pagepress

Can serum progranulin level be used as a prognostic biomarker in non-small cell lung cancer?

Nevin Taci Hoca[1], Ebru Ünsal[2], Koza Murat[3], Arzu Ertürk[4], Nermin Çapan[5]

[1]Department of Pulmonology, Gazi University, Ankara; [2]Department of Pulmonology, Yıldırım Beyazıt University, Ankara; [3]Department of Biochemistry, Saglik Bilimleri University, Ataturk Sanatoryum Education and Research Hospital, Ankara; [4]Department of Pulmonology, Ahi Evran University, Kırsehir; [5]Department of Pulmonology, Saglik Bilimleri University, Ataturk Sanatoryum Education and Research Hospital, Ankara, Turkey

Abstract

Progranulin has been considered to be a poor prognostic biomarker for some types of malignancies. However, the clinical significance of serum progranulin level and the prognostic value are still not explored in advanced stages of lung cancer. The current study investigates the prognostic significance of progranulin serum levels in advanced-stage non-small cell lung cancer (NSCLC) patients. This study involved 94 subjects (70 advanced-stage NSCLC patients and 24 healthy controls). Serum progranulin level was measured by enzyme-linked immunosorbent assay (ELISA) and was correlated with patient outcome. The association between circulating progranulin level and clinicopathological parameters was detected. Serum progranulin cut-off level predicting six-month survival was determined. Serum progranulin level was found significantly elevated in NSCLC patients than in the control group ($p<0.001$). We did not determine a significant difference between stage IIIB and stage IV NSCLC patients for serum progranulin levels ($p=0.166$). When we evaluated the laboratory parameters, only serum LDH level was found significantly correlated with serum progranulin level ($p=0.043$), also bone and liver metastasis showed a significant correlation with progranulin level ($p=0.008$ and $p=0.024$, respectively). The cut-off level of serum progranulin in predicting six months of survival was determined as 16.03 ng/ml (AUC = 0.973, 95%Cl: 0.903-0.997, $p<0.001$) with 97.06% sensitivity and 88.89% specificity. Overall survival was determined shorter in patients with progranulin level ≥16 ng/ml than those with <16 ng/ml ($p<0.001$). Also, in the multivariate analysis using the Cox regression model serum progranulin level was found as an independent prognostic factor for NSCLC ($p=0.001$). Serum progranulin level may be a useful biomarker for predicting poor survival in advanced-stage NSCLC patients.

Correspondence: Nevin Taci Hoca, MD., Department of Pulmonology, Gazi University, Mevlana Blv 29, 06560 Ankara, Turkey.
Tel. +90.3122024444 - Mobile: +90.5327655405.
E-mail: nevintacihoca@yahoo.com

Key words: progranulin, cancer, prognostic factor, biomarker.

Contributions: all the authors made a substantive intellectual contribution, read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Ethical committee approval: the study protocol received Institutional Review Board approval (Keciören Education and Research Hospital Ethical Board of Clinical Research, no: 2012-KAEK-15/1138) and all participants provided informed consent.

Availability of data: all data generated or analyzed during this study are included in this published article

Received: 10 July 2022.
Accepted: 8 November 2022.
Early view: 14 November 2022.

Monaldi Archives for Chest Disease 2023; 93:2373
doi: 10.4081/monaldi.2022.2373

Introduction

Lung cancer is the most common cancer type seen in the world [1]. The prognosis of lung tumors mostly depends on the histology and disease stage at diagnosis [2]. There are two main histological subtypes of lung carcinoma that are defined non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) [3]. Non-small cell lung cancer is having a poor prognosis and high mortality rate. However, in some of these patients having a good prognosis the molecular background of NSCLC that favors longer survival has not been yet determined [4].

Many growth factors play an important role in the pathophysiology of tumorigenesis, tumor spread, and treatment response. Thus, these factors have been investigated for their prognostic role in tumor tissue and serum of lung cancer patients [5]. Progranulin, also called PC-cell-derived growth factor, granulin/epithelin precursor, or acrogranin is a secreted 88 kDa glycosylated protein (GP88), which is having important functions in inflammation,

tumorigenesis, and wound repair. It has been shown to mediate cell cycle progression, cell motility, and inflammatory processes [6,7]. The elevated progranulin expression has been demonstrated in some tumor types such as melanoma, breast, prostate, ovarian, renal, liver, and esophageal cancers, and also it has been recently identified as a poor prognostic biomarker in melanoma, ovarian and breast cancer [6,8-13].

High expression of progranulin in tumor tissues is having a role in cell proliferation, angiogenesis, malignant transformation, invasion, and resistance to anticancer drugs. Many studies in the last decades have investigated the importance of progranulin expression in cancer cells, but limited information is available on its significance in NSCLC. We determined the prognostic value of serum progranulin level in patients having advanced stages of NSCLC.

Materials and Methods

Our study was a prospective, case-control study in which patients were recruited consecutively from the tertiary referral hospital from July 2016 to July 2018. The patients were followed until January 2020. For this research, 70 patients diagnosed with locally advanced or metastatic NSCLC and 24 healthy volunteers were enrolled in the study. Study inclusion criteria were histologically confirmed diagnosis of NSCLC, age ≥18 years, stage IIIB or IV at primary diagnosis. Patients having other malignancies, autoimmune, neurodegenerative diseases or infection diseases, immunosuppressive drug usage, or pregnancy were excluded. The diagnosis of lung cancer was established according to the revised World Health Organization Classification of Lung Tumors and clinical staging was performed due to the TNM staging for lung cancer [2,3]. There were 16 (22.9%) patients with stage IIIB and 54 (77.1%) patients with stage IV. Patients were first evaluated with detailed clinical history and physical examination. Also, complete blood cell counts, biochemistry tests and chest roentgenograms, positron emission tomography (PET) imaging and cranial magnetic resonance imaging (MRI) were all performed before treatment. Patients received chemotherapy with/without radiotherapy (curative or palliative radiotherapy) or only supportive therapy depending on the stage of disease and Eastern Cooperative Oncology Group (ECOG) performance status.

Institutional ethics committee approval (Keciören Education and Research Hospital Ethical Board of Clinical Research, no: 2012-KAEK-15/1138) and informed consent from all participants were obtained. Also, the study was performed according to the ethical standards of the appropriate version of the Helsinki Declaration.

Approximately 5 mL of peripheral venous blood samples from all participants were collected in the morning in fasting status at the time of diagnosis before any therapeutic measures were started. The blood samples were centrifuged at 1000 × g for 15 min and the serum was stored at -80°C until analysis. All samples were assayed on the same day. The serum progranulin level was measured according to the manufacturer's instructions by using enzyme-linked immunosorbent assay (ELISA) kits (Human progranulin Elisa Kit). The results were reported as ng/mL.

The data were evaluated in a statistical package program IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA). The number of units (n), percentage (%), mean ± standard deviation ($\bar{x}$± ss), and median [interquartile range (IQR)] were all reported. The normal distribution of numerical variables was determined by the Shapiro-Wilk normality test and Q - Q graphs. In the comparison of the two groups, the variables showing the normal distribution were evaluated by the independent samples *t*-test, whereas the variables not normally distributed were examined by Mann-Whitney U analysis. Overall survival was reported as the time in months from the date of diagnosis to the date of death or last follow-up. Kaplan Meier method was used to determine the survival distribution. The difference in survival time was analyzed by the log-rank test. Variables related independently to mortality were determined by Cox proportional hazards model. ROC (receiver operating characteristics) curve analysis was performed to select the appropriate cut-off level for serum progranulin in predicting 6 months survival and its overall diagnostic accuracy was estimated by areas under ROC curves. Serum progranulin optimal cut-off values were determined according to the Youden method. A p-value <0.05 was accepted as statistically significant.

Results

A total of 70 patients diagnosed with locally advanced or metastatic NSCLC (65 male, 5 female) and 24 healthy volunteers (22 male, 2 female) were included in this study. The mean age of patients and healthy volunteers was 61.37 ± 8.47 and 62.08 ± 7.95 years. Thirty-four (48.5%) patients were diagnosed with adenocarcinoma, 23 (32.9%) patients with squamous cell carcinoma, and 13 (18.6%) patients with NSCLC not otherwise specified (NOS) respectively. In a 6-months period, 34 (48.6 %) patients were alive, whereas 36 (51.4 %) patients died. After 1 year period, 19 (27.1 %) patients were alive, whereas 51(72.9 %) patients died. At the end of the study, 9 (12.9 %) patients were alive, whereas 61 (87.1 %) patients died. The features of the patients were shown in Table 1.

Table 1. Clinical and pathological characteristics of patients.

Characteristics	n (%)
Age*	61.37±8.47 years
Smoking#	40 (30) pack/years
Female	5 (7.1)
Male	65 (92.9)
BMI*	22.96±3.99
Histopathological type	
Adenocarcinoma	34 (48.5)
NSCLC not otherwise specified	13 (18.6)
Squamous cell carcinoma	23 (32.9)
Stage	
Stage IIIB	16 (22.9)
Stage IV	54 (77.1)
Treatment modality	
Chemotherapy	22 (31.4)
Radiotherapy	7 (10)
Chemotherapy + radiotherapy	20 (28.6)
Supportive therapy	21 (30)
Survival	
6 months interval alive	34 (48.6)
6 months interval exitus	36 (51.4)
1-year interval alive	19 (27.1)
1-year interval exitus	51(72.9)
At the end of the study alive	9 (12,9)
At the end of the study exitus	61 (87.1)

*Mean±SD; #median (interquartile range); NSCL, non-small cell lung cancer.

We compared the NSCLC patients and healthy controls for serum progranulin levels. Serum progranulin level was found significantly more elevated in NSCLC patients than in healthy controls (p<0.001). Serum progranulin levels did not correlate with age. The association between serum progranulin level and clinicopathological parameters was summarized in Table 2. There was no statistically significant difference between stage IIIB (locally advanced stage) and stage IV (advanced stage) NSCLC patients (p=0.166), whereas bone and liver metastasis showed a significant correlation with pre-treatment serum progranulin level (p=0.008 and p=0.024, respectively). Also, the progranulin level did not show a statistically significant association with the histopathological type (p=0.700). Additionally, when we evaluated the laboratory parameters, only serum LDH level was found to have a statistically significant correlation with progranulin level (p=0.043).

The ROC curve analysis demonstrated the optimal cut-off level of serum progranulin in predicting 6 months' survival as 16.03 ng/mL. We found the area under the ROC curve (AUC) as 0.973 with a 95% confidence interval (95%Cl): 0.903-0.997 (p<0.001) (Figure 1). This cut-off level of progranulin showed 97.06% sensitivity, 88.89% specificity, 89.2% positive predictive value, and 97% negative predictive value respectively for estimating predicting 6 months of survival. Kaplan Meier's analysis revealed that NSCLC patients having progranulin levels ≥16 ng/mL had shorter overall survival (OS: 2.651 months, 95% CI: 1.377-3.925) than the patients with progranulin levels <16 ng/mL (OS: 14.326 months, 95% CI: 12.367-16.286) (Figure 2). There was a significant difference in survival of the two groups (p<0.001). In the multivariate analysis using the Cox regression test, liver metastasis (p=0.001), adrenal gland metastasis (p=0.012) and progranulin level (p=0.001) were found independently associated with short survival (Table 3).

Table 2. Association of serum progranulin level with clinicopathological variables.

Variables	Progranulin level, ng/mL, median (interquartile range)	p-value
Control (n=24)	5.69 (5.17)	<0.001*
Patients (n=70)	13.62 (45.30)	
Anemia		
No (n=64)	11.2 (41.03)	0.056
Yes (n=6)	51.75 (58.51)	
High LDH level		
No (n=40)	10.16 (38.91)	0.043*
Yes (n=30)	24.82 (43.93)	
High CRP level		
No (n=12)	10.5 (20.71)	0.629
Yes (n=58)	15.51 (45.47)	
Histopathology		
Adenocarcinoma (n=34)	16.55(43.75)	0.700
NSCLC not otherwise specified (n=13)	42.26 (48.67)	
Squamous cell carcinoma (n=23)	11.2 (11.57)	
Stage		
Stage IIIB (n=16)	9.99 (29.86)	0.166
Stage IV (n=54)	18.1 (44.95)	
Bone metastasis		
No (n=38)	10.16 (20.10)	0.008*
Yes (n=32)	30.51 (48.25)	
Cranial metastasis		
No (n=54)	11.2 (37.79)	0.097
Yes (n=16)	35.34 (55.66)	
Adrenal gland metastasis		
No (n=49)	11.2 (44.68)	0.058
Yes (n=21)	21.21 (46.60)	
Liver metastasis		
No (n=62)	11.2 (39.00)	0.024*
Yes (n=8)	51.74 (29.51)	

*Statistically significant; CRP, C-reactive protein; LDH, lactate dehydrogenase; NSCL, non-small cell lung cancer.

Discussion

Progranulin is a growth factor having significant biological effects on different cancer types, as it stimulates angiogenesis, migration, and invasion [6,7,12,13]. Pathological studies have shown that progranulin was not expressed in normal tissues and benign lesions, whereas it was overexpressed in tumor tissues [6,12,13]. Studies using cell lines and animal models provide evidence that progranulin induces tumor cell proliferation, migration, and drug resistance. As progranulin is an extracellular regulator of tumorigenesis, it is a potential biomarker of prognosis in various cancer types [7,8]. For example, in human breast adenocarcinoma cell lines, there was an association between progranulin expression and growth of tumor cells, furthermore, in mouse xenograft studies, inhibition of progranulin expression led to a 90% reduction in tumor cells growth [6,8]. Also, elevated serum progranulin levels have been reported in many cancer types [14-16]. In addition, pro-

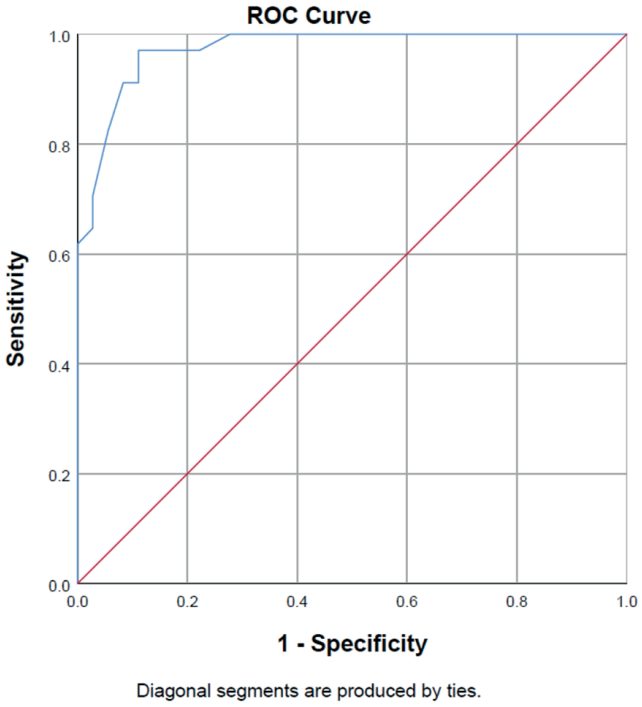

Figure 1. ROC curve for predicting 6 months survival by comparing patients (n=34) with serum progranulin level <16.03 ng/mL and patients (n=36) with ≥16.03 ng/mL (area under the ROC curve=0.973, 95%Cl; 0.903 to 0.997, p<0.001).

granulin has been considered to be a prognostic biomarker for some types of malignancies, as it has an important role in carcinogenesis. Carlson *et al*. reported that the serum level of progranulin was significantly elevated in patients having ovarian epithelial cancer with advanced stages, but not in the earlier stages as stages I and II [17]. Similarly, serum progranulin level was found higher in advanced NSCLC patients than in healthy controls in this study ($p<0.001$).

Serum progranulin level increases in systemic lupus erythematosus, rheumatoid arthritis, and some interstitial lung diseases. Because of this, the patients having other malignancies, autoimmune diseases, neurodegenerative diseases, or chronic infections were not included in this study [8,13,18,19]. Tanaka *et al*. showed that serum progranulin levels were correlated with the prognosis of dermatomyositis with interstitial lung diseases and also had a significantly positive association with serum LDH levels (r_S = 0.54, p=0.003) [19]. In another study, circulating progranulin level was associated with elevated serum LDH level in patients with liver enzyme dysfunction [20]. Similar to these previous studies, we also found serum LDH level significantly correlated with progranulin level.

To our knowledge, there are few studies evaluating the prognostic value of progranulin in lung cancer. Edelman *et al.* firstly demonstrated the progranulin expression in 70% of NCCLC patients whereas there was no expression in normal tissues and small cell lung carcinoma. They found high progranulin expression in tumor tissues correlated with poor survival in NSCLC patients with early stages as stage I, II, and also in stage IIIA. The same study demonstrated the utility of progranulin as a biomarker for NSCLC because the blood levels of progranulin were determined higher in patients with stages IIIB and IV than in healthy individuals. However, they did not evaluate the association between serum progranulin level and survival in advanced NSCLC patients, whereas progranulin expression was found to correlate with poor prognosis in early stages [15]. Patients having advanced-stage NSCLC and short survival expectancy were included in our study. Thus, we evaluated the association between serum progranulin levels and survival in advanced NSCLC patients. The cut-off level of serum progranulin in predicting 6 months survival was determined as 16.03 ng/mL with 97.06% sensitivity and 88.89% specificity. Also in this study, high progranulin concentrations were associated with a decrease in overall patients' survival. Patients having serum progranulin level ≥16 ng/mL had 2.6 months survival, whereas overall survival was 14.3 months for patients having progranulin level <16 ng/mL ($p<0.001$). In the multivariate analysis, liver metastasis (p=0.001) and adrenal gland metastasis (p=0.012) were found independently correlated with short survival. Also, serum progranulin level was determined as an independent prognostic factor for NSCLC in the multivariate analysis using the Cox regression model (p=0.001). Therefore, this study shows that the use of progranulin as a prognostic biomarker in NSCLC is promising.

Also, Naumnik *et al*. studied progranulin levels in bronchoalveolar lavage fluid (BALF) of forty-six patients having advanced NCSLC and they found progranulin levels elevated in cancer patients compared to the healthy control group. They suggested that BALF progranulin levels are associated with time to

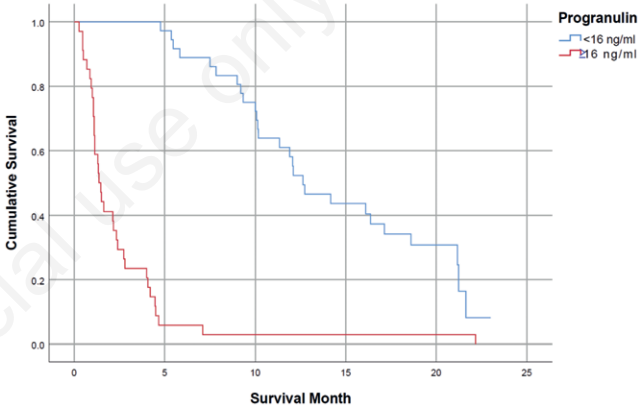

Figure 2. Survival analysis of patients based on serum progranulin level was evaluated by Kaplan–Meier survival curves. Survival was determined shorter in patients with progranulin levels ≥16 ng/mL than in those with <16 ng/mL ($p<0.001$).

Table 3. Multivariate analysis related to survival using Cox regression.

	HR	%95 CI	p-value
Age	1.035	0.991 - 1.081	0.124
Smoking	1.012	0.998 - 1.026	0.093
Gender	1.501	0.349 - 6.449	0.585
Tumor diameter	0.888	0.770 - 1.024	0.102
Adenocarcinoma			0.730
NSCLC not otherwise specified	0.751	0.291 - 1.936	0.553
Squamous cell carcinoma	1.121	0.516 - 2.433	0.774
Stage	0.737	0.257 - 2.118	0.571
Bone metastasis	1.786	0.796 - 4.009	0.160
Cranial metastasis	0.648	0.298 - 1.409	0.274
Contralateral lung metastasis	1.059	0.374 - 3.001	0.914
Pleural metastasis	1.371	0.616 - 3.053	0.439
Adrenal gland metastasis	2.103	1.177 - 3.756	0.012*
Liver metastasis	5.532	2.253 - 13.584	0.001*
Progranulin level	1.083	1.060 - 1.107	0.001*

HR, hazard ratio; *statistically significant.

tumor progression [21]. In another study, progranulin expression was found higher in the squamous cell carcinoma tissues than in adjacent non-tumorous tissues. They suggested that microRNA-588 expression suppressed the invasion of tumor cells by targeting progranulin in lung squamous cell carcinoma [22]. Progranulin is reported to have effects in promoting angiogenesis and metastasis through the regulation of vascular endothelial growth factors in lung cancer [23]. The mechanism of progranulin in contribution to carcinogenesis is unclear as the signaling receptor is still unclear. In recent years, some receptors such as sortilin, tumor necrosis factor receptor, and ephrin type-A receptor have been found as having potential links in the action of progranulin [24,25].

Since patients with advanced-stage lung cancer were included in this study, we do not know whether progranulin could be useful in predicting poor prognosis in early-stage lung cancer or other types of lung cancer patients. Also, the number of patients included in this study was relatively low. These are considered limiting factors of our study.

Conclusions

There is a significantly elevated serum progranulin level in NSCLC patients with advanced stages, and especially with bone and liver metastasis. Serum progranulin level seems to be associated with 6-month survival and overall patient survival. Baseline serum progranulin level may be a useful test for predicting the prognosis of NSCLC patients with advanced stage. The information that currently exists regarding progranulin is very exciting, but the studies with a higher number of patients which also include early-stage and other types of lung cancer are required to be able to make further comments.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: Cancer J Clin 2020;70:7-30.
2. Woodard GA, Jones KD, Jablons DM. Lung cancer staging and prognosis. Cancer Treat Res 2016;170:47-75.
3. Travis WD, Brambilla E, Nicholson AG, et al. The 2015 World Health Organization classification of lung tumors: Impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol 2015;10:1243-60.
4. Puderecki M, Szumiło J, Marzec-Kotarska B. Novel prognostic molecular markers in lung cancer. Oncol Lett 2020;20:9-18.
5. Salgia R. Prognostic significance of angiogenesis and angiogenic growth factors in NSCLC. Cancer 2011;117:3889-99.
6. Arechavaleta-Velasco F, Perez-Juarez CE, Gerton GL, Diaz-Cueto L. Progranulin and its biological effects in cancer. Med Oncol 2017;34:194.
7. Ong CHP, Bateman A. Progranulin (granulin-epithelin precursor, PC-cell derived growth factor, acrogranin) in proliferation and tumorigenesis. Histol Histopathol 2003;18:1275-88.
8. Serrero G. Potential of theranostic target mining in the development of novel diagnostic and therapeutic products in oncology: Progranulin/GP88 as a therapeutic and diagnostic target for breast and lung cancers. Rinsho Byori 2016;64:1296-309.
9. Berger K, Rhost S, Rafnsdóttir S, et al. Tumor co-expression of progranulin and sortilin as a prognostic biomarker in breast cancer. BMC Cancer 2021;21:185.
10. Purrahman D, Mahmoudian-Sani MR, Saki N, et al. Involvement of progranulin (PGRN) in the pathogenesis and prognosis of breast cancer. Cytokine 2022;151:155803.
11. Voshtani R, Song M, Wang H, et al. Progranulin promotes melanoma progression by inhibiting natural killer cell recruitment to the tumor microenvironment. Cancer Lett 2019;465:24-35.
12. Tanimoto R, Lu KG, Xu SQ, et al. Mechanisms of progranulin action and regulation in genitourinary cancers. Front Endocrinol (Lausanne) 2016;7:100.
13. Abella V, Pino J, Scotece M, et al. Progranulin as a biomarker and potential therapeutic agent. Drug Discov Today 2017;22: 1557-64.
14. Yamamoto Y, Goto N, Takemura M, et al. Association between increased serum GP88 (progranulin) concentrations and prognosis in patients with malignant lymphomas. Clin Chim Acta 2017;473:139-46.
15. Edelman MJ, Feliciano J, Yue B, et al. GP88 (progranulin): a novel tissue and circulating biomarker for non-small cell lung carcinoma. Hum Pathol 2014;45:1893-9.
16. Greither T, Fischer K, Theil G, et al. Expression of GP88 (progranulin) in serum of prostate cancer patients is associated with Gleason scores and overall survival. Cancer Manag Res 2018; 10:4173-80.
17. Carlson AM, Maurer MJ, Goergen KM, et al. Utility of progranulin and serum leukocyte protease inhibitor as diagnostic and prognostic biomarkers in ovarian cancer. Cancer Epidemiol Biomarkers Prev 2013;22:1730-5.
18. Cerezo LA, Kuklová M, Hulejová H, et al. Progranulin is associated with disease activity in patients with rheumatoid arthritis. Mediators Inflamm 2015;2015:740357.
19. Tanaka A, Tsukamoto H, Mitoma H, et al. Serum progranulin levels are elevated in dermatomyositis patients with acute interstitial lung disease, predicting prognosis. Arthritis Res Ther 2015;17:27.
20. Tanaka Y, Takahashi T, Tamori Y. Circulating progranulin level is associated with visceral fat and elevated liver enzymes: significance of serum progranulin as a useful marker for liver dysfunction. Endocr J 2014;61:1191-6.
21. Naumnik W, Panek B, Ossolińska M, Naumnik B. B Cell-attracting chemokine-1 and progranulin in bronchoalveolar lavage fluid of patients with advanced non-small-cell lung cancer: new prognostic factors. Adv Exp Med Biol 2019;1150:11-6.
22. Qian L, Lin L, Du Y, et al. MicroRNA-588 suppresses tumor cell migration and invasion by targeting GRN in lung squamous cell carcinoma. Mol Med Rep 2016;14:3021-8.
23. Zhou C, Huang Y, Wu J, et al. A narrative review of multiple mechanisms of progranulin in cancer: a potential target for anti-cancer therapy. Transl Cancer Res 2021;10:4207-16.
24. Kim WG. Association of serum progranulin levels with progression of papillary thyroid cancer. Endocrinol Metab (Seoul) 2020; 35:288-9.
25. Neill T, Buraschi S, Goyal A, et al. EphA2 is a functional receptor for the growth factor progranulin. J Cell Biol 2016;215:687-703.