Original Article / Özgün Makale

The role of galectin-3 and its genetic variants in tumor risk and survival of patients with surgically resected early-stage non-small cell lung cancer

Cerrahi rezeksiyon uygulanmış erken evre küçük hücreli dışı akciğer kanserli hastalarda galektin-3 ve genetik varyantlarının tümör riski ve sağkalım üzerindeki rolü

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ABSTRACT

Background: The aim of this study was to investigate the possible relationship between galectin-3 gene variants, serum level, gene expression level, and the risks and survivals of resectable non-small cell lung cancer patients.

Methods: The rs4644 and rs4652 variants of galectin-3 were genotyped by TaqMan single nucleotide polymorphism assay using genomic deoxyribonucleic acid isolated from the peripheral blood of 65 (54 males, 11 females; mean age: 60.1±11.9 years; range, 34 to 83 years) with Stage IA-IIIA non-small cell lung cancer who underwent primary surgical treatment and 95 healthy individuals (48 males, 47 females; mean age: 53.9±13.5 years; range, 32 to 87 years) between March 2017 and September 2018. Circulating galectin-3 levels in serum samples of the patient and control groups were assessed by enzyme-linked immunosorbent assay. Messenger ribonucleic acid expression of galectin-3 in tumor and surrounding tissues of the patient group was examined by real-time quantitative polymerase chain reaction. Both predictive and prognostic significance of the results were analyzed.

Results: The presence of angiolymphatic invasion was significant in the patients with rs4652 AA genotype (p=0.04). Serum galectin-3 levels were significantly higher in the patients than the controls (p<0.0001). The patients with rs4644 CA/CC (p<0.0001 and p<0.0001) and rs4652 AA/AC (p=0.001 and p<0.0001) genotypes had higher serum galectin-3 levels than their corresponding controls. Serum galectin-3 levels increased in the presence of vascular invasion in patients with both rs4644 AC (p=0.03) and rs4652 AC (p=0.019) genotypes. The receiver operating characteristic curve suggested serum galectin-3 level as a strong predictive marker for the patient group with a cut-off value of 17.089 ng/mL (area under the curve: 0.910±0.04; 95% confidence interval: 0.832-0.988; p<0.001). Univariate analysis revealed the association of lower serum galectin-3 levels with better survival (p=0.048). Multivariate survival analysis showed that only high serum galectin-3 levels tended to be related to survival of the patients (hazard ratio: 5.106; 95% confidence interval: 0.956-27.267; p=0.056).

Conclusion: The presence of galectin-3 gene variants may lead to histopathological differences among patients with non-small cell lung cancer. Serum galectin-3 level may be a valuable diagnostic biomarker and be associated with survival of these patients.

Keywords: Enzyme-linked immunosorbent assay, galectin-3, gene expression, non-small cell lung cancer, single nucleotide polymorphism genotyping.

ÖZ

Amaç: Bu çalışmada galektin-3 gen varyantları, serum düzeyi, gen ekspresyon düzeyi ile rezektabl küçük hücreli dışı akciğer kanseri hastalarının riskleri ve sağkalımları arasındaki olası ilişki araştırıldı.

Çalışma planı: Mart 2017 - Eylül 2018 tarihleri arasında galektin-3'ün rs4644 ve rs4652 varyantları, primer cerrahi tedavi yapılan küçük hücreli dışı akciğer kanserli 65 hastanın (54 erkek, 11 kadın; ort. yaş: 60.1±11.9 yıl; dağılım, 34-83 yıl) ve 95 sağlıklı bireyin (48 erkek, 47 kadın; ort. yaş: 53.9±13.5 yıl; dağılım, 32-87 yıl) periferik kanından izole edilen genomik deoksiribonükleik asitler kullanılarak TaqMan tek nükleotid polimorfizm testi ile genotiplendi. Hasta ve kontrol gruplarının serum örneklerinde dolaşımdaki galektin-3 düzeyi, enzim bağlı immünosorbent testi ile değerlendirildi. Hasta grubunda tümör ve çevre dokulardaki galektin-3'ün haberci ribonükleik asit ekspresyonu, gerçek zamanlı kantitatif polimeraz zincir reaksiyonu ile incelendi. Bu sonuçların hem prediktif, hem de prognostik önemi analiz edildi.

Bulgular: Rs4652 AA genotipi olan hastalarda anjiyolenfatik invazyon varlığı anlamlı idi (p=0.04). Kontrol grubuna kıyasla, hastalarda serum galektin-3 düzeyleri anlamlı düzeyde yüksekti (p<0.0001). Rs4644 CA/CC (p<0.0001 ve p<0.0001) ve rs4652 AA/AC (p=0.001 ve p<0.0001) genotipleri olan hastalarda, karşılık gelen kontrollere kıyasla, serum galektin-3 düzeyleri daha yüksekti. Hem rs4644 (p=0.03) hem de rs4652 (p=0.019) AC genotipleri olan hastalarda, vasküler invazyon varlığında daha yüksek serum galektin-3 düzeyleri izlendi. Alıcı işletim karakteristik eğrisi, 17.089 ng/mL (eğri altında kalan alan: 0.910±0.04; %95 güven aralığı: 0.832-0.988; p<0.001) kesme değeri ile serum galektin-3 düzeyinin hasta grubu için güçlü bir prediktif belirteç olduğunu gösterdi. Tek değişkenli analiz, daha düşük serum galektin-3 düzeylerinin daha iyi sağkalım ile ilişkili olduğunu ortaya koydu (p=0.048). Çok değişkenli sağkalım analizi, yalnızca yüksek serum galektin-3 düzeylerinin hastaların sağkalımı ile ilişkili olma eğiliminde olduğunu gösterdi (risk oranı: 5.106; %95 güven aralığı: 0.956-27.267; p=0.056).

Sonuç: Küçük hücreli dışı akciğer kanserli hastalarda galektin-3'ün genetik varyantlarının varlığı histopatolojik farklılıklara yol açabilir. Serum galektin-3 düzeyi, bu hastalar için değerli bir tanısal biyobelirteç olabilir ve sağkalım süreleri ile ilişkili olabilir.

Anahtar sözcükler: Enzim bağlı immünosorbent testi, galektin-3, gen ekspresyonu, küçük hücreli dışı akciğer kanseri, tek nükleotid polimorfizm genotiplemesi.

Lung cancer is one of the most devastating diseases with an approximately 15% of cure rate globally.[1] The two main types of lung cancer are common which are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC).[2] The former one accounts for the vast majority of lung cancer cases, of which almost two-thirds are detected at advanced stages.[3] Surgery remains the primary option for curative therapy; however, most of patients die from poor diagnosis, as patients with the same pathological stage may have different genetic aberrations.[4] In addition, response against platinum-based chemotherapy as the standard first-line chemotherapy among patients of advanced NSCLC is quite different.[5] Hence, it is particularly important to elucidate the underlying genetic differences and, thus, early detection and diagnosis would be enhanced for high-risk populations and patients with NSCLC.

Galectin-3 is an approximately 30 kDa beta-galactoside-binding protein which is encoded by the LGALS3 gene located on chromosome 14q21-q22 in human and is composed of six exons and five introns.[6] As a multifunctional protein, galectin-3 plays a pivotal role in cancer contributing to tumor progression, angiogenesis, and metastasis.[7-9] Furthermore, alterations in expression and plasma levels of galectin-3 have been considered as potential biomarkers with a clinical value, particularly in cancer.[10,11]

There are two most common single nucleotide polymorphism (SNP) sites located in exon 3 of LGALS3, namely rs4644 and rs4652 variants. The variant of rs4644 +191 C>A substitutes histidine to proline at residue 64, whereas the variant of rs4652 +292 A>C changes threonine at residue 98 to proline.[12] Double-sided roles of different variants of galectin-3 have been studied in different types of cancer. In a study, a polymorphism at rs4644 variant resulted in susceptibility to matrix metalloproteinase (MMP) cleavage and the acquisition of resistance to drug-induced apoptosis; thus, it was correlated with the incidence of breast cancer.[13] In another study, a possible protective role of rs4644 variant of galectin-3 was suggested in prostate cancer.[14] In addition, rs4652 variant, but not rs4644 variant, was associated with the tumor grade and prognosis of glioma in Chinese population.[15] Besides, rs4652 variant was thought to contribute to the susceptibility and chemotherapeutic drug resistance in gastric carcinoma.[16] Recently, Fang et al.[17] recorded that allele C of rs4652 variant might be risk factor for cervical cancer.

Despite the fact that SNPs play a key role in the occurrence and development of lung cancer, there has yet no a report showing specifically a correlation of galectin-3 gene variants with the risk and prognosis of NSCLC. In the present study, we, for the first time, aimed to investigate the association of galectin-3 and its genetic variants with the risk development and prognosis for NSCLC.

PATIENTS AND METHODS

Study design and study population

This retrospective study was conducted at Istanbul University-Cerrahpaşa, Cerrahpaşa Medical School, Department of Thoracic Surgery between March 2017 and September 2018. A total of 65 patients (54 males, 11 females; mean age: 60.1±11.9 years; range, 34 to 83 years) with Stage IA-IIIA NSCLC who underwent primary surgical treatment and 95 healthy individuals (48 males, 47 females; mean age: 53.9±13.5 years; range, 32 to 87 years) were included. The selection criteria for the control participants were negative family history of cancer and having no tumor. The participants were questioned in detail, if they had a genetic disorder or any other malignancies. None of the control individuals were diagnosed with any type of cancer and they were chosen to be numerically matched to case patients on the basis of age and sex. The NSCLC patients were followed up with telephone calling or nation-wide population registry system data. A written informed consent was obtained from each participant. The study protocol was approved by Clinical Research Ethics Committee of Bezmialem Vakif University (No: 71306642-050.01.04). The study was conducted in accordance with the principles of the Declaration of Helsinki. In the expression analysis, the control group referred to as tumor surrounding tissue which was distant from the tumor without any findings of the macroscopic invasion. The diagnosis of the NSCLC patients was confirmed by the pathological examinations of the resected tumor tissues according to the 7th edition of the pathological Tumor, Node, Metastasis (TNM) staging system.[18] Tumor tissue and tumor-free surrounding tissue samples from 18 NSCLC patients were collected for expression analysis.

TaqMan SNP genotyping

The peripheral blood samples from all participants were collected into the ethylenediaminetetraacetic acid (EDTA)-containing tubes. Genomic deoxyribonucleic acid (DNA) was isolated from peripheral circulating lymphocytes by salting-out process.[19] The distribution of SNPs rs4644 and rs4652 were performed using the TaqMan SNP genotyping assays C___7593635_1_ and

C___7593636_30, respectively. Briefly, approximately 50 ng of each DNA sample was subjected to a 5′-nuclease allelic discrimination assay using TaqMan Universal PCR Master Mix (Applied Biosystems Inc., CA, USA) and the primer-probe mix consisting of two SNP region-specific pre-validated primers, as well as mutation-specific fluorescence-labeled TaqMan minor groove binder (MGB) probes. In this study, one probe was labeled with VIC dye to detect the allele A; the second probe was labeled with fluorescein amidite dye to detect the allele C. The reaction was amplified by an initial denaturation step of 2 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. Endpoint analysis was performed on an ABI Prism 7900 Sequence Detection System (Applied Biosystems Inc., CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

Blood serum was obtained from participants by centrifugation and stored -80°C. Serum levels of galectin-3 concentrations were measured in patients and controls by using human galectin-3 platinum ELISA kit (Invitrogen, Bender MedSystems, Vienna, Austria) (sensitivity: 0.29 ng/mL; intra-assay coefficient of variation [CV]: 7.5% and inter-assay CV: 5.4%) according to the manufacturer's instructions.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Total ribonucleic acid (RNA) was isolated from the tumor and non-malignant surrounding lung tissue specimens of the same patient by the TRizol (Invitrogen, CA, USA) method. Validation of RNA concentrations were performed by NanoDrop2000 (Thermo Fisher Scientific Inc., MA, USA). First-strand complementary DNA (cDNA) was synthesized using high-capacity cDNA reverse transcription kit (Applied Biosystems Inc., CA, USA). The quantitative real-time PCR was conducted by Stratagene Mx3005P (Agilent Technologies Inc., CA, USA) system in the presence of SensiFAST TM SYBR® No-ROX green dye (Bioline Reagents Ldt., TN, USA). All PCR primers used were as follow: LGALS3 forward, 5′GTGCCTCGCATGCTGATAAC3′ and reverse, 5′GCAACCTTGAAGTGGTCAGG3′; GAPDH primer, 5′TGCACCACCAACTGCTTAGC3′ and reverse, 5′GGCATGGACTGTGGTCATGAG3′. Gene cards were analyzed using the threshold cycle (CT) relative quantification method. The CT values were normalized for endogenous reference [ΔCT=CT (GAPDH)-CT (LGALS3)] and compared with the control using the $\Delta\Delta$CT formula [$\Delta\Delta$CT=ΔCT (tumor tissue)-ΔCT (surrounding healthy tissue as control)]. Relative messenger RNA (mRNA) expression (fold change) was calculated by 2−$\Delta\Delta$Ct method (GAPDH for the internal control). Each sample was tested in duplicate.

Statistical analysis

Statistical analysis was performed using the PASW version 17.0 software (SPPS Inc., Chicago, IL, USA) and GraphPad Prism version 8.0.0 (GraphPad, La Jolla, CA, USA). The distribution of rs4644 and rs4652 genotypes and the frequency of alleles between the patients and control groups were compared using the chi-square, Fisher's exact, and Student's t-tests. Serum galectin-3 levels and galectin-3 mRNA expression analysis results between NSCLC patients and controls were analyzed using the Mann-Whitney U test. The inter-group differences of ELISA and pathological parameters with different genotypes were compared using the Kruskal-Wallis test. Multivariate logistic regression analysis was adjusted by risk factors in terms of galectin-3, sex, and smoking status. The receiver operating characteristic (ROC) curve analysis calculated the area under the curve (AUC) to check the diagnostic performance of serum galectin-3 level in NSCLC. The optimal cut-off value on ROC curve was determined with respect to AUC value by maximizing both specificity and sensitivity, as well as their summation.[20] Prognostic factors were also evaluated in NSCLC patients. Differences in survival were investigated using the log-rank test in the univariate analysis, and multivariate analysis was done using the Cox proportional hazards regression model. A p value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics of patients and healthy controls are summarized in Table 1. There were no significant differences in the mean age at the time of diagnosis between the groups (p=0.146). The incidence rate was higher in men (83.1%) than in women (16.9%) (p<0.001). The rate of smokers was higher in both groups (84.6% in patient group *vs.* 70.5% in the control group than non-smokers (15.4% in the patient group *vs.* 29.5% in the control group) (p=0.04). The median follow-up was 26 (range, 12 to 48) months. On the other hand, no significant differences in the distribution of genotypes and allele frequencies between the patients and controls were observed for both rs4644 and rs4652 variants (Table 2).

The clinical features of the patients with genotypes of rs4644 and rs4652 variants were presented in Table 3. Regarding disease`s characteristics including tumor stage, lymph node metastasis, perineural invasion,

Table 1. Baseline characteristics of study population

Characteristics	Control group (n=95)			Patient group (n=65)			*p*
	n	%	Mean±SD	n	%	Mean±SD	
Age (year)			53.9 ± 13.5			60.14 ± 11.9	0.146
Sex							<0.001*
Female	47	49.5		11	16.9		
Male	48	50.5		54	83.1		
Smoke							0.04*
Yes	67	70.5		55	84.6		
No	28	29.5		10	15.4		

SD: Standard deviation; * p<0.05.

angiolymphatic invasion and vascular invasion, no association was observed among NSCLC patients with genotypes of rs4644 variant. However, there was a markedly suggestive evidence of the presence of angiolymphatic invasion for NSCLC patients who had AA genotype of rs4652 variant (p=0.04).

The mean serum galectin-3 levels were significantly higher in NSCLC patients (26.05±1.77 ng/mL), compared to healthy controls (11.62±1.30 ng/mL) (p<0.0001) (Figure 1a). The patients with CA and CC genotypes of rs4644 variant were associated a higher serum galectin-3 level compared to the controls (for CA genotype: 26.02±1.72 *vs*. 12.08±2.05 ng/mL, respectively; p<0.0001; for CC genotype: 28.8±3.43 *vs*. 12.27±1.80 ng/mL; respectively; p<0.0001)

(Figure 1b). Moreover, the patients with AC and AA genotypes of rs4652 variant were correlated with higher serum galectin-3 levels, compared to controls (for AC genotype: 26.50±1.86 *vs*. 12.08±1.76 ng/mL, respectively; p<0.0001; for AA genotype: 28.76±3.43 *vs*. 11.70±2.36 ng/mL, respectively; p=0.001) (Figure 1c). Moreover, serum galectin-3 levels of NSCLC patients with AC genotype of both rs4644 and rs4652 variants were found to be higher in the presence of vascular invasion than in the absence of it (for rs4644 variant: 30.81±2.04 ng/mL *vs*. 23.03±1.85 ng/mL, respectively; p=0.03; for rs4652 variant: 31.94±2.20 ng/mL *vs*. 22.87±1.37 ng/mL, respectively; p=0.019, respectively) (Tables 4 and 5).

Multivariate logistic regression analysis was also tested for a statistical interaction in which the

Table 2. Genotype and allele frequencies for rs4644 and rs4652 variants of galectin-3 in study population

	Control group		Patient group		*p*
	n	%	n	%	
			rs4644		
AA	15	15.8	4	6.2	
CA	32	33.7	30	46.2	0.099
CC	48	50.5	31	47.7	
C allele	128	67.4	92	70.8	0.519
A allele	62	32.6	38	29.23	
			rs4652		
AA	37	38.9)	27	41.5	
AC	39	41.1	29	44.6	0.602
CC	19	20	9	13.8	
A allele	113	59.5	83	63.8	0.430
C allele	77	40.5	47	36.2	

Table 3. The genotype distribution for rs4644 and rs4652 variants of galectin-3 according to histopathological features of NSCLC patients

| | rs4644 | | | | | | rs4652 | | | | | |
| | AA | | AC | | CC | | AA | | AC | | CC | |
Features	n	%	n	%	n	%	n	%	n	%	n	%
Tumor stage												
T3+T4	1	9.1	4	36.4	6	54.5	6	54,5	3	27.3	2	18.2
T1+T2	3	9.4	13	40.6	16	50	15	46,9	11	34.4	6	18.8
Lymph node metastasis												
N1, N2, N3	0	0	5	35.7	9	64.3	9	64.3	4	28.6	1	7.1
N0	4	13.8	12	41.4	13	44.8	12	41.4	10	34.5	7	24.1
Perineural invasion												
No	3	6.5	22	47.8	21	45.7	17	37	24	52.2	5	10.9
Yes	1	5.3	8	42.1	10	52.6	10	52.6	5	26.3	4	21.1
Angiolymphatic invasion												
No	2	6.9	16	55.2	11	37.9	*8	27.6	18	62.1	3	10.3
Yes	2	5.6	14	38.9	20	55.6	*19	52.8	11	30.6	6	16.7
Vascular invasion												
No	2	4.3	23	50	21	45.7	18	39.1	23	50	5	10.9
Yes	2	10.5	7	36.8	10	52.6	9	47.4	6	31.6	4	21.1

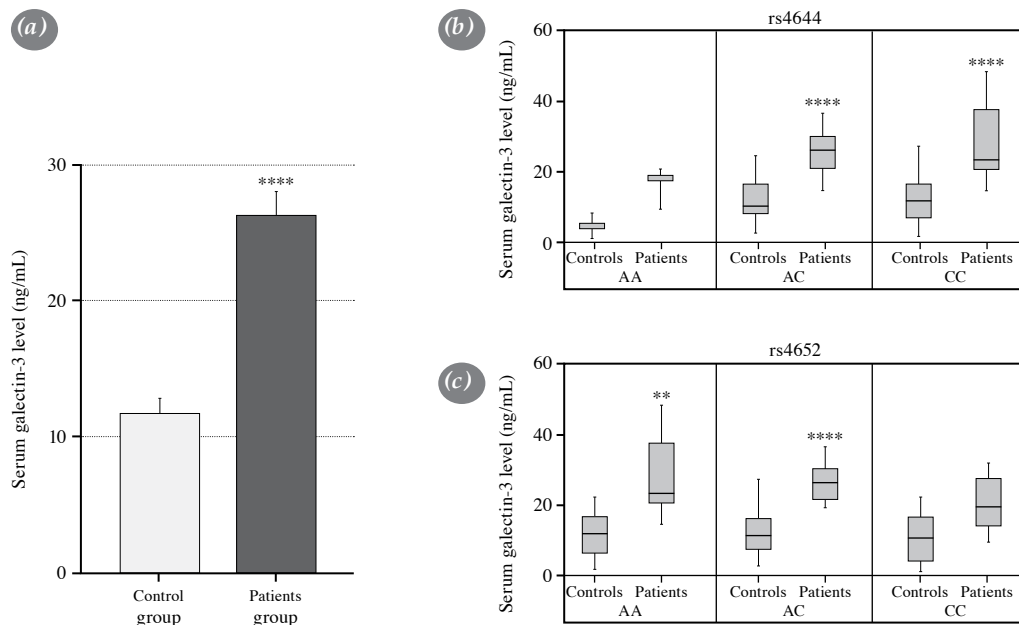NSCLC: non-small cell lung cancer; * p<0.05.

Figure 1. The comparison of serum galectin-3 levels **(a)** in controls vs. NSCLC patients **(b)** among NSCLC patients having genotypes of rs4644 variant **(c)** among NSCLC patients having genotypes of rs4652 variant. The data are given in mean ± standard error.

NSCLC: Non-small cell lung cancer; ** p<0.01; **** p<0.0001.

Table 4. Distribution of serum galectin-3 levels (ng/mL) according to histopathological features of NSCLC patients among genotypes of rs4644 variant

Features	AA Mean±SE	AA Mean	*p*	AC Mean±SE	*p*	CC Mean±SE	*p*
Tumor stage							
T3+T4		9.72	0.667	27.6±2.7	0.913	32.9±6.2	0.315
T1+T2	19.4±1.2			26.0±2.4		26.4±4.1	
Node metastasis							
Yes		-		27.0±2.9	1.00	33.9±5.8	0.247
No	16.2±3.3			26.4±2.3		24.5±3.6	
Perineural invasion							
Yes		9.72	0.667	27.9±1.9	0.445	29.6±5.0	1.00
No	19.4±1.2			24.4±2.7		27.4±4.5	
Vascular invasion							
Yes		9.72	0.667	30.8±2.0	0.03*	24.5±3.6	0.247
No	19.4±1.2			23.0±1.9		33.9±5.8	
Angiolymphatic invasion							
Yes		9.72	0.667	26.4±1.9	0.641	28.76±3.43	0.667
No	19.4±1.2			24.0±4.4		-	

NSCLC: Non-small cell lung cancer; SE: Standard error; * p<0.05.

Table 5. Distribution of serum galectin-3 levels (ng/mL) according to histopathological features of NSCLC patients among genotypes of rs4652 variant

Features	AA Mean±SE	*p*	AC Mean±SE	*p*	CC Mean±SE	CC Mean	*p*
Tumor stage							
T3+T4	32.9±6.2	0.315	28.1±3.8	1.00	18.0±8.3		0.80
T1+T2	26.4±4.1		26.9±2.4		21.5±3.6		
Node metastasis							
Yes	33.9±5.8	0.247	24.6±2.7	0.50		31.72	
No	24.5±3.6		28.0±2.3		18.02±2.75		
Perineural invasion							
Yes	29.6±5.0	1.00	27.3±2.8	0.914	22.6±6.6		0.70
No	27.4±4.5		25.9±2.7		18.0±1.5		
Vascular invasion							
Yes	24.5±3.6	0.247	31.9±2.2	0.019*	18.0±8.3		0.80
No	33.9±5.8		22.9±1.4		21.5±3.6		
Angiolymphatic invasion							
Yes	28.8±3.4	0.533	27.1±2.2		20.7±5.0		1.00
No	-		24.0±4.4		19.4±1.2		

NSCLC: Non-small cell lung cancer; SE: Standard error; * p<0.05.

Table 6. Multivariate logistic regression analysis for the risk of development of NSCLC

	Parameters (controls/patients)		
	OR	95% CI	p
Serum galectin-3 level (higher *vs.* lower)	57.431	9.085-363.046	<0.001
Sex (male *vs.* female)	2.002	0.201-19.948	0.554
Smoking (smoker *vs.* non-smoker)	21.730	1.357-348.056	0.030

NSCLC: Non-small cell lung cancer; OR: Odds ratio; CI: Confidence interval; * p<0.05.
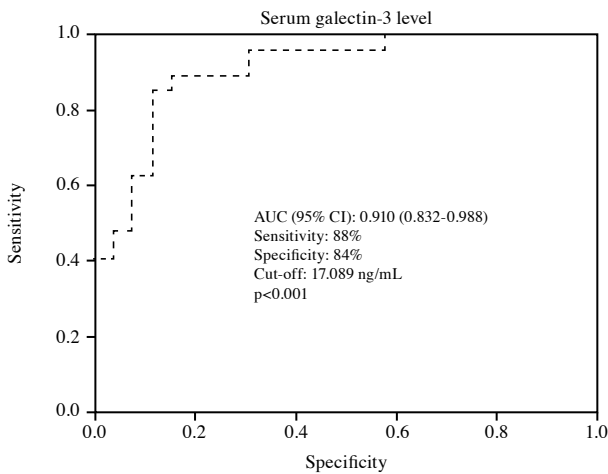

Figure 2. Receiver operating characteristics (ROC) analysis for serum galectin-3 level in NSCLC patients.

NSCLC: Non-small cell lung cancer.

association of serum galectin-3 level, sex, and smoking status with NSCLC occurrence. It was shown that serum galectin-3 level (odds ratio [OR]: 57.431; 95% confidence interval [CI]: 9.085-363.046; p=0.000) and smoking (OR: 21.730; 95% CI: 1.357-348.056; p=0.03) could be predictive risk factors of NSCLC influencing the diagnosis independent from variant distribution of galectin-3 (Table 6).

To further evaluate the diagnostic value of serum galectin-3 level in NSCLC patients, the ROC curve was plotted (Figure 2). The cut-off value of serum galectin-3 level was calculated as 17.089 ng/mL with the maximal sensitivity (88%) and specificity (84%) (AUC: 0.910±0.04; 95% CI: 0.832-0.988; p<0.001).

Galectin-3 mRNA expression level was determined in the tumor tissue, compared to the corresponding surrounding healthy tissue of the same participant for each NSCLC patient by the RT-qPCR. The mean galectin-3 mRNA expression was higher in surrounding healthy tissue referred to as control tissue (0.09±0.039 fold) (0.09±0.039 fold) than tumor tissue (0.04± 0.012), although this difference was not statistically significant (p=0.4959) (Figure 3).

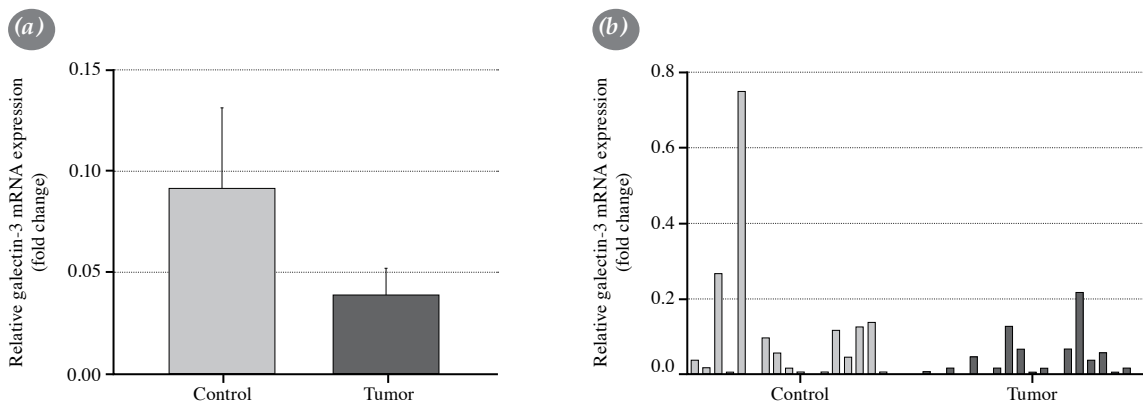**Figure 3.** The comparison of galectin-3 mRNA expression levels **(a)** in control (surrounding healthy tissue) *vs.* tumor tissues of NSCLC patients (p=0.4959) **(b)** individual distribution of galectin-3 mRNA expression level between control and tumor tissues.

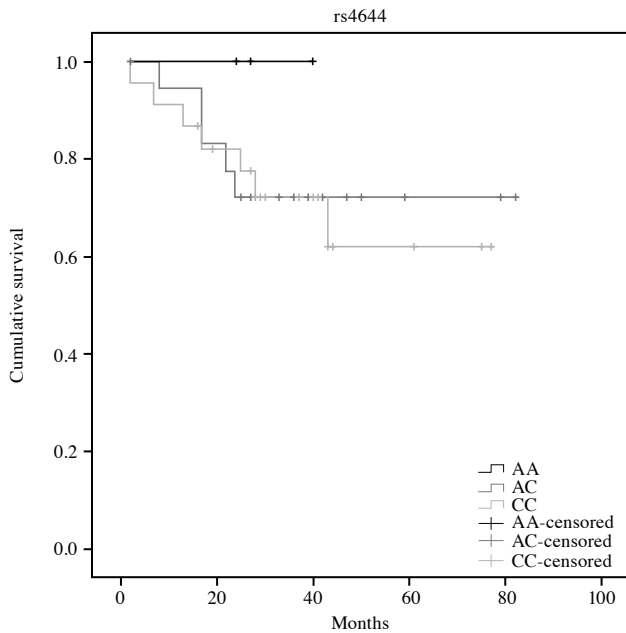NSCLC: Non-small cell lung cancer; mRNA: Messenger ribonucleic acid.

Figure 4. Survival of NSCLC patients according to genotypes with rs4644 variant of galectin-3. No statistically significant difference was found between groups (p=0.625).
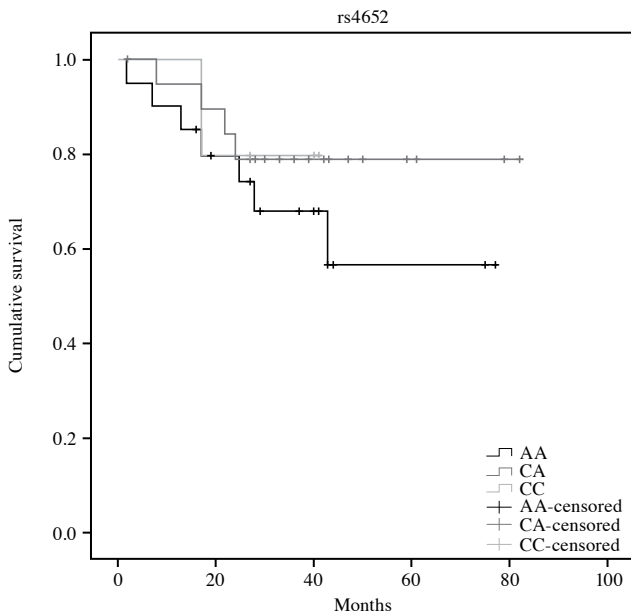
NSCLC: Non-small cell lung cancer.

Figure 5. Survival of NSCLC patients according to genotypes with rs4652 variant of galectin-3. No statistically significant difference was found between groups (p=0.329).

NSCLC: Non-small cell lung cancer.

The five-year overall survival rate of operated NSCLC patients was 68.2% and the median survival time was 62.7 months (95% CI: 53.4-72.1). While analyzing survival using log-rank comparison of survival data with regards to genotypes of galectin-3 gene variants, there was no statistically significantly different survival rates between the patients with genotypes of rs4644 variant (p=0.625) (Figure 4). Similarly, no statistically significant difference in the survival rates was found between patients with regards to rs4652 genotypes (p=0.349) (Figure 5). In terms of galectin-3 serum levels, the five-year survival rate of patients who had higher serum galectin-3 levels (>17.089 ng/mL) was 44.4%, whereas it was 64.3% in the patients with lower serum galectin-3 levels (p=0.048) (Figure 6). The N factor, T factor, angiolymphatic involvement, and serum galectin-3 levels were subjected to the multivariate Cox proportional analysis. It revealed that only high levels of serum galectin-3 tended to be associated with survival of the patients (hazard ratio [HR]=5.106; 95% CI: 0.956-27.267; p=0.056) (Table 7).

DISCUSSION

Many driver mutations and SNPs have been identified to elucidate the causative molecular mechanism underlying NSCLC. However, extensive clinical studies of lung cancer are still needed to use the genetic variations as a useful clinical diagnostic and prognostic marker. In our study, we examined the role of galectin-3 gene variants (rs4644 and rs4652) in tumor risk and survival of patients with surgically resected early-stage NSCLC. The patients and control groups were comparable in terms of sex and smoking status. However, males were found to have 4.9 times higher risk of having lung cancer consistent with the results of Kacan et al.[21] Besides, smoking, particularly
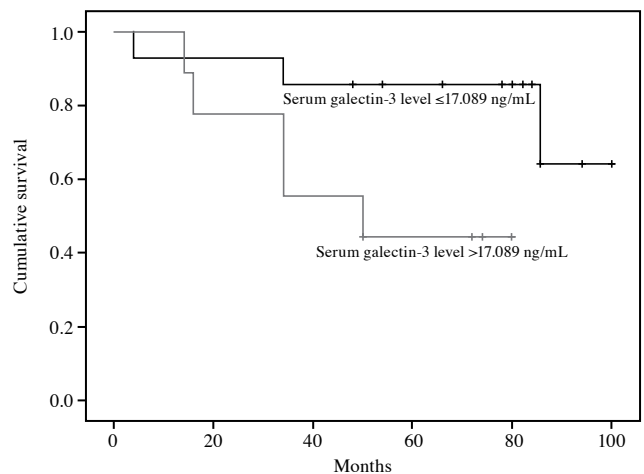

Figure 6. Survival of NSCLC patients according to serum galectin-3 levels. The difference was statistically significant (p=0.048).

NSCLC: Non-small cell lung cancer.

Table 7. Multivariate cox regression model for death hazard estimation in NSCLC patients

Parameters	Hazard ratio	95% CI	p
Nodal metastasis (N1-2 *vs.* N0)	2.45	0.471-12.79	0.287
T factor (T2-3 *vs.* T1)	1.092	0.97-12.30	0.943
Lymphatic invasion (presence *vs.* absence)	0.678	0.33-13.79	0.800
High serum galectin-3 level (>17.089 ng/mL) (higher *vs.* lower)	5.106	0.956-13.79	0.056

NSCLC: Non-small cell lung cancer; CI: Confidence interval.

in parallel to male sex, is one of the most causes for lung cancer. In our study, 84.6% of the patients were smokers.

In terms of SNPs, the distribution of rs4644 and rs4652 variants were not found to be exclusive between the patients and controls. However, rs4652 variant of galectin-3 was reported to be associated with platinum-based chemotherapy response and prognosis in patients with NSCLC rather than rs4644 variant.[22] However, we did not analyze the response to chemotherapy of our patients, since all patients underwent resection surgery. Regarding the possible roles of rs4644 and rs4652 variants in the incidence of several cancer types,[13-17] the sample size of the present study could be enlarged to reach statistical significance for the genotype and allele distribution between the patients and controls.

In our study, the frequency of patients with AA homozygous genotype of rs4652 variant was significantly increased in the presence of angiolymphatic invasion. In the studies including NSCLC patients with Stage I-IIA, having angiolymphatic invasion was found to be a negative prognostic factor for the development of long-term survival and relapse.[23] However, we obtained no association between the genotypes of galectin-3 variants (rs4644 and rs4652) and survival in NSCLC patients.

Serum galectin-3 levels were higher in patients with AC and CC genotypes of rs4644 variant than in healthy controls. Moreover, rs4652 variant showed higher serum galectin-3 levels in patients with AA and AC genotypes, compared to the control group. There has been no study exemplifying the distribution of serum galectin-3 levels with respect to genotypes of galectin-3 variants in NSCLC patients. However, it has been evidenced that polymorphic amino acid changes in both the rs4644 and rs4652 positions along with the distribution of genotypes affect the secretion of serum galectin-3 in the body through taking an active role in various pathological processes which contribute

to the cancer and other diseases.[12] Nevertheless, the exact mechanism of how amino acid change alters the protein expression and function is still unclear. It is known that rs4644 in the 64th residue which harbors proline than histidine has a more stable structure which is evolutionarily conserved, any change in this sequence is likely to affect the function of this molecule.[24] Besides, proline amino acid at the 98th residue of rs4652 variant is also known as critical in protein transportation.[22] Studies on hamsters, N-terminal sequence containing residues 89-96 (Tyr-Pro-Ser-Ala-Pro-Gly-Ala-Tyr) in the galectin-3 gene was found to play a critical role in protein secretion.[25] Also, N-terminal region of galectin-3 is evolutionary conserved in mammalians.[22] Therefore, human N-terminal region located on the human rs4652 variant of galectin-3, which is highly homologous to hamsters, may alter the intracellular galectin-3 level in the body.

Considering the pathological features of patients, in the presence of vascular invasion, serum galectin-3 levels were found to be higher in patients with AC genotypes of both rs4644 and rs4652 variants. The main molecular mechanisms proposed for the galectin-3 promoted angiogenesis include the binding of galectin-3 carbohydrate recognition domain (CRD) to αv3 integrins on endothelial cells, and subsequently inducing the signaling pathways related to angiogenic activity such as vascular endothelial growth factor, fibroblast growth factor, activation of focal adhesion kinase phosphorylation.[8] Shi et al.[16] hypothesized that an A>C alteration at rs4652 site located on the CRD of galectin-3 might strengthen the interaction between CRD and fibroblast surface receptors, thereby, leading to pathway the myofibroblast promotion of angiogenesis and the growth of gastric cancer cells,[16] as activated fibroblasts, also known as myofibroblasts, were thought to increase angiogenesis by interacting with cancer cells.[26]

Multivariate regression analyses have confirmed that smoking contributed to the lung cancer risk matching variables such as age and sex.[27] To extend

the work of literature, serum galectin-3 level was also identified as a promising predictive risk factor for NSCLC in our study. Furthermore, the cut-off value of serum galectin-3 level (17.089 ng/mL) was proposed for assessing the discriminatory accuracy between NSCLC patients and healthy individuals.

Finally, in the survival analysis, we found that rs4644 and rs4652 genotypes of galectin-3 were not associated with survival. However, we found that high serum galectin-3 level was marginally associated with lower survival among NSCLC patients. Multivariate analysis indicated that high serum galectin-3 level tended to be an independent surrogate marker for worse survival. This finding may help to select patients for possible adjuvant, despite having early-stage tumors. It is also plausible to propose that the patients with higher serum galectin-3 levels could be followed more frequently for possible earlier recurrences. Similarly, Kataoka et al.[28] found that higher expressions of galectin-3 in tumor cells were associated with tumor recurrence. However, they did not perform multivariate analysis to prove that whether high galectin-3 level was independently related with worse survival.

The main limitations to our study include relatively low number of patients and the matching problems for sample from the same study population, resulting from the exclusion of certain patients, particularly for the expression analysis. Therefore, a heterogeneous gene expression profile of galectin-3 was obtained among tumor tissues of NSCLC patients. A larger series with the analysis of higher number of patients could have revealed a statistically significant association between serum galectin-3 level and survival. Thus, the outcomes of this study warrant further confirmation with larger groups.

In conclusion, the present study may provide a basis for the literature investigating the role of galectin-3 variants in lung cancer by shedding light into whether galectin-3 may be one of the important biomarkers to be suggested in disease risk and progression. Serum galectin-3 level may be utilized to better identify patients with early-stage lung cancer who can benefit from adjuvant therapy after surgical resection. Further *in vitro* studies are needed to elucidate cellular functional changes in cancer signaling pathways related to rs4644 and rs4652 variants of galectin-3.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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