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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

A 3′-Untranslated Region Polymorphism in *IGF1* Predicts Survival of Non–Small Cell Lung Cancer in a Chinese Population

Mingfeng Zhang[1], Zhibin Hu[1], Jinlin Huang[6], Yongqian Shu[1,2], Juncheng Dai[1], Guangfu Jin[1], Rong Tang[6], Jing Dong[1], Yijiang Chen[3], Lin Xu[4], Xinen Huang[5], and Hongbing Shen[1,2]

Abstract

Purpose: Disruption of the balance of insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP3) has been implicated in the etiology and progression of lung and other cancers. Single nucleotide polymorphisms (SNP) in *IGF1* and *IGFBP3* have been reported to be associated with the expression of the IGF-I/IGFBP3 axis. Therefore, we hypothesized that SNPs in these two genes were associated with lung cancer survival.

Experimental Design: We selected and genotyped 21 tagging and potentially functional SNPs in *IGF1* and *IGFBP3* by using Illumina Goldengate Genotyping Chip in a case cohort of 568 patients diagnosed with non–small cell lung cancer (NSCLC) in a Chinese population. Log-rank test and Cox proportional hazard models were used for the survival analyses.

Results: We found that rs5742714C/G in the 3′-untranslated region of *IGF1* was associated significantly with NSCLC survival after adjustment for demographic and clinicopathologic factors, showing an improved median survival time in patients carrying variant CG/GG genotypes [median survival time, 28.5 months for CG/GG and 23.0 for CC; crude hazard ratio (HR), 0.74; 95% confidence interval (95% CI), 0.57-0.95, and adjusted HR, 0.77; 95% CI, 0.60-0.99]. This protective effect was more predominant for patients receiving surgical operation (HR, 0.58; 95% CI, 0.40-0.85; *P* for heterogeneity test = 0.045), along with a significant multiplicative interaction between variant genotypes and operation status (*P* = 0.028).

Conclusions: Our findings suggest that rs5742714 in *IGF1* may be a genetic modifier for NSCLC prognosis in this Chinese population, especially among patients with surgical operation. *Clin Cancer Res; 16(4); 1236–44. ©2010 AACR.*

Lung cancer, predominantly non–small cell lung cancer (NSCLC), is the leading cause of cancer-related deaths worldwide (1). Despite some advances achieved in the diagnosis and treatment in the last decades, the prognosis of lung cancer remains poor (1). Many studies have revealed that application of specific prognostic biomarkers, incorporated with traditional factors such as age, sex, histologic type, and stage at diagnosis, could largely help to improve prognosis prediction and guide medical care of lung cancer (2).

Authors' Affiliations: [1]Department of Epidemiology and Biostatistics, Cancer Center, Nanjing Medical University, [2]Department of Oncology, Jiangsu Key Discipline of Medicine, and [3]Department of Thoracic Surgery, The First Affiliated Hospital of Nanjing Medical University, and Departments of [4]Thoracic Surgery and [5]Oncology, Jiangsu Cancer Hospital, Nanjing, China; and [6]Berkeley Biotech Inc, Taizhou, Jiangsu, China

Corresponding Author: Hongbing Shen, Department of Epidemiology and Biostatistics, Cancer Center, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, China. Phone: 86-25-8686-2756; Fax: 86-25-8652-7613; E-mail: hbshen@njmu.edu.cn.

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The insulin-like growth factor (IGF) signaling system plays a central role in cellular growth, differentiation, and proliferation (3, 4). Studies have shown that IGF-I is a potent proliferative molecule, affecting almost every cell type via the RAS-mitogen-activated protein kinase signaling pathway, and a powerful antiapoptotic molecule through activation of the phosphatidylinositol-3 kinase–AKT pathway, which ultimately activates downstream transcription factors that regulate gene expression of proliferative, differentiation, and antiapoptotic factors (5, 6). Besides, the bioactivity of IGF-I is modulated by a group of high-affinity IGF-binding proteins (IGFBP1 to 6; refs. 7, 8), and among them, IGFBP3 is the most abundant circulating binding partner for IGFs and shows strong growth-inhibitory effects by binding and subsequently sequestrating IGF-I in the extracellular milieu (9, 10).

Clinical and epidemiologic evidence further indicate that disruption of the balance of IGF-I and IGFBP3 is implicated in the etiology and progression of multiple cancers, revealing that increased levels of IGF-I, reduced levels of IGFBP3, or an increased ratio of IGF-I to IGFBP3 in the circulation is associated with an increased risk as

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Translational Relevance

Although many studies have been conducted to explore the prognostic biomarkers of non–small cell lung cancer (NSCLC), stable markers for clinical outcome prediction are still scarce. Epidemiologic and laboratory-based studies have identified the disruption of the balance of insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP3) as a prognostic factor of lung and other cancers. In this study, we found that variant genotypes of rs5742714C/G in the 3′-untranslated region of *IGF1* were associated with significantly improved NSCLC survival, especially among patients after surgical operation. Our findings offer an opportunity to use *IGF1* polymorphism as a biomarker in prediction of NSCLC outcome and in the identification of individuals with poor prognosis for more aggressive treatments. In addition, this discovery also supports the concept that IGF-I contributes to lung cancer progression and suggests that it may be a potential target for the treatment of lung cancer.

well as a poor prognosis of several common cancers (11–17), and genetic studies have further correlated the single nucleotide polymorphisms (SNP) of *IGF1* and *IGFBP3* with the prognosis of breast cancer (18). With regard to lung cancer, cumulative experimental evidence *in vivo* and *in vitro* suggests that suppression of IGF-I signaling can induce apoptosis and inhibit invasion or metastasis of NSCLC cells (19–22). However, clinical and epidemiologic data are still rare (23, 24), with a doubtful result on IGF-I that high plasma level is associated with a favorable prognosis (23), which seems to conflict with the laboratory studies and the result from some other cancer (16). In addition, no epidemiologic studies to date have evaluated the role of SNPs in the genes of the IGF axis with lung cancer survival.

Given the important role of IGFs in stimulating cellular growth, influencing apoptotic pathway, and inhibiting invasion and metastasis, it is conceivable that SNPs in these genes may have an effect on progression and prognosis of lung cancer. To test this hypothesis, we comprehensively selected 21 common genetic variations in *IGF1* and *IGFBP3* by a strategy of integrating both tagging SNPs and potentially functional SNPs, and evaluated the associations with NSCLC survival among a cohort of 568 NSCLC cases in a Chinese population.

Subjects and Methods

Study population. Our study was approved by the Institutional Review Board of Nanjing Medical University, Nanjing, China. All the patients were newly diagnosed and histopathologically confirmed without prior history of other cancers or previous chemotherapy or radiotherapy, and were prospectively recruited into an ongoing study of lung cancer molecular epidemiology from July 2003 to April 2008. Overall, 828 NSCLC patients with complete follow-ups and clinical information were prospectively recruited from the Cancer Hospital of Jiangsu Province and the First Affiliated Hospital of Nanjing Medical University, Nanjing, China (25, 26). All patients were face-to-face interviewed to collect demographic data and exposure information, including age, sex, and cigarette smoking. Those who smoked <1 cigarette per day and <1 year in their lifetime were defined as nonsmokers; otherwise, they were considered as smokers. Each patient donated 5 mL venous blood after providing a written informed consent. Patients were followed up prospectively every 3 mo from the time of enrollment by personal or family contacts until death or last time of follow-up. The maximum follow-up time was 72 mo (last follow-up in July 2009) and the median follow-up time was 18.8 mo. We selected the patients with adequate DNA sample by checking quality and quantity for Illumina genotyping assay. As a result, 568 NSCLC patients were included and genotyped in this study.

SNP selection and genotyping. Polymorphisms were selected by an approach combining both tagging SNPs and potentially functional SNPs of the *IGF1* and *IGFBP3* genes. All common (minor allele frequency >0.05 in Asians) polymorphisms were searched within nucleotide -2,000 upstream from the start codon of each gene and their 3′-untranslated regions (3′UTR) using the HapMap (last search date, February 2008) and the National Center for Biotechnology Information dbSNPs public databases (build 127). Potentially functional polymorphisms were identified to meet the following criteria: (*a*) located in the 5′-flanking regions, 5′-UTR, 3′-UTR, and coding regions with amino acid changes; (*b*) were shown to be of biological significance according to the literature review; and (*c*) were associated with gene expression and/or cancer risk/survival in previous studies. Tagging SNPs (tSNP) were chosen from genotyped SNPs of Chinese Han Beijing (CHB) in the HapMap database (minor allele frequency ≥0.05, Hardy-Weinberg equilibrium $P \geq 0.05$, and call rate ≥90%) in Haploview 4.1 software on the basis of pairwise linkage disequilibrium (r^2 threshold = 0.8) and with a priority of forcing the potentially functional SNPs in the evaluation. Among the 24 SNPs (13 in *IGF1* and 11 in *IGFBP3*) selected, three SNPs (rs5031032 and rs6220 in *IGF1* and rs6413441 in *IGFBP3*) could not be genotyped because of Illumina design quantity scores <0.5. Therefore, a total of 21 SNPs (11 in *IGF1* and 10 in *IGFBP3*) were genotyped by using the Illumina GoldenGate platform in Taizhou, Jiangsu province, China. Information on assay conditions, primers, and probes is available upon request. Quality control was followed according to the quality criteria (i.e., one blank well and three repeated samples) in our previous studies (27). In the end, 17 SNPs were successfully genotyped with call rates >95% and consistent with those expected from the Hardy-Weinberg equilibrium ($P > 0.05$), whereas three SNPs (rs12579108 of *IGF1*, rs2854744 and rs34480712 of *IGFBP3*) that could

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Table 1. Patient characteristics and clinical features

Variable	Patients *n* = 568 (%)	Deaths *n* = 311	MST (mo)	Log-rank *P*	HR (95% CI)
Age (y)				0.690	
≤60	285 (50.2)	155	24.7		1.00
>60	283 (49.8)	156	25.0		1.05 (0.84-1.31)
Sex				0.805	
Male	434 (76.4)	241	24.7		1.00
Female	134 (23.6)	70	27.8		0.97 (0.74-1.26)
Smoking				0.792	
Never	201 (35.4)	111	24.8		1.00
Ever	367 (64.6)	200	24.3		1.03 (0.82-1.30)
Histology				0.519	
Adenocarcinoma	353 (62.2)	192	26.2		1.00
Squamous cell	184 (32.4)	99	24.0		0.97 (0.76-1.23)
Others*	31 (5.5)	20	17.9		1.28 (0.81-2.03)
Clinical stage				<0.001	
I	144 (25.4)	46	60.4		1.00
II	71 (12.5)	29	47.6		1.37 (0.86-2.19)
III	221 (38.9)	133	19.6		3.04 (2.17-4.26)
IV	132 (23.2)	103	13.4		5.75 (4.03-8.21)
Surgical operation				<0.001	
None	200 (35.2)	155	15.3		1.00
Yes	368 (64.8)	156	36.8		0.33 (0.26-0.41)
Chemotherapy or radiotherapy				0.102	
None	106 (18.7)	47	33.0		1.00
Yes	462 (81.3)	264	23.5		1.30 (0.95-1.77)

*Other carcinomas include large cell, undifferentiated and mixed-cell carcinomas.

not be genotyped and one (rs13241830 in *IGFBP3*) with a call rate of 79.5% and that deviated from Hardy-Weinberg equilibrium ($P = 0.01$) were removed from our further analysis.

Statistical analysis. Deviation of genotype distribution from the Hardy-Weinberg equilibrium for each SNP was tested by a goodness-of-fit χ^2 test. Survival time was calculated from the date of lung cancer diagnosis to the date of death or to last follow-up. The different survival times according to demographic characteristics, clinical features, and *IGF1* and *IGFBP3* SNPs were estimated by using the Kaplan-Meier method and compared by the log-rank test. Mean survival time was presented when the median survival time (MST) could not be calculated. Univariate or multivariate Cox regression analysis was done to determine predictive factors of NSCLC prognosis by estimating the crude hazard ratios (HR), adjusted HRs, and their 95% confidence intervals (95% CI), with adjustment for age, sex, smoking status, histology, stage, surgical operation, and chemotherapy or radiotherapy status. In addition, we used the PHASE 2.1 Bayesian algorithm (28) to infer haplotypes based on the observed genotypes of the SNPs. All the statistical analyses were carried out by Statistical Analysis System software (version 9.1.3, SAS Institute).

Results

Patient characteristics and clinical features. The demographic characteristics and clinical information for the 568 NSCLC patients retained in the study are summarized in Table 1. The median age was 60 years (range, 25-83 years), and there were 434 males (76.4%) and 367 smokers (64.6%). Among these patients, 353 (62.2%) were adenocarcinomas, 184 (32.4%) were squamous cell carcinomas, and the others (31 patients, 5.5%) were large cell, undifferentiated, and mixed-cell carcinomas. In a period of up to 72 months of follow-up, 311 patients died from NSCLC, and 3 died from other causes. For disease-specific survival analysis, the latter were considered as censored data in the analyses. Clinical stage and surgical operation status, but not chemotherapy or radiotherapy status, were significantly associated with survival time (log-rank $P < 0.001$). As the stage increased, the risk of death for NSCLC showed a significant increase in a dose-response manner by using univariate Cox regression analysis (compared with stage I, HR, 1.37; 95% CI, 0.86-2.19 for stage II; HR, 3.04; 95% CI, 2.17-4.26 for stage III; HR, 5.75; 95% CI, 4.03-8.21 for stage IV; and $P < 0.001$ for the trend test). Patients with surgical operation (MST, 36.8 months) had a 67% significantly decreased risk of death (HR, 0.33; 95%

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Table 2. Genotyping of selected SNPs of *IGF1* and *IGFBP3* and their associations with NSCLC survival in genetic models

Gene	SNPs*	Location	No. (%) of genotyping	MAF in patients	HWE†	Cox *P*		
						Additive model	Dominant model	Recessive model
IGF1	rs9919733	5′ near gene	99.7	0.265	1.00	0.242	0.256	0.519
	rs35767	5′ near gene	99.3	0.318	0.53	0.560	0.673	0.566
	rs5742612	5′ near gene	99.8	0.271	0.72	0.329	0.330	0.621
	rs2288377	5′ near gene	97.7	0.272	0.84	0.240	0.238	0.565
	rs12821878	Intron	99.8	0.050	1.00	0.212	0.282	0.011
	rs4764699	Intron	99.8	0.252	0.84	0.432	0.678	0.248
	rs1520220	Intron	99.8	0.417	0.93	0.313	0.228	0.718
	rs6218	3′UTR	99.5	0.240	0.93	0.339	0.522	0.254
	rs6214	3′UTR	99.5	0.478	0.28	0.211	0.760	0.078
	rs5742714	3′UTR	96.4	0.170	0.87	0.013	0.018	0.170
IGFBP3	rs2132572	5′ near gene	100.0	0.198	0.79	0.006	0.037	0.004
	rs2854746	A32G	99.8	0.242	0.65	0.123	0.394	0.026
	rs9282734	P164H	99.8	0.043	1.00	0.540	0.580	0.624
	rs2471551	Intron	100.0	0.044	0.61	0.173	0.192	0.490
	rs3110697	Intron	100.0	0.260	0.48	0.206	0.473	0.076
	rs10255707	Intron	99.5	0.178	0.49	0.023	0.060	0.045
	rs2453839	Intron	99.7	0.226	0.70	0.056	0.048	0.502

Abbreviations: MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.
*All the SNPs served as tagging SNPs except for rs9919733 in *IGF1* and rs2854746 in *IGFBP3*.
†Hardy-Weinberg equilibrium was examined by goodness-of-fit χ^2 test.

CI, 0.26-0.41), compared with those without surgical operation (MST, 15.3 months).

Effects of genetic variants of* IGF1 *and* IGFBP3 *on NSCLC survival. Cox regression analyses were used to access the associations of *IGF1* and *IGFBP3* genotypes with NSCLC survival in different genetic models (Table 2). In the additive model, three polymorphisms were significantly associated with the survival of NSCLC (P = 0.013, 0.006, and 0.023 for rs5742714 of *IGF1*, and rs2132572 and rs10255707 of *IGFBP3*, respectively). As shown in Table 3, however, rs2132572 and rs10255707 of *IGFBP3* were associated with higher risk of death only before adjustment for demographic and clinicopathologic factors (crude HR, 1.28; 95% CI, 1.02-1.61 for rs2132572 variant genotypes in a dominant model, and crude HR, 1.74; 95% CI, 1.01-2.97 for rs10255707 variant genotype in a recessive model). Log-rank test and Cox regression analyses revealed that variant genotypes of *IGF1* rs5742714 CG/GG had a significantly improved survival (MST, 28.5 months; crude HR, 0.74; 95% CI, 0.57-0.95, and adjusted HR, 0.77; 95% CI, 0.60-0.99) compared with the homozygote CC (MST, 23.0 months; Fig. 1A and B).

We carried out haplotype inference using the PHASE 2.1 based on the known genotypes of the SNPs for *IGF1* and *IGFBP3*, respectively. For statistical consideration, we ignored all haplotypes with a frequency <0.05. However, no statistically significant association between the haplotypes of *IGF1* and *IGFBP3* and lung cancer survival was evident (data not shown).

Stepwise cox regression model for NSCLC survival. We further did a multivariate stepwise Cox regression analysis for the effects of demographic characteristics, clinical features, and *IGF1* and *IGFBP3* genotypes on NSCLC survival. Five variables (stage, surgical operation status, chemotherapy or radiotherapy status, smoking status, and *IGF1* rs5742714) were included in the regression model with a significance level of 0.05 for entering and 0.051 for removing a variable (P < 0.001 for stage and surgical operation status; P = 0.014, 0.046, and 0.036 for chemotherapy or radiotherapy status, smoking status, and *IGF1* rs5742714, respectively).

Stratified analyses and interaction. The associations between *IGF1* rs5742714 polymorphism and NSCLC survival were further evaluated by stratified analysis of smoking

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status, histology, stage, surgical operation, and radiotherapy or chemotherapy status. As shown in Table 4, there was no obvious evidence of differentiated association between the protective effects of variant genotypes of rs5742714 and NSCLC survival among different subgroups of smoking status, histology, stage, and radiotherapy or chemotherapy status. However, the decreased risk was more predominant among subjects receiving surgical operation (HR, 0.58; 95% CI, 0.40-0.85, *P* for heterogeneity test = 0.045). Therefore, a gene- surgical operation interaction analysis was carried out (Table 5), and there was a statistically significant multiplicative interaction between the genotypes of rs5742714 and operation status on NSCLC survival (*P* for multiplicative interaction = 0.028). Kaplan-Meier plots of survival by combination of *IGF1* genotypes and operation status in NSCLC-specific survival are shown in Fig. 1C.

We also evaluated the interactions between the genotypes of *IGF1* rs5742714, and *IGFBP3* rs2132572 and rs10255707, but no statistical significance was observed (data not shown).

Discussion

In this lung cancer study with a relatively comprehensive selection of SNPs in *IGF1* and *IGFBP3*, we investigated the effects of 17 tagging and potentially functional SNPs on NSCLC-specific survival. The results indicated that the *IGF1* rs5742714 G allele was associated with a significantly improved survival of NSCLC in a dominant model (crude HR, 0.74; 95% CI, 0.57-0.95 and adjusted HR, 0.77; 95% CI, 0.60-0.99), and this protective effect was more predominant for patients receiving

Table 3. Polymorphisms of *IGF1* and *IGFBP3* genes and NSCLC patients' survival

Locus	Genotype	Patients	Deaths	MST (mo)	Log-rank *P*	Crude HR (95% CI)	Adjusted HR (95% CI)*
IGF1 rs5742714		*n* = 547	*n* = 301				
Codominant model	CC	377	219	23.0		1.00	1.00
	CG	153	76	28.2		0.77 (0.60-1.01)	0.82 (0.63-1.08)
	GG	17	6	26.5†	0.043	0.54 (0.24-1.21)	0.48 (0.21-1.10)
Dominant model	CC	377	219	23.0		1.00	1.00
	CG/GG	170	82	28.5	0.017	0.74 (0.57-0.95)	0.77 (0.60-0.99)
Recessive model	CC/CG	530	295	24.3		1.00	1.00
	GG	17	6	26.5†	0.163	0.57 (0.25-1.27)	0.50 (0.22-1.12)
Additive model	G allele‡					0.75 (0.60-0.94)	0.77 (0.61-0.96)
	Trend *P*					0.010	0.023
IGFBP3 rs2132572		*n* = 568	*n* = 311				
Codominant model	GG	366	194	25.9		1.00	1.00
	AG	178	97	24.8		1.19 (0.93-1.51)	1.04 (0.81-1.34)
	AA	24	20	16.7	0.005	2.07 (1.31-3.30)	1.43 (0.90-2.29)
Dominant model	GG	366	194	25.9		1.00	1.00
	AG/AA	202	117	22.1	0.037	1.28 (1.02-1.61)	1.09 (0.86-1.39)
Recessive model	GG/AG	544	291	25.9		1.00	1.00
	AA	24	20	16.7	0.003	1.96 (1.25-3.10)	1.39 (0.88-2.20)
Additive model	A allele‡					1.31 (1.08-1.58)	1.12 (0.92-1.36)
	Trend *P*					0.006	0.247
IGFBP3 rs10255707		*n* = 565	*n* = 309				
Codominant model	GG	383	200	25.5		1.00	1.00
	AG	161	95	24.8		1.20 (0.94-1.53)	1.03 (0.80-1.32)
	AA	21	14	15.0	0.046	1.84 (1.07-3.16)	1.47 (0.84-2.56)
Dominant model	GG	383	200	25.5		1.00	1.00
	AG/AA	182	109	23.1	0.060	1.25 (0.99-1.58)	1.08 (0.84-1.37)
Recessive model	AA/AG	544	295	25.1		1.00	1.00
	AA	21	14	15.0	0.042	1.74 (1.01-2.97)	1.47 (0.85-2.54)
Additive model	A allele‡					1.26 (1.03-1.54)	1.11 (0.90-1.36)
	Trend *P*					0.023	0.338

*Adjusted for age, gender, smoking status, histology, stage, surgical operation, and chemotherapy or radiotherapy status.
†Mean survival time was provided when MST could not be calculated.
‡Assuming an additive effect of the variant alleles.

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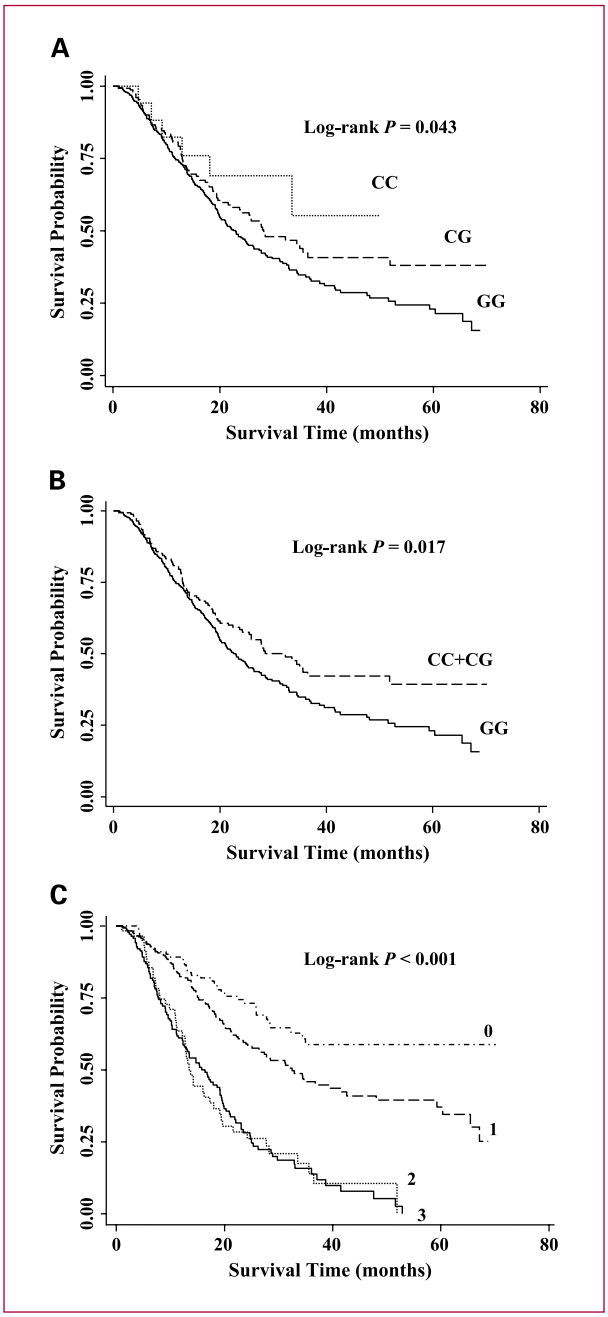

Fig. 1. Kaplan-Meier plots of survival by *IGF1* genotypes in NSCLC-specific survival. A, *IGF1* rs5742714 genotypes and NSCLC-specific survival (log-rank $P = 0.043$) in a codominant model. B, *IGF1* rs5742714 genotypes and NSCLC-specific survival (log-rank $P = 0.017$) in a dominant model. C, Kaplan-Meier plots of survival by combination of *IGF1* genotypes and operation status in NSCLC-specific survival (log-rank $P < 0.001$). 0, patients with variant genotypes (CC or CG) and receiving operation; 1, those with common genotype (GG) and receiving operation; 2, those with variant genotypes but without operation; 3, those with common genotypes and without operation.

surgical operation (HR, 0.58; 95%CI, 0.40-0.85; P for heterogeneity test = 0.045), along with a significant interaction of gene-surgical operation (adjusted P for multiplicative interaction = 0.028). To the best of our knowledge, this is the first report to evaluate the role of genetic variations in the genes of *IGF1-IGFBP3* on NSCLC survival.

According to previous studies, IGF-I and its binding proteins play key roles in the genesis of many types of tumors including lung cancer (29), and evidence of the influence on disease progression and prognosis has accumulated recently (19–22, 30). Following the initial *in vitro* experiment showing a dose-dependent increase of breast cancer cell proliferation with increased IGF-I concentration (30), a series of laboratory studies on NSCLC cell lines revealed that inhibition of IGF-I receptor led to a decrease of cell survival and an increase of apoptosis (19–21), and suppression of IGF-I signaling inhibited A549 lung cancer cell invasion *in vitro* as well as metastasis in a mice model *in vivo* (22). Besides, epidemiologic studies have shown that high circulating level of IGF-I was a risk factor for disease progression and was associated with poor prognosis of breast, ovarian, and prostate cancers (16, 29, 31), and genetic studies have reported that variation of *IGF1* was associated with progression and prognosis of common cancers, including breast and prostate cancers (16, 18, 32).

In the current study, by systematically evaluating the association of SNPs in *IGF1* and *IGFBP3* with NSCLC survival in a Chinese population, we found a significant association between the rs5742714 in the 3′UTR of *IGF1* with NSCLC survival. Given the position of 3′UTR where rs5472714 located, it is biologically plausible that such a variation at this position may have an effect on mRNA stability and lead to altered binding activity to microRNAs, which might downregulate gene expression by mRNA cleavage or translational repression (33). It should be noted, however, that the association of this polymorphism with lung cancer prognosis might also be mediated by linkage disequilibrium with other causal loci. According to the HapMap database, there was another polymorphism (rs6219) in 3′UTR of *IGF1* in complete linkage disequilibrium with rs5742714, which is also a candidate for the true association. In stratified analyses, the association was more predominant among patients receiving surgical operation, and there was a significant multiplicative interaction between risk genotypes and operation status. Both experimental and clinical studies have shown that the IGF-I receptor is overexpressed in lung and other carcinomas compared with normal tissues (34–37), which might eliminate the downregulation effect of the variant genotypes of rs5742714 among patients without operation.

We conducted this study in a relative large cohort, and with such a sample size the statistical power for the estimated crude and adjusted effects (HR) of rs5742714 in a dominant model was 74.19% and 62.63%, respectively, and for the interaction of gene-surgical operation, it was 97.7% and 98.9%, respectively, assuming the significant level $P = 0.05$. However, we failed to find significant associations between any SNPs of *IGFBP3* and the prognosis of

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Table 4. Stratified analysis of rs5742714 genotypes associated with NSCLC patients survival

Variables	rs5742714 (deaths/patients)		Adjusted HR (95% CI)*	Heterogeneity *P*
	CC	CG/GG		
Smoking				0.173
Nonsmokers	75/138	30/55	0.98 (0.63-1.53)	
Smokers	144/239	52/115	0.67 (0.49-0.93)	
Histology				0.729
Adenocarcinoma	137/237	48/100	0.77 (0.55-1.08)	
Squamous cell	69/120	27/59	0.75 (0.48-1.18)	
Others†	13/20	7/11	1.25 (0.38-4.15)	
Stage				0.535
Early stage (I-II)	52/138	18/67	0.68 (0.39-1.18)	
Late stage (III-IV)	167/239	64/103	0.83 (0.61-1.11)	
Surgical operation				0.045
None	107/139	45/57	0.99 (0.69-1.42)	
Yes	112/238	37/113	0.58 (0.40-0.85)	
Chemotherapy or radiotherapy				0.356
None	31/72	14/31	1.01 (0.51-2.03)	
Yes	188/305	68/139	0.71 (0.53-0.94)	

*Adjusted for age, sex, smoking, histology, stage, surgical operation and chemotherapy or radiotherapy status.
†Other carcinomas include large cell, undifferentiated and mixed-cell carcinomas.

NSCLC, although some of the polymorphisms evaluated in our study were linked to prognosis of other cancers or altered circulating levels of IGFBP3 (18, 38, 39). For example, rs3110697 and rs2854746 were shown to be associated with IGFBP3 levels in a multiethnic population in America (38, 39), and rs3110697 and rs2471551 were associated with breast cancer survival in premenopausal women in a Chinese population (18). Besides the diverse genetic background, the discrepancy of these findings may be explained by different tumor sites or therapeutic issues. For instance, reduced IGFBP3 was correlated with poor survival with lung and ovarian cancers (17, 23, 24), whereas an inverse prognostic effect was revealed in prostate cancer (40), indicating the variation among different tumors. Additionally, it has been reported that rs35767 in *IGF1* was associated with the circulating IGF-I level in Americans (41), but this variant revealed no significance with relation to NSCLC survival in our study, which may result from the ethnic difference.

Besides, there were still some limitations in this study. Firstly, after adjusting for multiple comparisons, none of the 17 SNPs attained significant level (0.003), so our findings need to be confirmed by additional studies with large sample size. Secondly, among the cohort of 828 NSCLC patients, only 568 patients (68.6%) with adequate DNA sample for Illumina genotyping assay were included in the current study, which could potentially influence the final estimation due to selection bias. Thirdly, in this study, 4 of 21 SNPs were removed from further analysis due to the failure in genotyping, which may result in losses of

Table 5. Interaction between rs5742714 genotypes and surgical operation

Variables	Patients	Deaths	MST (mo)	Crude HR (95% CI)	Adjusted HR (95% CI)*
CC without operation	139	107	16.4	1.00	1.00
CG/GG without operation	57	45	13.7	1.01 (0.71-1.44)	0.99 (0.69-1.42)
CC with operation	238	112	32.5	0.38 (0.29-0.50)	0.67 (0.48-0.94)
CG/GG with operation	113	37	28.2†	0.24 (0.16-0.35)	0.34 (0.21-0.56)
P for multiplicative interaction				0.043	0.028

*Adjusted for age, sex, smoking, histology, stage and chemotherapy or radiotherapy status.
†Mean survival time was provided when MST could not be calculated.

information. However, it was due to technical limitation and may not lead to selection bias.

In summary, our findings suggest that rs5742714 in *IGF1* may be a genetic modifier for NSCLC prognosis in this Chinese population, especially among patients after surgical operation. Further validation in larger cohort or independent population and functional characterizations are needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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