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ORIGINAL ARTICLE

Development of a prognosis-prediction model incorporating genetic polymorphism with pathologic stage in stage I non-small cell lung cancer: A multicenter study

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Keywords
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Abstract

Background: This multicenter study was performed to develop a prognosis-prediction model incorporating genetic polymorphism with pathologic stage for surgically treated non-small cell lung cancer (NSCLC) patients.

Methods: A replication study including 720 patients and a panel of eight single nucleotide polymorphisms (SNPs), which predicted the prognosis of surgically treated NSCLC in our previous study, was conducted. Using the combined cohort of current and previous studies including 1534 patients, a nomogram for predicting overall survival was made using Cox proportional hazards regression.

Results: Among the eight SNPs, *C3* rs2287845, *GNB2L1* (alias *RACK1*), and rs3756585 were significantly associated with overall survival. A nomogram was constructed based on pathologic stage and the genotypes of the two SNPs, and the risk score was calculated for each patient in the combined cohort. Using the prognosis-prediction model, we categorized patients into low, intermediate, and high-risk groups, which had greater accuracy in predictive ability (log-rank statistics = 54.66) than the conventional tumor node metastasis staging (log-rank statistics = 39.56). Next, we generated a prognosis-prediction model for stage I to identify a subgroup of potential candidates for adjuvant chemotherapy. Notably, 97 out of 499 stage IB patients were classified as high-risk patients with a similar prognosis to stage II patients, suggesting the benefit of adjuvant chemotherapy.

Conclusions: This prognosis-prediction model incorporating genetic polymorphism with pathologic stage may lead to more precise prognostication in surgically resected NSCLC patients. In particular, this model may be useful in selecting a subgroup of stage IB patients who may benefit from adjuvant chemotherapy.

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Introduction

Lung cancer is the leading cause of cancer death worldwide, with an average five-year survival rate of 18%.[1] Although surgery is the treatment of choice for potential cure in early stages of non-small lung cancer (NSCLC), a large proportion of patients die from lung cancer recurrence, even after complete resection.[2] Pathologic stage is the most important predictor of survival after surgical resection of NSCLC. However, patients at the same pathologic stage are at varying risk of recurrent disease and death[2]; therefore, pathologic stage alone is not a perfect tool for prognosis. Recently, investigators have focused on prognostic biomarkers in cancer patients.[3] Incorporating validated biomarkers into the current staging system may allow more accurate prognosis-prediction in lung cancer. Given that effective adjuvant chemotherapy is available, developing a reliable risk scoring model for surgically treated NSCLC patients is even more important because it may more precisely select subgroups of patients who will benefit from adjuvant treatment.[4]

Genetic polymorphisms have been investigated for prognostic/predictive biomarkers to guide therapeutic decisions in several cancers, including lung cancer.[5,6] For example, patients with certain genotypes may have a higher risk of poor prognosis after curative resection, and thereby may benefit from adjuvant chemotherapy. During the past several years, our research has focused on single nucleotide polymorphisms (SNPs) for prognostic biomarkers in lung cancer patients who have undergone curative surgical resection. In our previous study, we reported that a panel of eight SNPs in genes potentially involved in carcinogenesis could predict prognosis in NSCLC patients after surgery.[7]

The aim of this study was to develop a prognosis-prediction model incorporating pathologic stage and genetic polymorphisms to predict overall survival (OS) in surgically treated NSCLC patients by constructing a nomogram using Cox proportional hazards regression.

Methods

Study population

A total of 720 patients with pathologic stage I, II, or IIIA (micro-invasive N2) NSCLC who underwent curative surgical resection at Chonnam National University Hwasun Hospital (CNUHH, n = 337), Seoul National University Bundang Hospital (SNUBH, n = 168), Keimyung University Dongsan Medical Center (KUDMC, n = 142), and Pusan National University Hospital (PNUH, n = 73) were enrolled in the study. None of the patients received chemotherapy or radiotherapy prior to surgery. All patients included in this study were ethnic Koreans. The pathologic stage of the tumors was determined according to the International System for Staging Lung Cancer.[2] Written informed consent was obtained from all patients prior to surgery at each of the participating institutions. The institutional review boards of CNUHH, SNUBH, KUDMC, and PNUH approved the research protocol of this study. For combined cohort analysis, 814 patients from our previous study were included.[7]

Selection of single nucleotide polymorphisms (SNPs) and genotyping

In a previous study, we reported that a panel of the following eight SNPs could predict prognosis in surgically treated NSCLC patients: CD3e molecule, epsilon associated protein (*CD3EAP*) rs967591G>A; tumor necrosis factor receptor superfamily; member 10b (*TNFRSF10B*) rs1047266C>T; v-akt murine thymoma viral oncogene homolog 1 (*AKT1*) rs3803300A>G; complement component 3 (*C3*) rs2287845T>C; guanine nucleotide binding protein, beta polypeptide 2-like 1 (*GNB2L1*) rs3756585T>G; homer protein homolog 2 (*HOMER2*) rs1256428A>G; a disintegrin-like and metalloprotease domain with thrombospondin type 1-like 3 (*ADAMTSL3*) rs11259927C>T; and CD3d molecule, delta (CD3-TCR Complex, [*CD3D*]) rs3181259T>C.[7] In this study, the same eight SNPs were investigated in 720 surgically treated NSCLC patients to replicate our previous results. Genotyping was performed using Sequenom's MassARRAY iPLEX assay (Sequenom Inc., San Diego, CA, USA) or restriction fragment length polymorphism assay. Duplicate samples and negative controls were included to ensure the accuracy of genotyping. Approximately 5% of the samples were randomly selected to be genotyped again with a restriction fragment length polymorphism assay by a different investigator and the results were 100% concordant.

Statistical analysis

Overall survival was measured from the day of surgery until the date of death or the date of the last follow-up. The survival estimates were calculated using the Kaplan–Meier method. The differences in OS rates across different genotypes were compared using the log-rank test. For the association between genetic polymorphisms and survival, hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using multiple Cox proportional hazard models, with adjustment for age, gender, smoking status, tumor histology, and pathologic stage. For the computation of risk score, a Cox's proportional hazard regression using pathologic stage and *C3* rs2287845 and *GNB2L1* rs3756585 genotypes was established. The cut-off

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values of risk score for risk grouping were chosen so that the sample sizes of each risk group (low, intermediate, and high) would be similar to those of corresponding tumor node metastasis (TNM) stages (I, II, and IIIA). In the prognosis-prediction model for stage I patients, the optimal cut-off value for grouping of high and low-risk stage IB was determined by the Contal and O'Quigley technique based on an algorithm for the maximization of hazard ratio.[8,9] For all tests, a two-sided P value < 0.05 was considered statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and the figure plot was calculated using RMS package for R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics and clinical predictors

The clinical and pathologic characteristics of the patients and the associations with OS are shown in Table 1. Upon univariate analysis, age, gender, smoking, pack-years of smoking, histologic type, and pathologic stage were associated with OS (log-rank P [$P_{L\text{-}R}$] for OS 0.02, 6.0×10^{-4}, 0.004, 0.03, 0.007, and 1.7×10^{-8}).

Associations between SNPs and survival outcomes

Among the eight SNPs (*CD3EAP* rs967591, *TNFRSF10B* rs1047266, *AKT1* rs3803300, *C3* rs2287845, *HOMER2* rs1256428, *GNB2L1* rs3756585, *ADAMTSL3* rs11259927, and *CD3D* rs3181259), *C3* rs2287845 and *GNB2L1* rs3756585 were replicated in the current study (Table S1). In the combined cohort including 1534 patients from current and previous studies, the two SNPs exhibited significantly poorer OS (adjusted hazard ratio [aHR] for OS 2.84, 95% CI 1.60–5.05, $P = 0.0004$ in recessive model for *C3* rs2287845; aHR for OS 1.32, 95% CI 1.14–1.52, $P = 0.0002$, in additive model for *GNB2L1* rs3756585; Table 2, Fig 1).

Nomogram and computation of risk score for overall survival

To investigate whether adding these genetic determinants to the pathologic stage would improve the prediction of prognosis, we performed an exploratory analysis evaluating a novel prognosis-prediction model incorporating the *C3* rs2287845 and *GNB2L1* rs3756585 genotypes with pathologic stage. The total score was calculated from the results of the Cox proportional hazard model, as:

$$\begin{aligned}&S(t,X) = [S_0(t)]^{\exp(LP)}, \text{Linear Predictor}(LP)\\ &= [0(\text{if x1} = \text{TT}) \text{ or } 0.2216 \times 0.7525(\text{if x1} = \text{TC}) \text{ or } 1.2436\\ &\times 0.9827(\text{if x1} = \text{CC})] + [0(\text{if x1} = \text{TT}) \text{ or } 0.4439\\ &\times 0.5650(\text{if x2} = \text{TG}) \text{ or } 0.5588 \times 0.9124(\text{if x2} = \text{GG})]\\ &+ [0(\text{if stage} = \text{I}) \text{ or } 0.4755 \times 0.7724(\text{if stage} = \text{II}) \text{ or}\\ &0.9028 \times 0.7870(\text{if stage} = \text{IIIA})]\end{aligned}$$

where S(t,X) denotes survival probability for a given time (year) and X (SNP and stage information), $S_0(t)$ denotes baseline survival probability for a given time (year), and x1 and x2 refers to rs2287845 and rs3756585 genotypes, respectively.

The baseline one-year survival probability is $S_0(1 = 0.9525)$, the three-year survival probability is $S_0(3) = 0.8368$, and the five-year survival probability is $S_0(5 = 0.7216)$.

Values were obtained for all patients included in the combined cohort. A nomogram was constructed based on these variables (Fig 2a). We could predict one, three, and five-year OS for each patient by applying the total score to the nomogram. To compare the model with the TNM staging system, we categorized the patients into low, intermediate, and high-risk groups (55.6%, 24.9%, and 19.5%, respectively), so that the sample sizes of each group were similar to those of stage I, II, and III (55.9%, 22.8%, and 21.3%, respectively, Table 3). The cut-off points for risk grouping were 50 and 80 (Fig 3).

The prognosis-prediction model had more accurate predictive ability (log-rank statistics = 54.66) than conventional TNM staging (log-rank statistics = 39.56) (Fig 3). According to our model, patients at the same TNM stage were classified into different risk groups. Subgroups of patients with stage I and II disease were predicted to have worse survival compared with some of the patients with higher stages. Interestingly, of 843 stage I patients, 12 (1.4%) were classified into the high-risk group (Table 3).

We then performed further analysis by generating a prognosis-prediction model for 843 stage I patients involving stage (i.e. stages IA and IB) and the two SNPs to identify patients at high risk of poor survival and who may benefit from adjuvant chemotherapy. The total score was calculated from the results of the Cox proportional hazard model using the following formula and a nomogram was made using those variables (Fig 2b):

$$\begin{aligned}&S(t,X) = [S_0(t)]^{\exp(LP)}, \text{Linear Predictor}(LP)\\ &= [0(\text{if x1} = \text{TT}) \text{ or } 0.0966 \times 0.7521(\text{if x1} = \text{TC}) \text{ or } 0.6902\\ &\times 0.9858(\text{if x1} = \text{CC})] + [0(\text{if x1} = \text{TT}) \text{ or } 0.2545\\ &\times 0.5623(\text{if x2} = \text{TG}) \text{ or } 0.5560 \times 0.9169(\text{if x2} = \text{GG})]\\ &+ [0(\text{if stage} = \text{IA}) \text{ or } 1.0707 \times 0.4081(\text{if stage} = \text{IB})]\end{aligned}$$

The baseline one-year survival probability is $S_0(1) = 0.9638$, the three-year survival probability is $S_0(3)$

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Table 1 Univariate analysis for overall survival by clinicopathologic features

Variables	Overall survival			
	No. of patients	No. of deaths (%)†	Five-year OS (%)‡	Log-rank *P*
Overall	720	174 (24.2)	64	
Age, years				
≤64	323	74 (22.9)	69	0.02
>64	397	100 (25.2)	59	
Gender				
Male	472	135 (28.6)	60	6.0×10^{-4}
Female	248	39 (15.7)	72	
Smoking status				
Never	250	42 (16.8)	69	0.004
Ever	470	132 (28.1)	61	
Pack-years§				
<40	249	54 (21.7)	68	0.03
≥40	221	78 (35.3)	54	
Histological types				
SCC	244	69 (28.3)	60	0.007
AC	435	89 (20.5)	68	
LCC	41	16 (39.0)	46	
Pathologic stage				
I	365	62 (17.0)	75	1.7×10^{-8}
II–IIIA	355	112 (31.6)	51	

†Row percentage; ‡five-year overall survival (OS), proportion of survival derived from Kaplan–Meier analysis; §in ever-smokers. AC, adenocarcinoma; LCC, large cell carcinoma; SCC, squamous cell carcinoma.

= 0.8978, and the five-year survival probability is $S_0(5) = 0.7973$.

Adjuvant chemotherapy is currently strongly recommended for most stage II and III and is not indicated for stage IA disease; however, stage IB is the only stage in which there are suggested high-risk factors to consider in determining the use of adjuvant chemotherapy. Therefore, we sought to define a high-risk group in stage IB using our prognosis-prediction model. Based on the calculated risk score, the optimal cut-off value for grouping of low and high-risk stage IB was 134, which was determined using an algorithm for maximization of HR. Stage IA and low-risk stage IB were separated at risk score 100 without overlap. Finally, we classified stage I patients into stage IA, low-risk stage IB, and high-risk stage IB (40.8%, 47.7%, and 11.5%, respectively) (Table 4). The patients in low and high-risk stage IB had significantly poorer OS compared with stage IA patients (aHR for OS 2.62, 95% CI 1.65–4.16, $P = 4.7 \times 10^{-5}$; and aHR for OS 3.99, 95% CI 2.30–6.92, $P = 8.1 \times 10^{-7}$, respectively) (Table 4). Notably, the prognosis of high-risk stage IB was similar to that of stage II (aHR for OS, compared with stage IA 3.45, 95% CI 2.18–5.46; $P = 1.2 \times 10^{-7}$) compared with those in low-risk stage IB (Fig 4).

Discussion

This study was conducted to develop a prognosis-prediction model incorporating genetic polymorphisms into pathologic stage using Cox proportional hazard regression to predict the prognosis of surgically treated NSCLC patients. Risk grouping by calculated risk scores

Table 2 Overall survival according to *C3* rs2287845 and *GNB2L1* rs3756585 genotypes

Polymorphism	Genotype	No. of cases (%)†	No. of deaths (%)‡	Overall survival		
				Five-year OS (%)§	HR (95% CI)¶	*P*¶
C3 rs2287845						
	TT	1120 (73.7)	289 (25.8)	66	1.00	—
	TC	374 (24.6)	113 (30.2)	59	1.27 (1.02–1.58)	0.03
	CC	26 (1.7)	12 (46.2)	36	3.03 (1.70–5.41)	0.0002
	Dominant	—	—	—	1.35 (1.09–1.66)	0.01
	Recessive	—	—	—	2.84 (1.60–5.05)	0.0004
	Additive	—	—	—	1.39 (1.15–1.69)	0.001
GNB2L1 rs3756585						
	TT	724 (47.7)	163 (22.5)	70	1.00	—
	TG	662 (43.6)	200 (30.2)	60	1.38 (1.12–1.70)	0.003
	GG	133 (8.7)	47 (35.3)	59	1.67 (1.20–2.31)	0.002
	Dominant	—	—	—	1.43 (1.17–1.74)	0.001
	Recessive	—	—	—	1.42 (1.04–1.92)	0.03
	Additive	—	—	—	1.32 (1.14–1.52)	0.0002

†Column percentage; ‡row percentage; §five-year overall survival (OS), proportion of survival derived from Kaplan–Meier analysis; ¶hazard ratios (HRs), 95% confidence intervals (CIs), and their corresponding P values were calculated using multivariate Cox proportional hazard models, adjusted for age, gender, smoking status, tumor histology, and pathologic stage.

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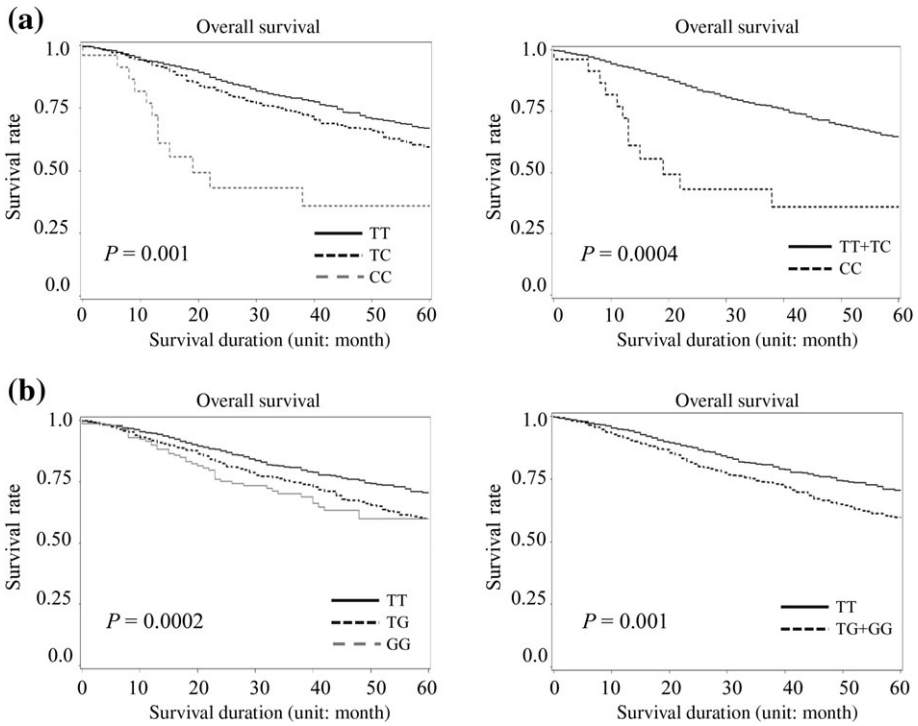

Figure 1 Kaplan–Meier plots of overall survival according to (**a**) *C3* rs2287845 and (**b**) *GNB2L1* rs3756585 genotypes. *P* values in the multivariate Cox proportional hazard model.

could more accurately classify patients compared with TNM stage in terms of OS. More importantly, the prognosis-prediction model could identify stage IB patients with a high risk of poor survival who may benefit from adjuvant chemotherapy. Our novel prognostic model may be useful for the more precise prediction of clinical outcome in early stage NSCLC patients who have undergone surgical resection. Specifically, this model may help to determine the use of adjuvant chemotherapy for stage IB NSCLC patients.

Although pathologic stage is the most powerful prognostic indicator after lung cancer surgery, patients with the same stage have markedly different prognoses. Incorporating relevant clinicopathological factors or validated

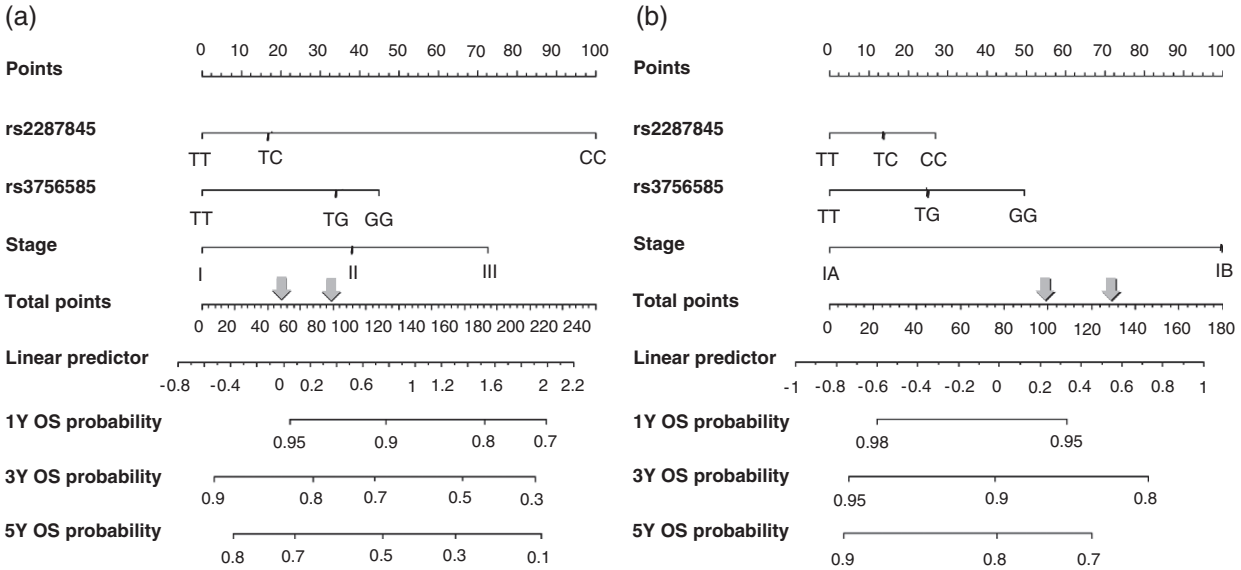

Figure 2 Nomograms for prediction of overall survival (OS) probability using the prognosis-prediction model in (**a**) all patients and (**b**) stage I patients. Arrows indicate cut-off points for risk grouping.

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Table 3 Risk groups according to the prognosis-prediction model and correlation with tumor node metastasis staging

Risk group/stage no. (%)	Stage I 843 (55.9)†	Stage II 343 (22.8)	Stage III 321 (21.3)	HR (95% CI)‡	*P*‡
Low					
838 (55.6)§	719 (85.3)§	119 (34.7)	0 (0.0)	1.00	—
Intermediate					
375 (24.9)	112 (13.3)	144 (42.0)	119 (37.1)	1.72 (1.30–2.29)	1.8×10^{-4}
High					
294 (19.5)	12 (1.4)	80 (23.3)	202 (62.9)	2.78 (2.09–3.69)	1.5×10^{-12}
HR (95% CI)	1.00	1.65 (1.24–2.20)	2.38 (1.79–3.16)	—	—
P§		5.8×10^{-4}	1.9×10^{-9}	—	—

†Row percentage; ‡hazard ratios (HRs), 95% confidence intervals (CIs), and their corresponding P values were calculated using Cox proportional hazard models; §column percentage.

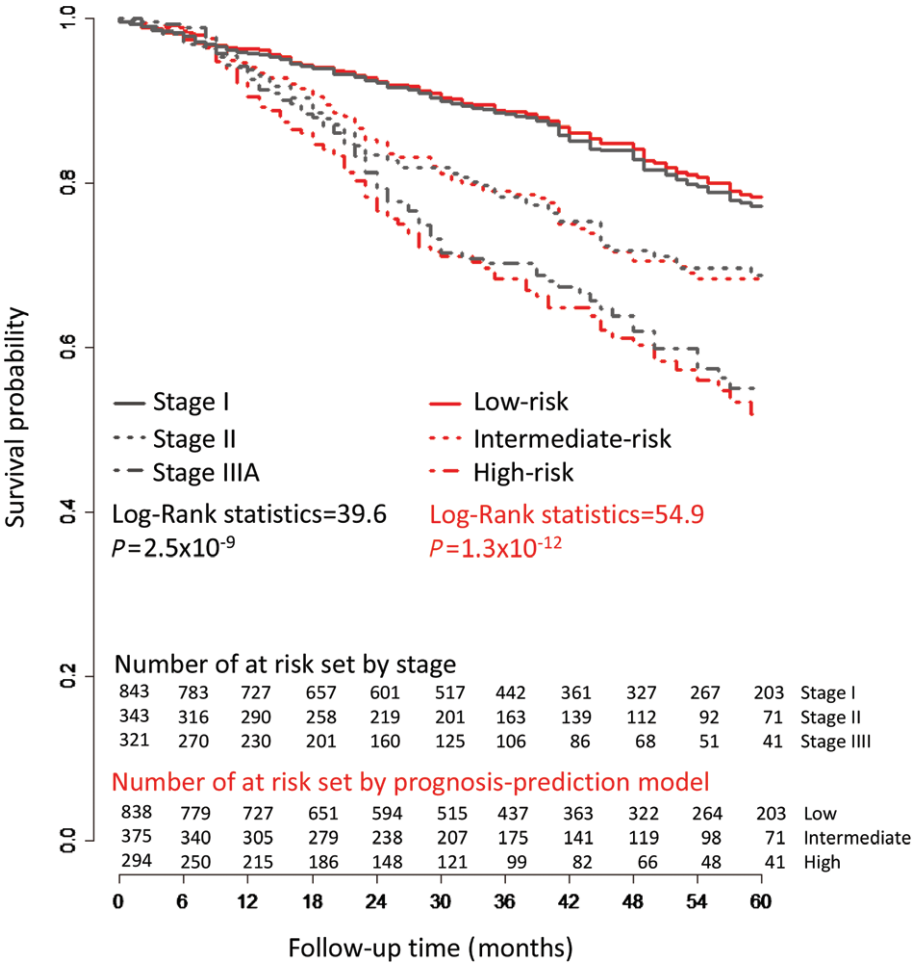

Figure 3 Comparison of survival curves by tumor node metastasis (TNM) staging and the prognosis-prediction model. *P* values by log-rank test.

biomarkers into the current staging system may compensate for the limitations, to allow more accurate prognosis-prediction in lung cancer patients. Our novel approach could enhance the prognostic value of the current pathologic staging system by adding two validated genetic polymorphisms, *C3* rs2287845 and *GNB2L1* rs3756585, which were subject to previous research and replicated for the current study. The fusion of stage and genetic biomarker led to significantly better resolution in predicting the prognosis of surgically treated stage I-IIIA NSCLC patients. In addition, we could identify a subgroup of stage I patients whose prognosis was as poor as or even worse than those at higher stages. This led us to further analyze the prognosis of stage I patients using this novel approach to

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Table 4 Risk groups in stage I non-small cell lung cancer by the prognosis-prediction model

Risk group (n = 843)	No. (%)†	HR (95% CI)‡	P‡
Stage IA	344 (40.8)	1.00	—
Stage IB	499 (59.2)	2.89 (1.85–4.52)	3.2×10^{-7}
Stage IB, low risk	402 (47.7)	2.62 (1.65–4.16)	4.7×10^{-5}
Stage IB, high risk	97 (11.5)	3.99 (2.30–6.92)	8.1×10^{-7}

†Row percentage. ‡Hazard ratios (HRs), 95% confidence intervals (CIs), and their corresponding P values were calculated using Cox proportional hazard models. HR (95% CI) for stage II vs. stage IA = 3.45 (2.18–5.46), $P = 1.2 \times 10^{-7}$.

investigate whether we could identify a subgroup of stage IB patients who could be considered as high-risk patients and, thus, candidates for adjuvant chemotherapy. There are only suggested high-risk factors in stage IB for determining treatment of adjuvant chemotherapy, in contrast to stage IA where adjuvant chemotherapy is not recommended and most stage II and III for which adjuvant chemotherapy is the current standard management. According to National Comprehensive Cancer Network guidelines version 3.2017, high-risk factors may include poorly differentiated tumors (including lung neuroendocrine tumors unless well-differentiated), vascular invasion, wedge resection, tumors >4 cm, visceral pleural involvement, and unknown lymph node status (Nx).[10] The guidelines suggest that these factors may not be independent indications but may be considered when determining whether to treat with adjuvant chemotherapy, indicating the relatively low level of evidence for those high-risk factors and potentially inconsistent clinical application of adjuvant chemotherapy in stage IB. In addition to these potential high-risk factors, our results suggest that *C3* rs2287845 and *GNB2L1* rs3756585 genotypes combined with pathologic stage may help to identify stage IB patients at high-risk for poor survival. In this study, stage IB patients were categorized into low and high-risk groups. The prognosis in high-risk stage IB patients was similar to that of stage II patients compared with low-risk stage IB patients, suggesting these patients should be considered for adjuvant chemotherapy.

The complement system has a major role in innate and adaptive immunity. The *C3* protein is a key player in the

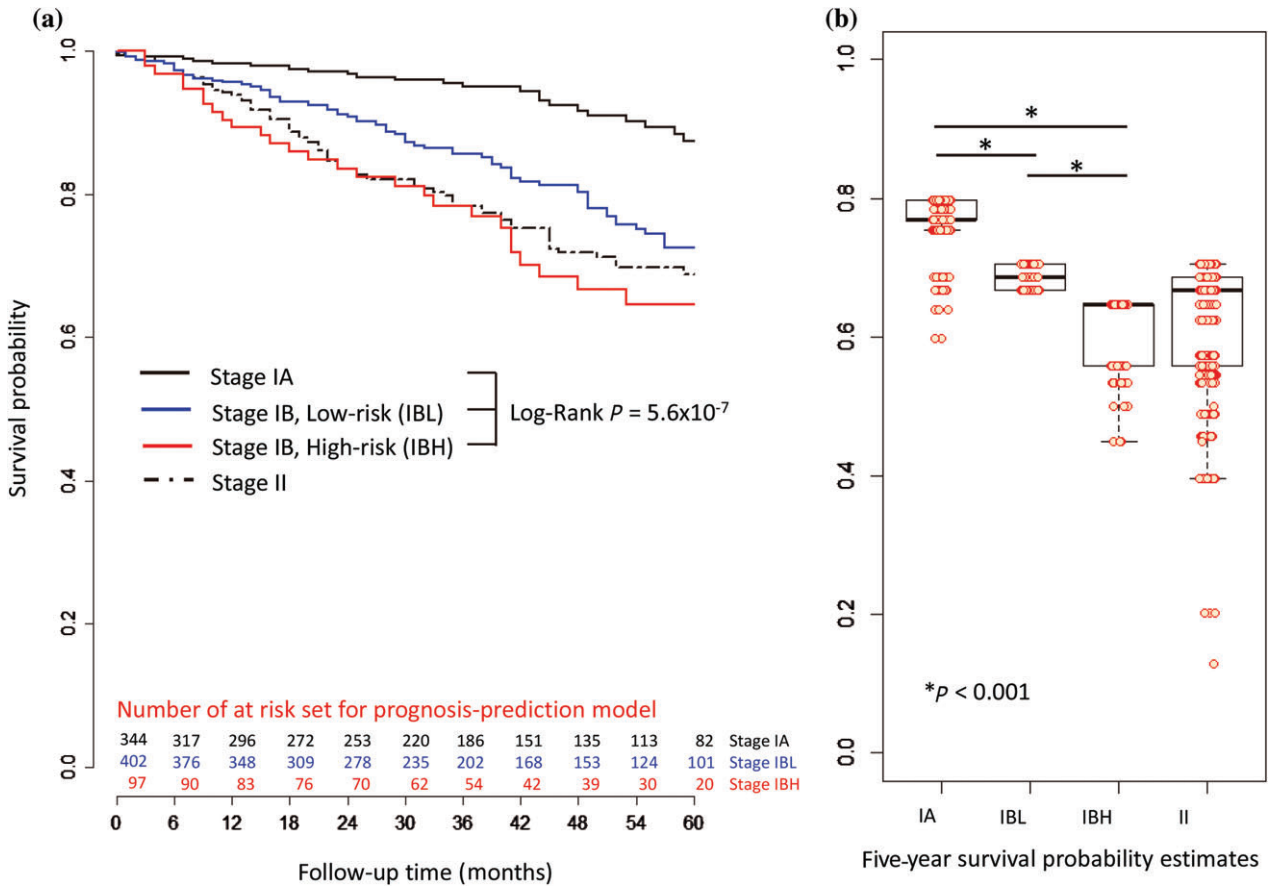

Figure 4 (**a**) Kaplan–Meier plots of overall survival in stage I. Stage IB patients were divided into low and high-risk groups. (**b**) Box plots of five-year survival probability estimates. Stage II patient data is displayed for reference. *P* values by log-rank test.

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activation of the complement pathways.[11,12] It has been reported that the complement system is activated in many cancers, including lung cancer.[12–14] Although complements have been linked to immunosurveillance against tumors,[12] there is growing evidence that complements play oncogenic roles.[15,16] *GNB2L1* (alias *RACK1*), belongs to a WD40 protein family that includes the β subunit of G-proteins. As a scaffold protein, GNB2L1 interacts with various signaling molecules, such as cyclic AMP-specific phosphodiesterase 4D isoform 5, β integrins, and PKC, playing a pivotal role in a wide range of biologic responses, including cell growth, adhesion, and migration.[17–19] Studies have indicated that GNB2L1 plays an important role in cancer progression and that its expression is upregulated during angiogenesis in some types of cancers, including lung cancer.[20–22] In addition, *GNB2L1* overexpression is strongly associated with poor clinical outcomes in cancer patients.[22,23] In our previous study, promoter assay and electrophoretic mobility shift assay (EMSA) revealed that the rs3756585 T-to-G change increased transcription factor binding and promoter activity of *GNB2L1*.[24] This study suggests that polymorphisms of the two genes enhance the prognostic ability of pathologic stage in the novel prognosis-prediction model. Further studies are needed to understand the roles of the two genes in lung cancer and to clarify the association between the SNPs and prognosis.

In conclusion, this prognosis-prediction model incorporating genetic polymorphisms into pathologic stage may lead to more precise prognostication of patients with surgically resected NSCLC. Specifically, this model may be useful to select a subgroup of stage IB patients who may benefit from adjuvant chemotherapy.

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Disclosure

No authors report any conflict of interest.

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Supporting Information

Additional Supporting Informationmay be found in the online version of this article at the publisher's website:

Table S1 Overall survival according to genotypes of eight polymorphisms.

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