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Systematic evaluation of apoptotic pathway gene polymorphisms and lung cancer risk

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We adopted a two-stage study design to screen 927 single nucleotide polymorphisms (SNPs) located in 73 apoptotic-pathway genes in a case-control study and then performed a fast-track validation of the significant SNPs in a replication population to identify sequence variations in the apoptotic pathway modulating lung cancer risk. Fifty-five SNPs showed significant associations in the discovery population comprised of 661 lung cancer cases and 959 controls. Six of these SNPs located in three genes (*Bcl-2*, *CASP9* and *ANKS1B*) were validated in a replication population with 1154 cases and 1373 controls. Additive model was the best-fitting model for five SNPs (rs1462129 and rs255102 of *Bcl-2*, rs6685648 of *CASP9* and rs1549102, rs11110099 of *ANKS1B*) and recessive model was the best fit for one SNP (rs10745877 of *ANKS1B*). In the analysis of joint effects with subjects carrying no unfavorable genotypes as the reference group, those carrying one, two, and three or more unfavorable genotypes had an odds ratio (*OR*) of 2.22 [95% confidence interval (*CI*) = 1.08–4.57, *P* = 0.03], 2.70 (95% *CI* = 1.33–5.49; *P* = 0.006) and 4.13 (95% *CI* = 2.00–8.57; *P* = 0.0001), respectively (*P* for trend = 6.05E-06). The joint effect of unfavorable genotypes was also validated in the replication population. The SNPs identified are located in or near key genes known to play important roles in apoptosis regulation, supporting the strong biological relevance of our findings. Future studies are needed to identify the causal SNPs and elucidate the underlying molecular mechanisms.

Introduction

Lung cancer is the most common cancer and the leading cause of cancer-related deaths in USA. It was estimated in 2012 that there were a total of 226,160 new incidences of lung cancer and 160,340 deaths from lung cancer (1). Eighty-seven percentage of lung cancer deaths are attributed to cigarette smoking (2). The fact that only a fraction of smokers develop lung cancer points to genetic susceptibility of the disease. During the past several decades, genetic susceptibility to lung cancer has been extensively studied in molecular epidemiologic studies. Interindividual variation in susceptibility to lung cancer may be mediated by genetic variations in multiple cancer-related pathways. The apoptotic pathway is one of such key pathways, and genetic variants in this pathway have been demonstrated to contribute to increased cancer risk, including lung cancer (3,4). Apoptosis is a geneticaly controlled cell suicide mechanism that enables multicelluar organisms to regulate cell number in tissues and to eliminate unnecessary or damaged cells (5,6). The activation of apoptosis signaling is through an intrinsic Bcl-2 pathway and an extrinsic or TNF-related apoptosis inducing ligand pathway (7–11). The extrinsic pathway acts via death receptors, whereas the intrinsic pathway acts via the release of mitochondrial proteins. In both pathways, initiator caspases are activated and the initiated caspases activate executioner caspases, which cleave death substrates, leading to cell death.

Although apoptosis is evolutionarily conserved, there may be inter-individual variation in apoptotic capacity in the general population. In literature, the association between genetic variants in the apoptotic pathway and lung cancer risk has been reported (12–19). However, the associations published so far are limited to a few candidate single nucleotide polymorphisms (SNPs) and there is a lack of comprehensive evaluations of a large panel of SNPs in this pathway. Moreover, few of the previous studies considered replication of the associations in their study design to scrutinize false-positive findings. With the aim to identify sequence variations in the apoptotic pathway modulating lung cancer risk, we took a systematic approach to evaluate the associations between a large panel of SNPs in the apoptotic pathway and lung cancer risk in a large case-control study. Furthermore, we confirmed the significant associations in the discovery population in a replication population.

Abbreviations: CI, confidence interval; FADD, Fas-associated death domain; GWAS, genome-wide association study; OR, odds ratio; SNP, single nucleotide polymorphism.

Materials and methods

Discovery and replication populations

In this study, cases in the discovery stage were identified from an ongoing lung cancer case-control study at the MD Anderson Cancer Center (20). Cases were newly diagnosed, histologically confirmed lung cancer patients presenting at the registration of the Thoracic Medical Oncology Clinic of the MD Anderson Cancer Center, and patients were previously untreated by chemotherapy or radiotherapy. There was no age, sex, ethnicity, or cancer stage restrictions on recruitment. The controls in the discovery stage were identified from a pool of control participants recruited in ongoing case-control studies of cancer in collaboration with the Kelsey Seybold Clinics, Houston's largest private multispecialty group practice in the Houston metropolitan area, with 18 clinics and more than 325 physicians. The control subjects were healthy individuals without prior history of cancer (except for non-melanoma skin cancer). The majority of control participants were healthy individuals seen at the clinic for annual physical exams or to address health concerns. A total of 661cases and 959 controls were included in the discovery stage. The replication population consisted of participants previously participated in a genome-wide association study (GWAS) of lung cancer at MD Anderson Cancer Center (21) with a total of 1154 cases and 1073 controls. The recruitment period for the replication population was from June 1996 to July 2007. The subjects of the discovery phase were recruited primarily after July 2007 till November 2008. There was no overlap of subjects in the discovery phase and the replication phase.

All patients and controls gave written informed consent before participation, and the studies were approved by the MD Anderson Institutional Review Board and the Kelsey Seybold Clinics. All participants completed an in-person interview administered by MD Anderson staff interviewers using a structured questionnaire. Demographic characteristics, history of tobacco use, family history of cancer, environmental exposures and other epidemiologic data were collected and recorded. At the end of the interview, each participant donated 40 ml blood sample for molecular analysis.

An individual who had never smoked or had smoked less than 100 cigarettes in his or her lifetime was defined as a never smoker. An individual who had smoked at least 100 cigarettes in his or her lifetime, but had quit more than 12 months before diagnosis (for cases) or before the interview (for controls) was classified as a former smoker. Current smokers were those who were currently smoking or quit less than 12 months before diagnosis (for cases) or before the interview (for controls).

Gene and SNP selection

We compiled the gene list using Gene Ontology (http://www.geneontology.org) and performed an extensive literature review to refine the gene list in the apoptosis pathway. A total of 73 genes were selected (Supplemental Table 1 available at *Carcinogenesis* Online). The SNPs in this pathway were extracted from previously genotyped SNPs as part of our GWAS in order to comprehensively screen genetic variation within this pathway. SNPs extracted in the discovery stage of the current study were originally genotyped using

Table I. Host characteristics of the discovery and replication populations

	Discovery		Replication	
	Cases, *n*(%)	Controls, *n*(%)	Cases, *n*(%)	Controls, *n*(%)
	N = 661	*N* = 959	*N* = 1154	*N* = 1073
Age, mean(SD)	62.43 (11.63)	64.52 (11.13)	62.08 (10.78)	60.48 (8.75)
Sex, *N*(%)				
Male	299 (45.23)	775 (80.81)	658 (57.02)	581 (54.15)
Female	362 (54.77)	184 (19.19)	496 (42.98)	492 (45.85)
Smoking status, *N*(%)				
Never smoker	328 (49.62)	408 (42.54)	0 (0%)	0 (0%)
Former smoker	183 (27.69)	475 (49.53)	600 (51.99)	595 (55.45)
Current smoker	150 (22.69)	76 (7.92)	554 (48.01)	478 (44.45)
Pack year of smoking, mean (SD)	50.59 (32.48)	32.51 (29.38)	51.49 (31.41)	44.45 (29.90)
Asbestos exposure, *N*(%)				
Yes	212 (32.07)	87 (9.07)	368 (31.89)	243 (22.65)
No	416 (62.93)	864 (90.09)	754 (65.34)	826 (76.98)
Unknown or not available	33 (4.99)	8 (0.83)	32 (2.77)	4 (0.37)
Hay fever, *N*(%)				
Yes	107 (16.19)	N/A	173 (14.99)	238 (22.18)
No	517 (78.21)	N/A	957 (82.93)	834 (77.73)
Unknown or not available	37 (5.60)	N/A	24 (2.08)	1 (0.09)
Lung cancer in first-degree relatives, *N*(%)				
Yes	191 (28.90)	202 (21.06)	280 (24.26)	180 (16.78)
No	470 (71.10)	755 (78.73)	874 (75.74)	893 (83.22)
Unknown or not available	0(0.00)	2(0.21)	0(0.00)	0(0.00)
Histology, *N*(%)				
Adenocarcinoma	408 (61.72)		543 (47.05)	
Squamous cell carcinoma	119 (18.00)		285 (24.70)	
Large cell carcinoma	8 (1.21)		39 (3.38)	
Adenosquamous carcinoma	4 (0.61)		11 (0.95)	
Bronchioloalveolar carcinoma	21 (3.18)		26 (2.25)	
Non-small cell carcinoma, NOS	93 (14.07)		174 (15.08)	
Carcinomas with other features	8(1.21)		76(6.59)	
Clinical stage, *N*(%)				
IA	54 (8.17)		155 (13.43)	
IB	38 (5.75)		141 (12.22)	
IIA	2 (0.30)		18 (1.56)	
IIB	13 (1.97)		76 (6.59)	
IIIA	127 (19.21)		172 (14.90)	
IIIB dry	100 (15.13)		156 (13.52)	
IIIB wet	27 (4.08)		41 (3.55)	
IV	300 (45.39)		395 (34.23)	
Grade, *N*(%)				
Well differentiated	48 (7.26)		56 (4.85)	
Moderately differentiated	154 (23.30)		248 (21.49)	
Poorly differentiated	234 (35.40)		524 (45.41)	
Undifferentiated	6 (0.91)		15 (1.30)	
Unknown or not available	219 (33.13)		311 (26.95)	

Illumina HumanHap660K BeadChips. We extracted tag SNPs within 10 kb upstream of transcriptional start site and 10 kb downstream of transcriptional stop site of each gene. The whole genome scan with Illumina HumanHap660K BeadChips provides excellent gene coverage of ≥80% for approximately 80% of the genes within the human genome of CEU population (22). The SNPs for the replication stage were originally genotyped using Illumina HumanHap300K BeadChips. GWAS sample and genotyping quality control were described in detail previously (21). Briefly, a sample was excluded (i) if suspected of being contaminated with different genomic DNA samples; (ii) was found not to be Caucasian on review; (iii) was a first-degree relative to another study subject; (iv) was a duplicate sample with discordant genotype; (v) or was found to have reported sex that did not match with X chromosome heterozygosity. After SNPs were extracted, in data analysis, we dropped out SNPs with low call rate (<90%), SNPs with minor allele frequency less than 1% in the study populations and SNPs not in Hardy–Weinberg equilibrium in controls. As a result, a total of 927 SNPs of the apoptosis pathway were included in the final statistical analysis (Supplemental Table 1 available at *Carcinogenesis* Online).

Statistical analysis

Hardy–Weinberg equilibrium was tested for each SNP using the goodness-of-fit Chi-Square test to compare the observed with the expected frequency of genotypes in controls. Pearson's χ^2 analysis or Fisher's exact test was used to test for genotype frequencies in cases and controls. Multivariate logistic regressions were performed to estimate odds ratios (ORs) of SNP main effects adjusting for age, sex, smoking status and pack year of smoking. To determine whether additional variables should be adjusted in the model, we checked the associations between other epidemiological risk factors and lung cancer in the two populations. In the discovery population, asbestos exposure and family history of lung cancer (lung cancer in first-degree relatives) were significant and in the replication population, emphysema, hay fever, asbestos and family history were significant. We therefore additionally adjusted these variables in the model to control for confounding in each population, respectively. Since the underlying model predisposing to cancer risk may follow dominant, recessive or additive models, we examined the SNPs risk by all three inheritance models. The best-fitting model was the one with the smallest *P* value among the three models. However, if

the genotype counts for the homozygous variant genotype were less than five in cases and controls combined, we only considered the dominant model that had the highest statistical power. To evaluate the cumulative effects from the genetic variants in the pathway, we summed up unfavorable genotypes (genotypes associated with significantly increased risk in the main effects analysis) for each subject. In the case when multiple SNPs within a haplotype block had significant main effect, only the most significant SNP with the smallest *P* value was selected for this analysis.

To see whether epidemiologic risk factors of lung cancer could confound the SNP-cancer associations, we performed an analysis to see if SNPs were correlated with smoking status, smoking intensity, asbestos exposure, family history, etc. The analyses were performed in controls, in cases and in all subjects.

All statistical tests were two sided. Statistical analyses were performed using the STATA software (Version 10, College Station, TX). Haplotype frequencies were analyzed using the HelixTree Genetics Analysis Software (Golden Helix, Bozeman, MT). Haplotypes were inferred using the expectation–maximization algorithm implemented in the Helix Tree software. The adjusted ORs and 95% confidence interval (*CI*) for each haplotype were calculated using multivariate logistic regression using the most abundant haplotype as the reference group.

Table II. SNPs significantly associated with lung cancer risk in the discovery population

Gene	SNP id	Minor allele	Cases[a]	Controls[a]	Genetic model	Adjusted *OR*[b]	***P* value**
ANKS1B	rs10459194	G	310\295\50	439\406\114	Recessive	0.50 (0.32–0.77)	0.002
ANKS1B	rs10745877	G	281\287\93	431\436\92	Recessive	1.60 (1.08–2.36)	0.019
ANKS1B	rs11109966	A	249\341\71	415\424\120	Dominant	1.37 (1.06–1.78)	0.017
ANKS1B	rs11110099	C	183\330\147	311\471\176	Additive	1.19 (0.99–1.43)	0.057
ANKS1B	rs1403506	G	418\224\19	561\344\54	Additive	0.72 (0.58–0.89)	0.003
ANKS1B	rs1523097	G	237\340\84	324\456\179	Recessive	0.55 (0.39–0.78)	<0.001
ANKS1B	rs1549102	C	195\341\125	274\456\229	Additive	0.80 (0.67–0.96)	0.015
ANKS1B	rs201363	A	474\174\13	749\195\15	Dominant	1.50 (1.12–2.01)	0.006
ANKS1B	rs4762543	A	244\335\82	333\449\177	Recessive	0.56 (0.39–0.79)	0.001
ANKS1B	rs7135384	G	192\346\123	335\450\174	Dominant	1.49 (1.13–1.96)	0.005
ANKS1B	rs7139028	A	236\347\78	339\445\175	Recessive	0.53 (0.37–0.76)	<0.001
ANKS1B	rs7301050	A	432\209\20	576\334\49	Additive	0.72 (0.57–0.89)	0.003
ANKS1B	rs7959046	A	421\217\23	554\348\57	Additive	0.71 (0.57–0.88)	0.002
ANKS1B	rs7963120	G	541\112\7	758\187\14	Dominant	0.63 (0.45–0.87)	0.005
ANKS1B	rs869032	A	418\220\23	554\348\57	Additive	0.72 (0.58–0.89)	0.003
Bcl-2	rs12454650	G	323\294\44	526\357\76	Dominant	1.28 (1.00–1.65)	0.05
Bcl-2	rs12454712	G	230\339\91	392\444\121	Dominant	1.42 (1.09–1.85)	0.009
Bcl-2	rs1462129	A	175\335\151	288\472\199	Additive	1.30 (1.08–1.55)	0.005
Bcl-2	rs1982673	C	459\185\17	707\228\24	Dominant	1.28 (1.02–1.69)	0.034
Bcl-2	rs7243091	A	409\212\38	561\358\39	Dominant	0.78 (0.60–0.99)	0.04
Bcl-2	rs8098848	G	319\295\47	522\359\78	Dominant	1.28 (1.00–1.65)	0.05
Bcl-2	rs2255302	G	315\309\37	540\355\64	Dominant	1.69 (1.31–2.18)	<0.001
Bcl-2	rs2551402	A	158\344\159	263\480\216	Additive	1.30 (1.08–1.55)	0.005
Bcl-2	rs2849371	A	315\309\37	534\360\65	Dominant	1.62 (1.26–2.09)	<0.001
Bcl-2	rs2849372	G	314\310\37	534\360\65	Dominant	1.62 (1.26–2.09)	<0.001
Bcl-2	rs2850767	C	263\317\81	425\427\107	Dominant	1.31 (1.01–1.69)	0.04
BID	rs366542	G	165\332\164	280\458\221	Dominant	1.31 (1.00–1.75)	0.05
BMF	rs11858141	G	233\332\96	285\492\182	Additive	0.78 (0.65–0.94)	0.008
BMF	rs16970325	A	531\122\8	816\138\5	Dominant	1.35 (1.00–1.88)	0.05
BMF	rs500804	A	293\300\68	515\366\78	Dominant	1.49 (1.15–1.92)	0.002
BMF	rs537455	C	332\272\54	552\337\70	Dominant	1.49 (1.16–1.92)	0.002
CAPN2	rs12080565	A	602\57\2	850\103\5	Dominant	0.75 (0.49–0.97)	0.035
CAPN2	rs1222145	C	396\242\23	564\333\61	Recessive	0.51 (0.30–0.88)	0.014
CAPN2	rs16842040	A	630\31\0	891\65\3	Dominant	0.59 (0.34–0.99)	0.049
CASP3	rs2019978	C	408\228\25	624\300\35	Dominant	1.29 (1.02–1.67)	0.03
CASP8	rs3769823	A	312\295\54	507\378\74	Dominant	1.37 (1.06–1.76)	0.014
CASP9	rs4645989	G	311\277\72	490\384\83	Recessive	1.48 (1.02–2.24)	0.038
CASP9	rs6685648	G	312\277\72	489\388\82	Additive	1.17 (0.97–1.429)	0.102
CFLAR	rs4487072	A	437\196\28	583\346\30	Dominant	0.79 (0.61–1.00)	0.049
CFLAR	rs7583529	A	442\191\28	588\334\36	Dominant	0.81 (0.62–1.00)	0.049
CASP2	rs17164250	A	404\212\44	606\308\43	Recessive	2.13 (1.24–6.67)	0.006
CASP2	rs2272256	A	371\258\31	539\353\67	Recessive	0.62 (0.35–0.99)	0.044
DAXX	rs2239839	A	346\269\45	470\389\100	Recessive	0.59 (0.37–0.93)	0.02
DAXX	rs2282851	A	347\269\45	469\390\100	Recessive	0.58 (0.37–0.92)	0.02
FADD	rs10898847	A	206\335\120	346\465\148	Dominant	1.30 (1.03–1.64)	0.03
FAS	rs2147419	C	345\259\57	451\422\86	Dominant	0.79 (0.61–1.01)	0.06
FOXO1A	rs17630266	A	600\58\2	902\55\2	Dominant	1.75 (1.09–2.80)	0.02
BAX	rs1042265	A	535\117\9	813\140\6	Dominant	1.53 (1.10–2.12)	0.011
BAX	rs2270937	A	274\315\72	445\419\95	Dominant	1.29 (1.00–1.67)	0.049
NCR3	rs2844480	A	406\224\31	632\284\43	Dominant	1.46 (1.12–1.90)	0.005
TNFRSF10A	rs2230229	G	498\149\14	690\241\28	Dominant	0.67 (0.50–0.89)	0.007
TNFRSF10D	rs3924519	G	365\252\44	474\409\76	Additive	0.81 (0.66–0.99)	0.04
TNFRSF1A	rs2228576	A	293\310\58	401\437\121	Recessive	0.72 (0.49–1.01)	0.06
TNFRSF21	rs2236037	A	518\134\9	774\178\7	Dominant	1.35 (1.00–1.85)	0.049
TRAF2	rs2811761	G	375\245\39	613\309\36	Additive	1.29 (1.04–1.60)	0.02

[a]The number of subjects of the WW (wild-type), WM (heterozygous) and MM (homozygous variant) genotypes: WW\WM\MM.
[b]Adjusted for age, gender, smoking status, pack year of smoking, asbestos exposure and lung cancer in first-degree relatives.

Table III. Lung cancer risk associated with significant SNPs in the discovery and in the replication populations

			Discovery population		Replication population		Pooled population	
Gene name	SNP	Model	*OR*[a] (95% *CI*)	*P* value	*OR*[a] (95% *CI*)	*P* value	*OR*[a] (95% *CI*)	*P* value
Bcl-2	rs1462129	Additive	**1.30 (1.08–1.55)**	0.0046	1.11 (0.98–1.25)	0.098	**1.16 (1.05–1.28)**	0.002
	rs2551402	Additive	**1.30 (1.08–1.55)**	0.0048	1.10 (0.97–1.24)	0.119	**1.16 (1.05–1.27)**	0.003
CASP9	rs6685648	Additive	1.17 (0.97–1.42)	0.102	**1.14 (1.00–1.31)**	0.046	**1.16 (1.04–1.28)**	0.005
ANKS1B	rs1549102	Additive	**0.80 (0.67–0.96)**	0.016	**0.86 (0.75–0.97)**	0.014	**0.85 (0.77–0.94)**	0.001
	rs11110099	Additive	1.19 (0.99–1.43)	0.057	**1.16 (1.03–1.32)**	0.018	**1.16 (1.06–1.28)**	0.002
	rs10745877	Recessive	**1.60 (1.08–2.36)**	0.019	**1.43 (1.08–1.90)**	0.013	**1.42 (1.15–1.77)**	0.001

Significant ORs in boldface.
[a]Adjusted by age, gender, smoking status and pack year of smoking, asbestos exposure, lung cancer in first degree relatives, prior history of emphysema and hay fever where appropriate.

Results

Characteristics of cases and controls were shown in Table 1. The discovery population included 661 lung cancer cases and 959 controls. We restricted the analysis to self-reported Caucasians to minimize confounding by ethnicity. The mean age of cases and controls were 62.43 and 64.52 years, respectively. Cases had higher percentage of current smokers than controls (22.69% versus 7.92%), higher percentage of cases reported exposure to asbestos (16.94% versus 9.07%) and higher percentage of lung cancer in first-degree relatives (28.9% versus 21.06%). The demographic and exposure profiles of the replication population were also shown in Table 1. Note that smoking status was matched in the replication population as part of the previous GWAS study design (21). In the discovery population, the majority of histology type was adenocarcinoma (61.72%) followed by squamous cell carcinoma (18.00%) (Table 1) and most patients had stages III and IV diseases. Similar distribution of clinical variables was observed in the replication population (Table 1).

A total of 927 SNPs located in 73 genes (Supplemental Table 1 available at *Carcinogenesis* Online) in the apoptotic pathway were screened individually for their association with lung cancer risk. Among the 927 SNPs, 55 showed significant or borderline significant association with lung cancer in multivariate logistic regression models in the discovery stage (Table 2). We then performed a fast-track validation of the top 55 SNPs in a case-control population previously reported in a lung cancer GWAS (21). The validation population comprised of 1154 Caucasian lung cancer cases and 1073 Caucasian healthy controls (21). The mean ages of the cases and controls were 62.08 and 60.48 years, respectively, and as part of the GWAS study design, the cases and controls were matched on sex and smoking status. Among the 55 SNPs, six SNPs were located in three genes: rs2551402 and rs1462129 of *Bcl-2*; rs6685648 of *CASP9*; rs1549102, rs10745877 and rs11110099 of *ANKS1B*, and were replicated showing significant association or borderline significant association. Additive model was the best-fitting model for five SNPs (rs1462129, rs2551402, rs6685648, rs1549102 and rs11110099), and recessive model was the best fit for one SNP (rs10745877) (Table 3). Two SNPs (rs1462129 and rs2551402) of the *Bcl-2* gene were in strong linkage disequilibrium (r^2 = 0.84) and each conferred significantly increased risk with an *OR* of 1.30 (95% *CI* = 1.08–1.55; *P* = 0.0046) in the discovery stage. The association was borderline significant in the replication population, but reached significance in the pooled dataset. The *OR* was 1.16 (95% *CI* = 1.05–1.28; *P* = 0.002) and 1.16 (95% *CI* = 1.05–1.27; *P* = 0.003) for rs1462129 and rs2551402, respectively (Table 3). One SNP (rs6685648) located in the *CASP9* was borderline significant in the discovery stage (*OR* = 1.17; 95% *CI* = 0.97–1.42) in an additive model, and the additive model reached significance in the replication stage (*OR* = 1.14; 95% *CI* = 1.00–1.31; *P* = 0.046), as well as in the pooled dataset (*OR* = 1.16; 95% *CI* = 1.04–1.28; *P* = 0.005) (Table 3). Three SNPs (rs1549102, rs1074587 and rs11110099) of the *ANKS1B* gene were associated with lung cancer risk. Specifically, subjects carrying the variant C allele of the rs1549102 SNP had an *OR* of 0.80 (95% *CI* = 0.67–0.96; *P* = 0.015) in the discovery population, 0.86 (95% *CI* = 0.75–0.97; *P* = 0.014) in the replication stage and 0.85 (95% *CI* = 0.77–0.94; *P* = 0.001) in the pooled dataset (Table 3). The C allele of the rs11110099 was borderline significant (*OR* = 1.19; 95% *CI* = 0.99–1.43; *P* = 0.057)

Table IV. Number of unfavorable genotypes and lung cancer risk

	Cases, n(%)	Controls, n(%)	*OR*[a] (95% *CI*)	*P* value
Discovery				
0	20 (3.03)	64 (6.68)	Ref.	
1	177 (26.82)	279 (29.12)	**2.22 (1.08–4.57)**	0.03
2	266 (40.30)	409 (42.69)	**2.70 (1.33–5.49)**	0.006
≥3	197 (29.85)	206 (21.50)	**4.13 (2.00–8.52)**	0.0001
P trend			6.05E-06	
Replication				
0	38 (3.32)	50 (4.72)	Ref.	
1	294 (25.70)	327 (30.85)	1.26 (0.78–2.01)	0.343
2	513 (44.84)	477 (45.00)	1.57 (0.99–2.48)	0.056
≥3	299 (26.14)	206 (19.43)	**2.13 (1.32–3.44)**	0.002
P trend			4.36E-06	
Pooled				
0	58 (3.22)	114 (5.65)	Ref.	
1	471 (26.11)	606 (30.03)	**1.61 (1.11–2.34)**	0.01
2	779 (43.18)	886 (43.90)	**1.87 (1.30–2.69)**	0.0008
≥3	496 (27.49)	412 (20.42)	**2.72 (1.87–3.97)**	1.89E-07
P trend			1.41E-10	

Significant ORs in boldface.
[a]Adjusted for age, gender, smoking status, pack year of smoking, asbestos exposure, lung cancer in first-degree relatives, prior history of emphysema and hay fever where appropriate.

in the discovery stage and the increased risk was observed in the replication stage (*OR* = 1.16; 95% *CI* = 1.03–1.32; *P* = 0.018). Recessive model was the best fit for rs10745877 with subjects carrying two copies of the G allele at 1.60-fold increased risk (95% *CI* = 1.08–2.36; *P* = 0.019) in the discovery population and a 1.49-fold increased risk in the replication population (95% *CI* = 1.08–1.90; *P* = 0.013). In the pooled dataset, the *OR* was 1.42 (95% *CI* = 1.15–1.77; *P* = 0.001) (Table 3).

To assess the cumulative effects of the unfavorable genotypes in the pathway, we performed a joint analysis of the replicated SNPs. The unfavorable genotypes were defined as following: rs1462129 (TC and CC), rs6685648 (TC and CC), rs1549102 (AA and AC), rs10745877 (GG) and rs11110099 (AC and CC). In the discovery stage, compared with the reference group of subjects carrying no unfavorable genotypes, those carrying one, two, three or more unfavorable genotypes conferred an increased risk of 2.22 (95% *CI* = 1.08– 4.57, *P* = 0.03), 2.70 (95% *CI* = 1.33–5.49; *P* = 0.006) and 4.13 (95% *CI* = 2.00– 8.52; *P* = 0.0001), respectively, with significant dose-response trend (*P* for trend = 6.05E-06) (Table 4). The joint effects of unfavorable genotypes were validated in the replication population. Specifically, using subjects carrying no unfavorable genotypes as the reference group, the risk progressively elevated in subjects carrying one (*OR* = 1.26; 95% *CI* = 0.78–2.01; *P* = 0.343), two (*OR* = 1.57; 95% *CI* = 0.99–2.48; *P* = 0.056), and three or more (*OR* = 2.13; 95% *CI* = 1.32–3.44; *P* = 0.002) unfavorable genotypes, with a significant dose-response trend (*P* for trend = 4.36E-06). In pooled dataset, the ORs for carrying one, two, and three and more unfavorable genotypes were 1.61 (95% *CI* = 1.11–2.34; *P* = 0.01), 1.87 (95% *CI* = 1.30–2.69; *P* = 0.0008) and 2.72 (95% *CI* = 1.87–3.97; *P* = 1.89E-07), respectively (*P* for trend = 1.41E-10).

We correlated the SNPs with lung cancer risk factors, such as smoking, asbestos exposure, family history etc., but did not observe any associations between SNPs and these variables (results data not shown).

Haplotypes of *Bcl-2 and ANKS1B* showed significant association with lung cancer risk in the discovery stage. Specifically, for *Bcl-2*, compared with the most common haplotype T_C (in the order of rs1462129 and rs2551402), the haplotype C_A conferred an increased risk of 1.31 (95% *CI* = 1.09–1.57; *P* = 0.0038) (Table 5). This association was consistently observed in the replication population with borderline significance (*OR* = 1.12; 95% *CI* = 0.98–1.27; *P* = 0.085) and significant in the pooled dataset (*OR* = 1.17; 95% *CI* = 1.06–1.30;

Table V. Haplotype association with lung cancer risk

Haplotype	Cases, n(%)	Controls, n(%)	*OR*[a] (95% *CI*)	*P* value
***Bcl-2* (rs1462129-rs2551402)**				
Discovery				
T_C	643 (48.64)	988 (51.51)	Ref.	
T_A	42 (3.18)	60 (3.13)	1.27 (0.76–2.12)	0.365
C_C	17 (1.29)	18 (0.94)	1.56 (0.68–3.60)	0.297
C_A	620 (46.90)	852 (44.42)	**1.31 (1.09–1.57)**	0.0040
Replication				
T_C	1104 (48.34)	1078 (50.61)	Ref.	
T_A	59 (2.58)	71 (3.33)	0.82 (0.57–1.19)	0.298
C_C	21 (0.92)	24 (1.13)	0.82 (0.44–1.53)	0.540
C_A	1100 (48.16)	957 (44.93)	1.12 (0.98–1.27)	0.085
Pooled				
T_C	1747 (48.45)	2066 (51.04)	Ref.	
T_A	101 (2.80)	131 (3.24)	0.98 (0.73–1.30)	0.879
C_C	38 (1.05)	42 (1.04)	1.06 (0.66–1.70)	0.821
C_A	1720 (47.70)	1809 (44.69)	**1.17 (1.06–1.30)**	0.001
***ANKS1B* (rs1549102-rs10745877-rs11110099)**				
Discovery				
A_A_A	299 (33.00)	459 (33.31)	Ref.	
A_A_C	43 (4.75)	60 (4.35)	0.84 (0.49–1.44)	0.518
A_G_A	1 (0.11)	1 (0.07)	2.63 (0.08–85.42)	0.586
A_G_C	179 (19.76)	214 (15.53)	1.33 (0.97–1.81)	0.072
C_A_A	210 (23.18)	393 (28.52)	**0.69 (0.52–0.92)**	0.012
C_A_C	46 (5.08)	61 (4.43)	0.88 (0.51–1.50)	0.629
C_G_A	2 (0.22)	4 (0.29)	0.30 (0.04–2.37)	0.254
C_G_C	126 (13.91)	186 (13.50)	0.96 (0.68–1.36)	0.837
Replication				
A_A_A	517 (32.27)	464 (31.22)	Ref.	
A_A_C	78 (4.87)	57 (3.84)	1.34 (0.92–1.95)	0.126
A_G_A	3 (0.19)	1 (0.07)	3.12 (0.31–30.76)	0.329
A_G_C	269 (16.79)	213 (14.33)	1.19 (0.95–1.50)	0.131
C_A_A	403 (25.16)	444 (29.88)	**0.79 (0.65–0.96)**	0.016
C_A_C	87 (5.43)	78 (5.25)	1.04 (0.74–1.47)	0.813
C_G_A	2 (0.12)	4 (0.27)	0.49 (0.08–2.95)	0.437
C_G_C	243 (15.17)	225 (15.14)	0.97 (0.77–1.22)	0.785
Pooled				
A_A_A	816 (32.54)	923 (32.23)	Ref.	
A_A_C	121 (4.82)	117 (4.09)	1.14 (0.85–1.52)	0.394
A_G_A	4 (0.16)	2 (0.06)	2.83 (0.50–16.10)	0.241
A_G_C	448 (17.86)	427 (14.91)	**1.22 (1.02–1.45)**	0.028
C_A_A	613 (24.44)	837 (29.22)	**0.76 (0.66–0.88)**	0.0004
C_A_C	133 (5.30)	139 (4.85)	1.00 (0.76–1.31)	0.981
C_G_A	4 (0.16)	8 (0.28)	0.51 (0.14–1.83)	0.301
C_G_C	369 (14.71)	411 (14.35)	0.93 (0.78–1.12)	0.460

Significant ORs in boldface.

[a]Adjusted for age, gender, smoking status, pack year of smoking, asbestos exposure, lung cancer in first-degree relatives, prior history of emphysema and hay fever where appropriate.

$P = 0.001$). For *ANKS1B*, compared with the most common haplotype A_A_A (in the order of rs1549102, rs10745877 and rs11110099), the OR of haplotypes C_A_A (*OR* = 0.69; 95% *CI* = 0.52–0.92; $P = 0.012$) were significant in the discovery stage. The association with haplotype C_A_A was validated in the replication population (*OR* = 0.79; 95% *CI* = 0.65–0.96; $P = 0.016$). When analyzed using the pooled dataset, the *OR* was 1.22 (95% *CI* = 1.02–1.45; $P = 0.028$) for haplotype A_G_C and 0.76 (95% *CI* = 0.66–0.88; $P = 0.0004$) for haplotype C_A_A, respectively (Table 5).

Discussion

In this study, we systematically assessed the associations of a large panel of SNPs in the apoptotic pathway and lung cancer risk. We first screened 927 SNPs located in 73 apoptotic-pathway genes in a case-control study and then performed a fast-track validation of the significant SNPs in a second study population. We found that 55 SNPs showed significant associations in the discovery population, and six of these SNPs located in three genes (*Bcl-2, CASP9* and *ANKS1B*) were validated in the replication population. The results from cumulative analysis and haplotype analysis further suggested that these genetic variants may influence lung cancer risk jointly, consistent with the polygenic etiology of lung cancer.

Apoptosis, or programmed cell death, is an essential cellular defense mechanism against cancer development (23–25). There are two principal signaling pathways: the extrinsic pathway and the intrinsic pathway (7–11), each regulated by an array of genes whose dysfunctions were commonly identified in various human malignancies. The intrinsic pathway is controlled by members of the Bcl-2 family and mediated by the release of cytochrome c from mitochondria. The release of cytochrome c from the intermembrane space of mitochondrion activates CASP9 through the signal transduced by APAF1. Released cytochrome c interacts with APAF1, proCASP-9 and dATP to form an apoptosome. Once bound to the apoptosome, CASP9 is activated, which subsequently triggers a cascade of effector caspases. The second pathway, the extrinsic pathway/the TNF-related apoptosis inducing ligand pathway is initiated by the binding of death receptors and their corresponding extracellular ligands. The interactions between the ligands and membrane receptors sequentially activate the downstream death-inducing signaling complex, primarily composed of the Fas-associated death domain (FADD) interacting with the death receptors through the homologous death domains on both molecules. This interaction further activates the death effector domain of FADD and activates CASP8, which, in turn, also activates CASP3, the converging effector caspase linking the intrinsic and extrinsic pathways to the same downstream signaling cascades leading to cellular suicide through the autoproteolytic processing of a series of apoptotic caspases.

Previous molecular epidemiologic studies identified SNPs in *CASP9* (12), *CASP8* (13,15,19), *CASP3* and *CASP7* (18), *TGFB1* (17), *CASP5* (15), *DR4* (15), *FASLG* and *IL1B* (14) and *TP53BP1* (16) as susceptibility loci for lung cancer. Most studies adopted a candidate gene approach to evaluate potential functional SNPs in apoptotic-related genes. However, without a replication stage to scrutinize the findings, large number of previous reported associations could be false-positive. Compared with the previous studies, one obvious strength of our study is that the associations found in the discover stage were further replicated in a large replication population. By adding the replication stage to the study design, our study is powered to differentiate true-positive from false-positive findings.

We identified and replicated SNPs located in the *Bcl-2* gene associated with lung cancer risk. The SNP rs1462129 is located in the intron region of the *Bcl-2* and the other SNP, rs2551402, is in strong linkage disequilibrium with rs1462129. *Bcl-2* genes are among the earliest genes that were identified as being involved in the regulation of apoptosis (25). The Bcl-2 protein interacts with a variety of proapoptotic factors to regulate the intrinsic pathway of apoptosis. *Bcl-2* functionally acts as proto-oncogene that promotes tumorigenesis by preventing cell death (26). SNPs and haplotypes of *Bcl-2* have been found to associate with susceptibility to chronic lymphocytic leukemia (27), chronic myeloid leukemia (28) and non-Hodgkin lymphoma (29,30). A total of 12 SNPs of *Bcl-2* were associated with the risk of non-Hodgkin's lymphoma in a large study with 1946 non-Hodgkin's lymphoma cases and 1808 controls (29). In a study of chronic myeloid leukemia (28), among 80 SNPs evaluated in pathways of apoptosis, angiogenesis, myeloid cell growth, interferon signaling and others, only SNPs of *Bcl-2* were found to be associated with disease susceptibility. Our current study is the first to report and replicate *Bcl-2* SNPs influencing lung cancer susceptibility, suggesting the possible etiologic relevance of *Bcl-2* SNPs in lung cancer. Over-expression of *Bcl-2* is observed in many cancers, including lung cancer (31,32). Bcl-2 is expressed relatively early during bronchial preneoplasia (33,34) and it is estimated that 20–50% of non–small cell lung cancer express *Bcl-2* (35–37). It was recently found that loss of Bcl-2 expression was correlated with a more aggressive behavior of non–small cell lung cancer tumors (38). These studies provide biological plausibility that *Bcl-2* is involved in lung carcinogenesis. However, since the two SNPs are all located in the intron region of the *Bcl-2* gene and are probably haplotype tagging SNPs, future fine-mapping and functional studies are warranted to identify the causal SNPs and elucidate the biological mechanisms underlying the observed *Bcl-2* SNP-lung cancer risk association.

We also identified and replicated one SNP (rs6685648) in *CASP9* as lung cancer susceptibility locus. The SNP, rs6685648, is also located in the intron region of *CASP9*. CASP9 is a pro-apoptotic protease integral to the intrinsic apoptotic pathway, responsible for effector caspase activation and apoptosis execution following activation by APAF1 bound to cytochrome c released from mitochondria (39). Inactivation of APAF1 or CASP9 could substitute for p53 loss in promoting the oncogenic transformation of Myc-expressiong cells, suggesting important roles of these proteins in controlling tumor development (40). Park et al. (12) examined four candidate SNPs in the *CASP9* promoter with the risk of lung cancer in a Korean population. Two SNPs (-1263 A > G and -712 C > T) exhibited significant associations with lung cancer risk in single SNP analysis as well as in haplotype analysis. By performing a functional assay, they further demonstrated that the *CASP9* promoter SNPs and their haplotypes had an influence on the *CASP9* promoter activity (12). Kelly et al. (30) identified *CASP9* and 4 other genes among 36 candidate genes in the apoptotic pathway as susceptible genes of non-Hodgkin's lymphoma in gene-based analysis. In SNP level analysis, among 226 SNPs examined, three SNPs in *CASP9* were identified as susceptibility loci. Our findings that a genetic variant in *CASP9* conferred lung cancer risk further strengthened the possible relevance of this gene in cancer etiology.

The third gene with three SNPs replicated is the *ANKS1B* gene (ankyrin repeat and sterile alpha motif domain containing 1B). All three SNPs (rs1549102, rs10745877 and rs11110099) reside in the intron region of the gene. There were no previous reports on whether genetic variants in this gene may be related to cancer risk. Ankyrin repeat is a motif mediating protein–protein interaction (41) and a construct of ankyrin repeat was reported to inhibit Caspase-2 in the complex biological apoptotic signaling network *in vitro* (42). On the other hand, *ANKS1B* is in close proximity to *APAF1* on 12q23. As discussed earlier, *APAF1* is a critical component of apoptosome. The 3′ end of *ANKS1B* is in a107 kb linkage disequilibrium block containing *APAF1*. It is possible that the three SNPs in *ANKS1B* are tagging SNPs that tag causal variants in the nearby *APAF1*, and *APAF1* is the gene in this region that is associated with lung cancer risk.

We performed haplotype analysis to identify additional independent markers for lung cancer that may not be revealed by single SNP association analysis. In our analysis, it appeared that the significant haplotypes in both *Bcl-2* and *ANKS1B* were driven by the significant single SNPs; for example, only the C_A haplotype, consisting of two variant allele that showed increased risk in single SNP analysis (Table 3), exhibited a significantly increased risk of lung cancer compared with the wild-type T_C haplotype; the C_A_A haplotype in *ANKS1B*, containing a variant allele at the first SNP (rs1549102), exhibited a significantly reduced lung cancer risk compared with the

A_A_A wild-type haplotype, which was consistent with the protective effect of variant C allele in rs1549102 in single SNP analysis (Table 3). Thus, the haplotype analysis further confirmed single SNPs identified and provided additional evidence to support the hypothesis that sequence variants in apoptosis pathway are associated with lung cancer risk in our study population.

Taken together, we took a pathway approach to systematically screen a large panel of SNPs in the apoptosis pathway with lung cancer risk. Currently, GWAS have become a favored approach to test the association between genetic variations and disease phenotypes. However, a pathway-based approach still has several advantages. Compared with GWAS, pathway-based approach restricts analyses to SNPs in specific pathways and reduces the number of multiple tests, thereby reducing the number of false-positive findings and promoting the effective power of the study. With large number of independent tests, GWAS requires very large sample size to detect true associations, whereas studies restricted to a pathway permit the use of study populations that are not large enough for use in GWAS.

To better control for confounding from other lung cancer risk factors, in addition to smoking, we adjusted asbestos exposure, lung cancer in first-degree relatives and prior lung diseases in the multivariate model. After adjustment for these factors, the significant associations between SNPs and lung cancer are still preserved, suggesting that the SNPs identified in this study are independent predictors of lung cancer risk. However, residual confounding from other unknown factors could still exist. As stated earlier, compared with previous studies of apoptotic pathway and lung cancer, one strength of current study is that the associations found in the discover stage were further validated in a replication population. By adding the replication stage to the study design, our study is sufficiently powered to scrutinize false-positive findings.

Further, in public health perspective, these SNPs could be incorporated into current lung cancer risk prediction models to refine risk prediction. Given that the magnitude of each single SNP association is only low to modest (with individual *ORs* of less than 1.5), the impact of any single SNP is minimal. Although the effect of any single SNP is trivial, a genomic risk profile combining set of SNPs identified from pathway-based analysis or GWAs could be developed to evaluate the prediction power of multiple SNPs (43). In this way, models with genomic risk panels could be compared with models that incorporate typical epidemiologic and clinical variables. However, to date, such comparisons have revealed a very modest, if any, added value to genomic risk profiles (43–45).

In conclusion, we systematically evaluated the association between genetic variants in or near apoptosis-related genes and lung cancer risk. We identified several putative variants in *Bcl-2*, *CASP9* and *ANKS1B* that affect lung cancer susceptibility. With the replication step, our findings are less probable to be false-positive. The SNPs identified are located in or near key genes known to play important roles in apoptosis regulation, supporting the strong biological relevance of our findings. All the identified variants are intronic SNPs that are probably tagging for other functional SNPs. Future fine-mapping and functional studies are needed to identify the causal variants and elucidate the molecular mechanisms underlying the association of these SNPs with lung cancer risk.

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Supplementary material

Supplementary materials can be found at http://carcin.oxfordjournals.org/.

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References

1. Siegel,R. *et al.* (2012) Cancer statistics, 2010. *CA Cancer J. Clin.*, **62**, 10–29.
2. Godtfredsen,N.S. *et al.* (2005) Effect of smoking reduction on lung cancer risk. *JAMA*, **294**, 1505–1510.
3. Miller,Y.E. *et al.* (2003) Genetic susceptibility to lung cancer. *Semin. Respir. Crit. Care Med.,* **24**, 197–204.
4. Chen,B. *et al.* (2009) TRAIL-R1 polymorphisms and cancer susceptibility: an evidence-based meta-analysis. *Eur. J. Cancer*, **45**, 2598–2605.
5. Thompson,C.B. (1995) Apoptosis is the pathogenesis and treatment of disease. *Science*, **267**, 1456–1462.
6. Raff,M. (1998) Call suicide for beginners. *Nature*, **396**, 119–122.
7. Li,H. *et al.* (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell*, **94**, 491–501.
8. Luo,X. *et al.* (1998) Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell*, **94**, 481–490.
9. Budihardjo,I. *et al.* (1999) Biochemical pathways of caspase activation during apoptosis. *Annu. Rev. Cell Dev. Biol*., **15**, 269–290.
10. Ashkenazi,A. *et al.* (1999) Apoptosis control by death and decoy receptors. *Curr. Opin. Cell Biol.*, **11**, 255–260.
11. Kischkel,F.C. *et al.* (2001) Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8. *J. Biol. Chem.*, **276**, 9–46.
12. Park,J.Y. *et al.* (2006) Caspase 9 promoter polymorphisms and risk of primary lung cancer. *Hum. Mol. Genet.*, **15**, 1963–1971.
13. Son,J.W. *et al.* (2006) Polymorphisms in the caspase-8 gene and the risk of lung cancer. *Cancer Genet. Cytogenet.,* **169**, 121–127.
14. Ter-Minassian,M. *et al.* (2008) Apoptosis gene polymorphisms, age, smoking and the risk of non-small cell lung cancer. *Carcinogenesis*, **29**, 2147–2152.
15. Ulybina,Y.M. *et al.* (2009) Coding polymorphisms in Casp5, Casp8 and DR4 genes may play a role in predisposition to lung cancer. *Cancer Lett.,* **278**, 183–191.
16. Truong,T. *et al.* (2010) International Lung Cancer Consortium: coordinated association study of 10 potential lung cancer susceptibility variants. *Carcinogenesis*, **31**, 625–633.
17. Teixeira,A.L. *et al.* (2011) Influence of TGFB1+869T>C functional polymorphism in non-small cell lung cancer (NSCLC) risk. *J. Cancer Res. Clin. Oncol*., **137**, 435–439.
18. Lee,S.Y. *et al.* (2010) Polymorphisms in the caspase genes and the risk of lung cancer. *J. Thorac. Oncol*., **5**, 1152–1158.
19. Hart,K. *et al.* (2011) A combination of functional polymorphisms in the CASP8, MMP1, IL10 and SEPS1 genes affects risk of non-small cell lung cancer. *Lung Cancer*, **71**, 123–129.
20. Spitz,M.R. *et al.* (2007) A risk model for prediction of lung cancer. *J. Natl. Cancer Inst.*, **99**, 715–726.
21. Amos,C.I. *et al.* (2008) Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. *Nat. Genet.*, **40**, 616–622.
22. Li,M. *et al.* (2008) Evaluation of coverage variation of SNP chips for genome-wide association studies. *Eur. J. Hum. Genet*., **16**, 635–643.
23. Evan,G. *et al.* (1998) A matter of life and cell death. *Science,* **281**, 1317–1322.
24. Evan,G.I. *et al.* (2001) Proliferation, cell cycle and apoptosis in cancer. *Nature*, **411**, 342–348.
25. Cotter,T.G. (2009) Apoptosis and cancer: the genesis of a research field. *Nat. Rev. Cancer*, **9**, 501–507.
26. Hanahan,D. *et al.* (2000) The hallmarks of cancer. *Cell*, **100**, 57–70.
27. Enjuanes,A. *et al.* (2008) Genetic variants in apoptosis and immunoregulation-related genes are associated with risk of chronic lymphocytic leukemia. *Cancer Res.*, **68**, 10178–10186.
28. Kim,D.H. *et al.* (2009) Genetic variants in the candidate genes of the apoptosis pathway and susceptibility to chronic myeloid leukemia. *Blood*, **113**, 2517–2525.
29. Morton,L.M. *et al.* (2009) Risk of non-Hodgkin lymphoma associated with germline variation in genes that regulate the cell cycle, apoptosis, and lymphocyte development. *Cancer Epidemiol. Biomarkers Prev.*, **18**, 1259–1270.
30. Kelly,J.L. *et al.* (2010) Germline variation in apoptosis pathway genes and risk of non-Hodgkin lymphoma. *Cancer Epidemiol. Biomarkers Prev.,* **19**, 2847–2858.
31. Shivapurkar,N. *et al.* (2003) Apoptosis and lung cancer: a review. *J. Cell Biochem.*, **88**, 885–898.
32. Thomadaki,H. *et al.* (2006) BCL2 family of apoptosis-related genes: functions and clinical implications in cancer. *Crit. Rev. Clin. Lab. Sci.*, **43**, 1–67.

33. Ferron,P.E. *et al.* (1997) Combined and sequential expression of p53, Rb, Ras and Bcl-2 in bronchial preneoplastic lesions. *Tumori*, **83**, 587–593.
34. Kalomenidis,I. *et al.* (2001) Combined expression of p53, Bcl-2, and p21WAF-1 proteins in lung cancer and premalignant lesions: association with clinical characteristics. *Lung*, **179**, 265–278.
35. Hu,Y. *et al.* (2004) Antitumor efficacy of oblimersen Bcl-2 antisense oligonucleotide alone and in combination with vinorelbine in xenograft models of human non-small cell lung cancer. *Clin. Cancer Res.*, **10**, 7662–7670.
36. Martin,B. *et al.* (2003) Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. *Br. J. Cancer*, **89**, 55–64.
37. Yaren,A. *et al.* (2006) Bax, bcl-2 and c-kit expression in non-small-cell lung cancer and their effects on prognosis. *Int. J. Clin. Pract.*, **60**, 675–682.
38. Anagnostou,V.K. *et al.* (2010) High expression of BCL-2 predicts favorable outcome in non-small cell lung cancer patients with non squamous histology. *BMC Cancer*, **10**, 186.
39. Allan,L.A. *et al.* (2009) Apoptosis and autophagy: regulation of caspase-9 by phosphorylation. *FEBS J.*, **276**, 6063–6673.
40. Soengas,M.S. *et al.* (1999) Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. *Science*, **284**, 156–159.
41. Li,J. *et al.* (2006) Ankyrin repeat: a unique motif mediating protein-protein interactions. *Biochemistry*, **45**, 15168–15178.
42. Schweizer,A. *et al.* (2007) Inhibition of caspase-2 by a designed ankyrin repeat protein: specificity, structure, and inhibition mechanism. *Structure,* **15**, 625–636.
43. Stadler,Z.K. *et al.* (2010) Genome-wide association studies of cancer. *J. Clin. Oncol.*, **28**, 4255–4267.
44. Gail,M. H. (2009) Value of adding single-nucleotide polymorphism genotypes to breast cancer risk model. *J. Natl. Cancer Inst.* **101**, 959–963.
45. Wacholder,S. *et al.* (2010) Performance of common genetic variants in breast-cancer risk models. *N. Engl. J. Med.,* **362**, 986–993.

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