GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

STAT3 gene polymorphisms and susceptibility to non-small cell lung cancer

B. Jiang[1], Z.Z. Zhu[2], F. Liu[3], L.J. Yang[4], W.Y. Zhang[3], H.H. Yuan[3], J.G. Wang[3], X.H. Hu[3] and G. Huang[1]

[1]Department of Nuclear Medicine, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, P.R. China
[2]Department of Oncology, No. 113 Hospital of People's Liberation Army, Ningbo, P.R. China
[3]Department of Oncology, No. 3 People's Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, P.R. China
[4]Department of Oncology, Changhai Hospital, Second Military Medical University, Shanghai, P.R. China

Corresponding author: G. Huang
E-mail: gang_huang@163.com

Genet. Mol. Res. 10 (3): 1856-1865 (2011)
Received September 20, 2010
Accepted May 2, 2011
Published August 26, 2011
DOI http://dx.doi.org/10.4238/vol10-3gmr1071

ABSTRACT. Signal transducer and activator of transcription protein 3 (STAT3) has been implicated in cancer development and is recognized as a type of oncogene. However, association studies of single nucleotide polymorphisms (SNPs) in the *STAT3* gene with cancer risk are rare and not available for lung cancer. We examined whether *STAT3* polymorphisms are associated with the risk of non-small cell lung cancer (NSCLC). Eight SNPs in the *STAT3* gene were genotyped by TaqMan assays in 326 NSCLC cases and 432 controls in a Chinese population. Significant decreased risk of NSCLC was observed for carriers of minor alleles rs4796793 (odds ratio (OR) = 0.68, 95% confidence interval (CI) = 0.51-0.92), rs7211777 (OR = 0.67, 95%CI = 0.50-0.90), rs12949918 (OR = 0.73, 95%CI = 0.54-0.97), rs744166 (OR = 0.69, 95%CI = 0.51-0.92), rs9912773 (OR = 0.75, 95%CI = 0.55-0.98), and rs3869550 (OR = 0.70, 95%CI = 0.53-0.94). The GGCGGC haplotype, comprised of minor alleles of the six NSCLC-associated SNPs,

had a 0.78-fold (95%CI = 0.62-0.97) significantly decreased risk of NSCLC, as compared to the most common haplotype of CATACT. Stratification analyses by clinical stage showed that the trend for the association between *STAT3* polymorphisms and NSCLC risk was present both for stage I/II and stage III/IV, and appeared moderately stronger for stage III/IV. We conclude that polymorphisms in the *STAT3* gene may have a protective role in the development of NSCLC, particular of stage III/IV NSCLC.

Key words: Non-small cell lung cancer; Polymorphisms; STAT3

INTRODUCTION

Lung cancer, predominantly non-small cell lung cancer (NSCLC), is the leading cause of cancer-related deaths worldwide (Parkin et al., 2005). In mainland China, the mortality rate for lung cancer is 41.8 for men and 19.3 for women per million individuals every year (Yang et al., 2004). Smoking has been established to be a major risk factor for lung cancer (Shields, 2002). However, not all individuals exposed to tobacco smoke develop lung cancer, suggesting that genetic susceptibility plays an important role in lung carcinogenesis (Scagliotti et al., 2009).

Signal transducer and activator of transcription protein 3 (STAT3), a member of a transcription factor family that mediates various biological responses induced by cytokines and growth factors (Turkson, 2004), is implicated in cancer development and progression and has, therefore, been recognized as a type of oncogene (Darnell, 2005; Fletcher et al., 2009; Yu et al., 2009). STAT3 participates in a series of tumorigenic cellular processes such as cell proliferation, survival, apoptosis, angiogenesis, and immune responses (Karin, 2006; Grivennikov and Karin, 2010). Constitutively activated STAT3 activity has been routinely observed in multiple human cancers including lung, gastric, skin, head and neck, ovarian, breast, colon, and prostate (Bromberg et al., 1999; Bowman et al., 2000; Yu and Jove, 2004; Hsieh et al., 2005; Lin et al., 2005; Yin et al., 2006; Sansone et al., 2007; Abdulghani et al., 2008). *In vitro* and animal models have shown that inactivation of STAT3 inhibited carcinogenesis and the growth of established tumors, while enhancing expression of this gene promoted tumor incidence and growth (Yu and Jove, 2004; Jing et al., 2006; Siddiquee et al., 2007a,b; Weerasinghe et al., 2007; Bollrath et al., 2009). Moreover, activation of STAT3 has been associated with advanced stages of prostate cancer (Horinaga et al., 2005) and metastatic progression of several different cancer types, including lung cancer (Dauer et al., 2005).

Despite the important role of STAT3 in cancer development and progression, association studies on single nucleotide polymorphisms (SNPs) in the *STAT3* gene with risk of cancer have been rare (Vaclavicek et al., 2007) and there is no data on *STAT3* polymorphisms and risk of lung cancer. It has been reported that overexpression of STAT3 is correlated with growth, survival, and radioresistance of NSCLC cells, and that STAT3 inhibition could inhibit tumorigenic ability and enhance the radiosensitivity of NSCLC cells both *in vitro* and *in vivo* (Yin et al., 2010; Hsu et al., 2011). Moreover, *STAT3* polymorphisms have been shown to correlate with STAT3 expression and therapy response in certain malignancies (Ito et al., 2007; Kreil et al., 2010). Therefore, identification of *STAT3* polymorphisms that are associated with NSCLC risk (either increased or decreased) is important, because it could help predict therapy response, particularly to EGFR inhibitors as STAT3 has been shown to be required

to mediate the oncogenic effects of EGFR in NSCLC (Lai and Johnson, 2010). In the present study, we examined the association between common polymorphisms in the *STAT3* gene and risk of NSCLC in a case-control study of 326 cases with NSCLC and 432 controls frequency-matched to the cases by gender, age and cigarette smoking in a Chinese population. We also examined whether the potential association of *STAT3* polymorphisms with NSCLC cancer risk differs according to clinical stage status.

MATERIAL AND METHODS

Subjects

This hospital-based case-control study consisted of 326 individuals with NSCLC and 432 as cancer-free controls. All cases were prospectively recruited from No. 3 People's Hospital, Shanghai Jiaotong University, and Changhai Hospital, Second Military Medical University, Shanghai, China, between July 2005 and April 2008. They were newly diagnosed and histopathologically confirmed, without prior history of other cancers or previous chemotherapy or radiotherapy. Controls were randomly selected from non-cancer patients admitted to the same hospitals during the same period when the case patients were recruited. The control subjects were frequency-matched to the cases by gender, age (±5 years) and cigarette smoking. Clinical data were systematically recorded at entry, including age, gender, smoking history, histology type, and clinical stage. An ever-smoker was defined as a smoker of at least 1 cigarette per day for 6 months or longer. Written informed consent was obtained from each subject. This study was approved by the Ethics Review Committee of the Institutional Review Board of the two participating hospitals.

SNP selection and genotyping

The SNPs in the *STAT3* gene that we examined included rs4796793, rs7211777, rs12949918, rs744166, rs9912773, rs3869550, rs2293152, and rs1053004, which span 76 kb of the *STAT3* gene locus from 5'-flanking region to 3'UTR. These SNPs were selected based on i) previously published associations with various diseases (Ito et al., 2007; Vaclavicek et al., 2007; Barrett et al., 2008), thus increasing the chance of selecting SNPs with functional consequences, and ii) minor allele frequency of ≥0.1 in the Chinese population, using HapMap and National Center for Biotechnology Information dbSNP public databases.

Genomic DNA was isolated from blood leukocytes by proteinase K digestion and phenol/chloroform extraction. All 8 SNPs in *STAT3* were genotyped using pre-designed TaqMan SNP genotyping assays, which were ordered from Applied Biosystems (Foster City, CA, USA): C_27977213_10 (rs4796793), C_1952182_10 (rs7211777), C_11628926_10 (rs12949918), C_3140282_10 (rs744166), C_11628917_10 (rs9912773), C_7530575_10 (rs3869550), C_3140302_1_ (rs2293152), and C_1795285_1_ (rs1053004). Information on assay conditions, polymerase chain reaction (PCR) primers, and probes is available upon request (https://products.appliedbiosystems.com/ab/en/US/adirect/ab). As a lab internal quality control, four human DNA controls as well as no-template controls were run with study samples for each assay. All genotyping assays were performed on the 7900HT Fast Real-Time PCR System, and the resulting data were analyzed using the SDS 2.2 software

(Applied Biosystems). The call rate was ≥99.6% for each SNP tested. Approximately 5% blind quality control samples from 2 individuals were interspersed with the study samples, achieving ≥99.5% concordance.

Statistical analyses

Differences in age, gender, and cigarette smoking between NSCLC cases and controls were evaluated using the χ^2 test. Departure from Hardy-Weinberg equilibrium (HWE) among controls was assessed using the asymptotic Pearson χ^2 test. The pair-wise linkage disequilibrium (LD) between the SNPs in *STAT3* was quantified using the Haploview 4.2 software (Barrett et al., 2005). The *STAT3* haplotypes were reconstructed from genotype data using the Phase 2.1 software (Stephens et al., 2001). The associations of *STAT3* genotypes and haplotypes with NSCLC risk were estimated by computing the odds ratios (ORs) and 95% confidence intervals (CIs) from multivariate logistic regression analyses with adjustment for age, gender and cigarette smoking. All tests were two-sided and a P value of less than 0.05 was considered to be significant. Statistical analyses were conducted using the Stata 10.1 software (Stata Corporation, College Station, TX, USA).

RESULTS

The general characteristics of the NSCLC cases and controls are shown in Table 1. As expected, no significant differences between cases and controls were found in the distributions of age, gender or cigarette smoking. A total of 202 adenocarcinomas, 98 squamous cell carcinomas and 26 large cell carcinomas were included in the study. The clinical stage distribution of these cases was as follows: 88 stage I, 56 stage II, 119 stage III, and 63 stage IV.

Table 1. Characteristics of NSCLC patients and control subjects.

Variable	Controls (N = 432)	Cases (N = 326)	P
Age (years)			
≤60	223 (51.6%)	167 (51.2%)	
>60	209 (48.4%)	159 (48.8%)	0.92
Gender			
Female	140 (32.4%)	104 (31.9%)	
Male	292 (67.6%)	222 (68.1%)	0.88
Cigarette smoking			
Never	189 (43.7%)	138 (42.3%)	
Ever	243 (56.3%)	188 (57.7%)	0.70
Histology type			
Adenocarcinoma		202 (61.9%)	
Squamous cell carcinoma		98 (30.1%)	
Large cell carcinoma		26 (8.0%)	
Clinical stage			
I		88 (27.0%)	
II		56 (17.2%)	
III		119 (36.5%)	
IV		63 (19.3%)	

Data are reported as number with percentage in parentheses.

All SNPs tested were in HWE among control subjects. As shown in Table 2, a significant decrease in NSCLC risk was observed among carriers of minor allele of

rs4796793 (OR = 0.68, 95%CI = 0.51-0.92), rs7211777 (OR = 0.67, 95%CI = 0.50-0.90), rs12949918 (OR = 0.73, 95%CI = 0.54-0.97), rs744166 (OR = 0.69, 95%CI = 0.51-0.92), rs9912773 (OR = 0.75, 95%CI = 0.55-0.98), and rs3869550 (OR = 0.70, 95%CI = 0.53-0.94), compared to their homozygote carriers of common alleles. No significant associations with NSCLC risk were observed for the remaining two SNPs, rs2293152 and rs1053004. The interactions between rs4796793, rs7211777, rs12949918, rs744166, rs9912773, or rs3869550 and NSCLC risk factors, including age, gender, and cigarette smoking, in relation to NSCLC risk, were not statistically significant (data not shown).

Table 2. Association of *STAT3* SNP genotypes and haplotypes with NSCLC risk.

SNP	Controls N (%)	Cases N (%)	OR (95%CI)†
rs4796793			
CC	159 (36.9)	150 (46.0)	1.00 (Reference)
CG	205 (47.6)	136 (41.7)	0.70 (0.51-0.96)
GG	67 (15.5)	40 (12.3)	0.63 (0.40-0.99)
CG or GG	272 (63.1)	176 (54.0)	0.68 (0.51-0.92)
rs7211777			
AA	158 (36.7)	151 (46.3)	1.00 (Reference)
AG	205 (47.5)	135 (41.4)	0.69 (0.50-0.94)
GG	68 (15.8)	40 (12.3)	0.61 (0.39-0.96)
AG or GG	273 (63.3)	175 (53.7)	0.67 (0.50-0.90)
rs12949918			
TT	161 (37.5)	147 (45.2)	1.00 (Reference)
TC	202 (47.1)	143 (44.0)	0.78 (0.57-1.06)
CC	66 (15.4)	35 (10.8)	0.58 (0.36-0.92)
TC or CC	268 (62.5)	178 (54.8)	0.73 (0.54-0.97)
rs744166			
AA	160 (37.0)	150 (46.0)	1.00 (Reference)
AG	206 (47.7)	137 (42.0)	0.71 (0.52-0.97)
GG	66 (15.3)	39 (12.0)	0.63 (0.40-0.99)
AG or GG	272 (63.0)	176 (54.0)	0.69 (0.51-0.92)
rs9912773			
CC	172 (39.8)	152 (46.6)	1.00 (Reference)
CG	198 (45.8)	136 (41.7)	0.78 (0.57-1.05)
GG	62 (14.4)	38 (11.7)	0.67 (0.42-1.07)
CG or GG	260 (60.2)	174 (53.4)	0.75 (0.55-0.98)
rs3869550			
TT	161 (37.3)	149 (45.7)	1.00 (Reference)
TC	205 (47.4)	137 (42.0)	0.72 (0.53-0.99)
CC	66 (15.3)	40 (12.3)	0.65 (0.41-1.02)
TC or CC	271 (62.7)	177 (54.3)	0.70 (0.53-0.94)
rs2293152			
CC	119 (27.6)	82 (25.2)	1.00 (Reference)
CG	211 (48.8)	165 (50.8)	1.14 (0.81-1.62)
GG	102 (23.6)	78 (24.0)	1.11 (0.74-1.68)
CG or GG	313 (72.4)	243 (74.8)	1.13 (0.81-1.57)
rs1053004			
AA	173 (40.1)	148 (45.4)	1.00 (Reference)
AG	205 (47.4)	136 (41.7)	0.78 (0.57-1.06)
GG	54 (12.5)	42 (12.9)	0.91 (0.57-1.44)
AG or GG	259 (59.9)	178 (54.6)	0.81 (0.60-1.08)
Haplotype‡			
CATACT	517 (59.8)	424 (65.0)	1.00 (Reference)
GGCGGC	312 (36.1)	199 (30.5)	0.78 (0.62-0.97)
GGCAGC	15 (1.8)	11 (1.7)	0.89 (0.40-1.96)
Other haplotypes§	20 (2.3)	18 (2.8)	1.10 (0.57-2.10)

†Adjusted for age, gender and cigarette smoking; ‡Order of polymorphisms = rs4796793, rs7211777, rs12949918, rs744166, rs9912773, and rs3869550; §All other haplotypes with a frequency of <1% in either cases or controls. SNP = single nucleotide polymorphism; OR = odds ratio; 95%CI = confidence interval at 95%.

LD analysis, based on genotype data of the control subjects, revealed that the six NSCLC-associated SNPs (rs4796793, rs7211777, rs12949918, rs744166, rs9912773, and rs3869550) were in strong LD (D' ≥ 0.96, $r^2 \geq 0.88$), and formed a haplotype block in the Chinese population (Figure 1). Haplotype association analysis showed that the GGCGGC haplotype, comprised of minor alleles of the six NSCLC-associated SNPs, was associated with a significantly decreased risk of NSCLC (OR = 0.78, 95%CI = 0.62-0.97), as compared to the most common haplotype of CATACT (Table 2).

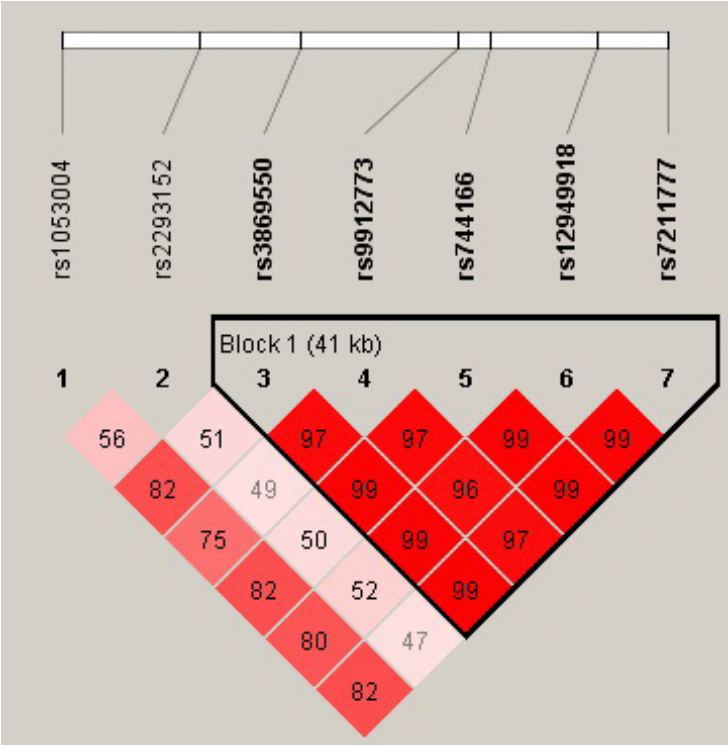

Figure 1. The linkage disequilibrium (LD) block structure observed in the *STAT3* gene using the genotype data from the control sample. Each square box indicates the pairwise magnitudes of LD.

Stratification analyses by clinical stage showed that the trend for the association between *STAT3* polymorphisms and NSCLC risk was present both for stage I/II and stage III/IV, and appeared moderately stronger for stage III/IV (Table 3). The ORs for stage III/IV NSCLC were 0.66 (95%CI = 0.46-0.94), 0.65 (95%CI = 0.46-0.93), 0.67 (95%CI = 0.47-0.96), 0.68 (95%CI = 0.48-0.97), 0.68 (95%CI = 0.48-0.98), and 0.67 (95%CI = 0.47-0.95) for carriers of minor allele of rs4796793, rs7211777, rs12949918, rs744166, rs9912773, and rs3869550, respectively. Similarly, the minor allele haplotype of GGCGGC had a 0.72-fold (95%CI = 0.55-0.94) decreased risk of stage III/IV NSCLC. No significant associations of individual SNPs or haplotypes with NSCLC were observed when stratified by age, gender, cigarette smoking, and histology type (data not shown).

Table 3. Association of *STAT3* gene polymorphisms with NSCLC risk, categorized by clinical stage.

SNP	Controls N (%)	Cases			
		Clinical stage I/II		Clinical stage III/IV	
		N (%)	OR (95%CI)†	N (%)	OR (95%CI)†
rs4796793					
CC	159 (36.9)	65 (45.1)	1.00 (Reference)	85 (46.7)	1.00 (Reference)
CG or GG	272 (63.1)	79 (54.9)	0.72 (0.49-1.06)	97 (53.3)	0.66 (0.46-0.94)
rs7211777					
AA	158 (36.7)	66 (45.8)	1.00 (Reference)	85 (46.7)	1.00 (Reference)
AG or GG	273 (63.3)	78 (54.2)	0.70 (0.47-1.02)	97 (53.3)	0.65 (0.46-0.93)
rs12949918					
TT	161 (37.5)	64 (44.8)	1.00 (Reference)	83 (45.6)	1.00 (Reference)
TC or CC	268 (62.5)	79 (55.2)	0.75 (0.51-1.11)	99 (54.4)	0.67 (0.47-0.96)
rs744166					
AA	160 (37.0)	66 (45.8)	1.00 (Reference)	84 (46.2)	1.00 (Reference)
AG or GG	272 (63.0)	78 (54.2)	0.71 (0.48-1.04)	98 (53.9)	0.68 (0.48-0.97)
rs9912773					
CC	172 (39.8)	65 (45.1)	1.00 (Reference)	87 (47.8)	1.00 (Reference)
CG or GG	260 (60.2)	79 (54.9)	0.82 (0.56-1.20)	95 (52.2)	0.68 (0.48-0.98)
rs3869550					
TT	161 (37.3)	64 (44.4)	1.00 (Reference)	85 (46.7)	1.00 (Reference)
TC or CC	271 (62.7)	80 (55.6)	0.76 (0.51-1.11)	97 (53.3)	0.67 (0.47-0.95)
Haplotype‡					
CATACT	517 (59.8)	182 (63.3)	1.00 (Reference)	242 (66.5)	1.00 (Reference)
GGCGGC	312 (36.2)	94 (32.6)	0.86 (0.64-1.14)	105 (28.9)	0.72 (0.55-0.94)
GGCAGC	15 (1.7)	3 (1.0)	0.62 (0.18-2.17)	8 (2.1)	1.07 (0.44-2.57)
Other haplotypes§	20 (2.3)	9 (3.1)	1.18 (0.52-2.65)	9 (2.5)	1.00 (0.45-2.23)

†Adjusted for age, gender and cigarette smoking; ‡Order of polymorphisms = rs4796793, rs7211777, rs12949918, rs744166, rs9912773, and rs3869550; §All other haplotypes with a frequency of <1% in either cases or controls.

DISCUSSION

STAT3 transcriptional factor has been found to play pivotal roles in various aspects of tumor development and progression in a number of malignancies (Darnell, 2005; Fletcher et al., 2009; Yu et al., 2009). However, there is only one article that has investigated *STAT3* polymorphisms in relation to cancer risk (Vaclavicek et al., 2007). Vaclavicek et al. (2007) reported that, although rs2293152 and rs7211777 in the *STAT3* gene were not individually associated with familial breast cancer, the haplotypes characterized by rs7211777 and a neighboring SNP rs6503691 in the *STAT5B* gene were found to modify the risk of familial breast cancer. In the present study, we showed for the first time that SNPs in the *STAT3* gene were associated with decreased NSCLC risk. In particular, carriers of the minor alleles at SNP rs4796793, rs7211777, rs12949918, rs744166, rs9912773, or rs3869550 had a decreased risk of NSCLC, with ORs ranging from 0.67 to 0.75. The GGCGGC haplotype comprised of all minor alleles at rs4796793, rs7211777, rs12949918, rs744166, rs9912773, and rs3869550 conferred a decreased risk of NSCLC with an OR of 0.78, supporting our findings based on individual SNPs. In a study of Crohn's disease, a significant association was observed between the rs744166 minor allele and decreased risk for Crohn's disease (Ferguson et al., 2010). Given that Crohn's disease has been recognized to have an increased risk of developing colorectal cancer, it is likely that rs744166 minor allele is a protective factor for colorectal cancer, which is consistent with the findings on NSCLC in the present study.

Six *STAT3* SNPs (rs4796793, rs7211777, rs12949918, rs744166, rs9912773, and

rs3869550) and the haplotype characterized by them were found to modify the risk of NSCLC in the present study. Due to the strong LD (D' ≥ 0.96, $r^2 \geq 0.88$), it was impossible to identify a single causative SNP among the six. The exact underlying mechanism(s) responsible for our findings that *STAT3* polymorphisms conferred decreased NSCLC risk remains unclear. All these SNPs are located in the 5' region upstream and the introns of the *STAT3* gene, suggesting that they may modify NSCLC risk by affecting gene transcriptional regulation and mRNA splicing. *In vitro* studies have shown that the genotypes of the SNP rs4796793 affected *STAT3* mRNA levels, with the minor allele having a lower *STAT3* expression (Ito et al., 2007). In the present study, rs4796793 was in strong LD (D' ≥ 0.97, $r^2 \geq 0.95$) with the other five NSCLC-associated SNPs (rs7211777, rs12949918, rs744166, rs9912773, and rs3869550). Thus, the *STAT3* haplotype containing the minor alleles of the six SNPs may also correlate with lower *STAT3* expression, and thus exert a protective effect on cancer development and progression. In line with this hypothesis, we observed that the six *STAT3* SNPs and the haplotype characterized by them had a decreased risk for NSCLC, particular for stage III/IV NSCLC.

Strengths of this study include a relatively large sample size, good reproducibility of the genotyping results and careful study design of frequency-matching controls to NSCLC cases by age, gender and cigarette smoking. Limitations in our study should be noted. Design of hospital-based case-control studies may result in selection bias of participants, especially for controls who were cancer-free subjects from the same hospitals as cases. However, considering the high similarity in genotype and allele frequency between our controls and that in the HapMap Chinese database, it is reasonable to assume that the controls of our study represent the general Chinese population. Additionally, although up to 8 SNPs across the *STAT3* gene were selected in the present study, the inclusion of SNPs was still limited and the SNPs examined may be in LD with untested genetic polymorphisms contributing to the results.

In summary, the *STAT3* SNPs rs4796793, rs7211777, rs12949918, rs744166, rs9912773, and rs3869550, and the haplotype characterized by them were associated with decreased NSCLC risk in a Chinese population. Our data provided the first evidence that polymorphisms in the *STAT3* gene may have a protective role in the development of NSCLC, particular of stage III/IV NSCLC. Because *STAT3* polymorphisms have been correlated with therapy response in certain malignancies (Ito et al., 2007; Kreil et al., 2010), the *STAT3* polymorphisms identified to be associated with decreased NSCLC risk in the present study could be helpful to predict how these patients would respond to therapy targeting STAT3 pathways. Future studies are needed to validate our findings and to investigate the potential effects of these SNPs on STAT3 expression and function.

ACKNOWLEDGMENTS

Research supported by the Shanghai Education Committee Foundation Grants (#08YZ47); National Natural Science Foundation of China (#30830038, #30970842); Key Project of Science and Technology Commission of Shanghai Municipality (#08JC1415000, #08410702000, #10JC1410000), and Shanghai Leading Academic Discipline Project (#S30203).

REFERENCES

Abdulghani J, Gu L, Dagvadorj A, Lutz J, et al. (2008). Stat3 promotes metastatic progression of prostate cancer. *Am. J. Pathol.* 172: 1717-1728.

Barrett JC, Fry B, Maller J and Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265.

Barrett JC, Hansoul S, Nicolae DL, Cho JH, et al. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat. Genet.* 40: 955-962.

Bollrath J, Phesse TJ, von Burstin VA, Putoczki T, et al. (2009). gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. *Cancer Cell* 15: 91-102.

Bowman T, Garcia R, Turkson J and Jove R (2000). STATs in oncogenesis. *Oncogene* 19: 2474-2488.

Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, et al. (1999). Stat3 as an oncogene. *Cell* 98: 295-303.

Darnell JE (2005). Validating Stat3 in cancer therapy. *Nat. Med.* 11: 595-596.

Dauer DJ, Ferraro B, Song L, Yu B, et al. (2005). Stat3 regulates genes common to both wound healing and cancer. *Oncogene* 24: 3397-3408.

Ferguson LR, Han DY, Fraser AG, Huebner C, et al. (2010). Genetic factors in chronic inflammation: single nucleotide polymorphisms in the STAT-JAK pathway, susceptibility to DNA damage and Crohn's disease in a New Zealand population. *Mutat. Res.* 690: 108-115.

Fletcher S, Drewry JA, Shahani VM, Page BD, et al. (2009). Molecular disruption of oncogenic signal transducer and activator of transcription 3 (STAT3) protein. *Biochem. Cell Biol.* 87: 825-833.

Grivennikov SI and Karin M (2010). Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. *Cytokine Growth Factor Rev.* 21: 11-19.

Horinaga M, Okita H, Nakashima J, Kanao K, et al. (2005). Clinical and pathologic significance of activation of signal transducer and activator of transcription 3 in prostate cancer. *Urology* 66: 671-675.

Hsieh FC, Cheng G and Lin J (2005). Evaluation of potential Stat3-regulated genes in human breast cancer. *Biochem. Biophys. Res. Commun.* 335: 292-299.

Hsu HS, Huang PI, Chang YL, Tzao C, et al. (2011). Cucurbitacin i inhibits tumorigenic ability and enhances radiochemosensitivity in nonsmall cell lung cancer-derived CD133-positive cells. *Cancer* 117: 2970-2985.

Ito N, Eto M, Nakamura E, Takahashi A, et al. (2007). STAT3 polymorphism predicts interferon-alfa response in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.* 25: 2785-2791.

Jing N, Zhu Q, Yuan P, Li Y, et al. (2006). Targeting signal transducer and activator of transcription 3 with G-quartet oligonucleotides: a potential novel therapy for head and neck cancer. *Mol. Cancer Ther.* 5: 279-286.

Karin M (2006). Nuclear factor-kappaB in cancer development and progression. *Nature* 441: 431-436.

Kreil S, Waghorn K, Ernst T, Chase A, et al. (2010). A polymorphism associated with STAT3 expression and response of chronic myeloid leukemia to interferon alpha. *Haematologica* 95: 148-152.

Lai SY and Johnson FM (2010). Defining the role of the JAK-STAT pathway in head and neck and thoracic malignancies: implications for future therapeutic approaches. *Drug Resist. Updat.* 13: 67-78.

Lin Q, Lai R, Chirieac LR, Li C, et al. (2005). Constitutive activation of JAK3/STAT3 in colon carcinoma tumors and cell lines: inhibition of JAK3/STAT3 signaling induces apoptosis and cell cycle arrest of colon carcinoma cells. *Am. J. Pathol.* 167: 969-980.

Parkin DM, Bray F, Ferlay J and Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J. Clin.* 55: 74-108.

Sansone P, Storci G, Tavolari S, Guarnieri T, et al. (2007). IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. *J. Clin. Invest.* 117: 3988-4002.

Scagliotti GV, Longo M and Novello S (2009). Nonsmall cell lung cancer in never smokers. *Curr. Opin. Oncol.* 21: 99-104.

Shields PG (2002). Molecular epidemiology of smoking and lung cancer. *Oncogene* 21: 6870-6876.

Siddiquee K, Zhang S, Guida WC, Blaskovich MA, et al. (2007a). Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proc. Natl. Acad. Sci. U. S. A.* 104: 7391-7396.

Siddiquee KA, Gunning PT, Glenn M, Katt WP, et al. (2007b). An oxazole-based small-molecule Stat3 inhibitor modulates Stat3 stability and processing and induces antitumor cell effects. *ACS Chem. Biol.* 2: 787-798.

Stephens M, Smith NJ and Donnelly P (2001). A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* 68: 978-989.

Turkson J (2004). STAT proteins as novel targets for cancer drug discovery. *Expert. Opin. Ther. Targets.* 8: 409-422.

Vaclavicek A, Bermejo JL, Schmutzler RK, Sutter C, et al. (2007). Polymorphisms in the Janus kinase 2 (JAK)/signal transducer and activator of transcription (STAT) genes: putative association of the STAT gene region with familial breast cancer. *Endocr. Relat. Cancer* 14: 267-277.

Weerasinghe P, Garcia GE, Zhu Q, Yuan P, et al. (2007). Inhibition of Stat3 activation and tumor growth suppression of non-small cell lung cancer by G-quartet oligonucleotides. *Int. J. Oncol.* 31: 129-136.

Yang L, Parkin DM, Li LD, Chen YD, et al. (2004). Estimation and projection of the national profile of cancer mortality in China: 1991-2005. *Br. J. Cancer* 90: 2157-2166.

Yin W, Cheepala S, Roberts JN, Syson-Chan K, et al. (2006). Active Stat3 is required for survival of human squamous cell carcinoma cells in serum-free conditions. *Mol. Cancer* 5: 15.

Yin ZJ, Jin FG, Liu TG, Fu EQ, et al. (2010). Overexpression of STAT3 potentiates growth, survival, and radioresistance of non-small-cell lung cancer (NSCLC) cells. *J. Surg. Res*. Doi: 10.1016/j.jss.2010.03.053.

Yu H and Jove R (2004). The STATs of cancer - new molecular targets come of age. *Nat. Rev. Cancer* 4: 97-105.

Yu H, Pardoll D and Jove R (2009). STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat. Rev. Cancer* 9: 798-809.

