOH and mutation status is necessary to reveal this association.

In conclusion, our data from a relatively large case–control study provide evidence that the SNPs in the promoter regions of *BAX* and *BCL2* may affect their regulation by *TP53* and their association with risk of SCCHN, particularly in *TP53* heterozygotes. Because this is a hospital-based case–control study and the findings of subgroups are limited due to small sample sizes in the strata, our results need to be validated by larger, preferably prospective, studies, in which the tumors from those who are heterozygous for the TP53 codon 72 SNP should be examined for the evidence of lacking TP53-LOH.

Funding

National Institutes of Health (ES11740 and CA100264) to Q.W; The University of Texas M. D. Anderson Cancer Center Support Grant (CA16672).

Acknowledgements

We thank Margaret Lung, Kathryn Patterson and Leanel Fairly for their assistance in recruiting the subjects and Yawei Qiao, Kejing Xu, Jianzhong He and Xiaodong Zhai for their technical assistance.

Conflict of Interest Statement: None declared.

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Received April 20, 2007; revised June 12, 2007; accepted July 20, 2007