

Elevated CEACAM5 Levels in Patients with Asthma

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Keywords

Asthma · Carcinoembryonic antigen-related cell adhesion molecule 5 · Induced sputum · Serum · Diagnosis

Abstract

Introduction: Asthma is a common chronic respiratory disease. This study aimed to explore the expression level of carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) in induced sputum supernatant, induced sputum cells, and serum of asthma patients. **Methods:** The protein levels of CEACAM5 in induced sputum supernatant and serum were detected by enzyme-linked immunosorbent assay. The expression of CEACAM5 in induced sputum cells was detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR). We analyzed the correlations between CEACAM5 expression and the clinical characteristics (FeNO and IgE) of asthma. The receiver operating characteristic (ROC) curve was used to analyze the diagnostic value of CEACAM5 in asthma. The expression level of CEACAM5 in 16HBE and BEAS-2B cells was detected by qRT-PCR. **Results:** The expression of CEACAM5 in induced sputum supernatant, induced sputum cells, and serum of asthma patients was significantly upregulated. Asthma patients with high CEACAM5 expression in induced sputum superna-

tant had higher levels of FeNO, IgE, and IL-13. The expression levels of CEACAM5 in induced sputum supernatant and induced sputum cells were positively correlated with FeNO and IgE. The ROC curve showed that CEACAM5 had a good diagnostic value in asthma. CEACAM5 expression was upregulated in BEAS-2B and 16HBE cells after IL-4 or IL-13 stimulation for 48 h. **Conclusion:** The expression levels of CEACAM5 in induced sputum supernatant, induced sputum cells, and serum of asthma patients were significantly increased. CEACAM5 may be involved in eosinophilic inflammation of asthma and may be used as a diagnostic biomarker and therapeutic target of asthma. © 2022 S. Karger AG, Basel

Introduction

Asthma is a common chronic respiratory disease that is characterized by chronic airway inflammation, mucus hypersecretion, and airway hyperresponsiveness [1]. Approximately 300 million people around the world are diagnosed with asthma. Moreover, the prevalence rate of

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Table 1. Characteristics of subjects

	Healthy subjects	Asthma patients	<i>p</i> value
<i>n</i>	34	106	
Sex, M:F (%F)	12/22 (64.7)	57/49 (46.2)	0.0765
Age, yr	32.97±13.61	44.32±15.73	0.0002
BMI, kg/m ²	21.50±3.087	23.27±3.292	0.0026
Lung function			
FEV1, % predicted	102.0±8.380	78.25±24.55	<0.0001
FVC, % predicted	101.3±10.80	91.28±20.63	0.0074
FEV1/FVC, %	86.97±6.345	70.09±14.00	<0.0001
Histamine PC20, mg/mL	na	1.998±2.247	–
FeNO, ppb	14.44±6.594	57.56±47.44	<0.0001
Blood			
Eosinophil rate, <i>n</i> (%)	2.656±3.612	4.610±3.816	0.0015
Neutrophil rate, <i>n</i> (%)	59.51±10.41	58.91±13.36	0.7776
Eosinophil count, <i>n</i> (10 ⁹ /L)	0.1788±0.3036	0.3138±0.2673	0.0005
Neutrophil count, <i>n</i> (10 ⁹ /L)	3.875±1.403	4.314±1.567	0.1713
Leukocyte count, <i>n</i> (10 ⁹ /L)	6.342±1.336	6.978±1.850	0.1158
IgE, IU/mL	97.10±102.70	214.70±206.20	0.0046
Others			
Family history (±)	0/34	17/89	–
Smoking history (±)	0/34	14/92	–

Values are presented as mean±SD. FeNO, fraction of exhaled nitric oxide; FEV1, forced expiratory volume in the first second; PC20, the dose at which FEV1 falls >20% of baseline FEV1.

asthma is increasing annually, and the number of cases is expected to reach 400 million by 2025 [2–4]. The pathogenesis of asthma is complex and involves innate immunity and adaptive immunity [5–7].

Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) has attracted considerable attention as a regulator of the immune system [8–10]. It is a glycoprotein with a molecular weight of 180 kDa and is widely expressed on various cell surfaces, including epithelial cells, endothelial cells, blood cells, and immune cells [11–13]. To date, studies on CEACAM5 have focused on tumors. For example, the high expression of CEACAM5 in the serum of patients with colorectal cancer, breast cancer, and lung cancer is closely related to tumor deterioration and subclinical metastasis [13–16]. CEACAM5 is also expressed in the human respiratory tract [17]. Nie et al. [18] integrated several asthma-related data sets and found that CEACAM5, the hub gene of asthma-related PPI network, was significantly upregulated in the bronchial epithelium from asthmatic patients. We also found a significant increase in the expression of CEACAM5 in asthma patients in GSE43696 in the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>). However, the protein expression of

CEACAM5 in induced sputum supernatant and serum remains unclear.

In this study, we aimed to explore the different expression of CEACAM5 in asthma patients and healthy people and analyze its association with eosinophilic inflammatory markers in asthma. The receiver operating characteristic (ROC) curve was used to analyze its diagnostic value. In addition, we induced an asthmatic cell model to further confirm CEACAM5 expression in vitro.

Materials and Methods

Study Subjects and Enrollment Criteria

A total of 140 subjects (34 healthy controls and 106 asthma patients) from the First Affiliated Hospital of Sun Yat-sen University were included in this study. Patients with asthma met the diagnostic criteria of the Global Initiative for Asthma (GINA) [2] guidelines and had no other respiratory diseases. The detailed inclusion and exclusion criteria are as follows. For the asthma group, the inclusion criteria were as follows: (1) in line with the diagnostic criteria of GINA: ① variable asthma symptom; ② variable airflow limitation test; ③ patients with variable asthma symptoms and those who meet any of the variable airflow limitation tests can be diagnosed with asthma. Atypical asthma without obvious wheezing symptoms should have at least one of the

above tests. (2) Diagnosed with asthma for the first time. (3) Age ≥ 16 years. The exclusion criteria for the asthma group were as follows: (1) complicated with other respiratory diseases or immune system-related diseases; (2) associated with circulatory, digestive, urinary, and neurological system diseases; (3) pregnant or lactating women; (4) has received standard drugs or anti-infective treatment for a long term or nearly 1 month. The inclusion criteria for the healthy group were as follows: (1) no obvious abnormality was found in chest X-ray or CT; the results of the pulmonary function test were normal. (2) No history of respiratory diseases such as asthma, chronic obstructive pulmonary disease, pulmonary infection, lung cancer, allergic rhinitis, eczema, allergic diseases, and autoimmune diseases. (3) Age ≥ 16 years. Among them, 23 patients were followed up after 1 month of treatment. Written informed consent was obtained from all participants, and all human-related studies were approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. The basic clinical information of the subjects is shown in Table 1.

Sample Collection

Sputum and peripheral blood were collected from the participants. The participants coughed deep sputum naturally or were induced by hypertonic saline, and 10 mL of peripheral blood was extracted. The sputum cell pellet was selected, weighed, and added with four times the weight of 0.1% DTT to dissolve [19]. Then, it was filtered by cell sieving. After centrifugation, the sputum supernatant was separated for further enzyme-linked immunosorbent assay (ELISA) detection, and 1 mL of TRIzol was added to the remaining cells for subsequent RNA extraction. Meanwhile, the serum was separated for subsequent ELISA detection.

Determination of FeNO

According to the recommendations of the American Thoracic Society/European Respiratory Society, an electrochemical analyzer (NIOX MINO Analytical Instruments; Aerocrine AB, Solna, Sweden) was used to determine FeNO [20–22]. In the process of measurement, the subjects were instructed to inhale air without NO to reach total vital capacity and then immediately exhale completely to the device at a constant expiratory flow rate of approximately 50 mL/s for 10 s.

Enzyme-Linked Immunosorbent Assay

The protein levels of CEACAM5 in sputum supernatant and blood serum were measured by using commercially available ELISA kits (HZbscience, Shanghai, China) in accordance with the manufacturer's instructions (the samples were diluted five times). The lower limit of CEACAM5 detection was 8 pg/mL, and the upper limit was 280 pg/mL. The protein levels of IL-5 and IL-13 in sputum supernatant were measured by using commercially available ELISA kits (MEIMIAN, Wuhan, China) in accordance with the manufacturer's instructions (the samples were diluted five times).

ICS Treatment

Patients with asthma were treated with a standard ICS regimen. We instructed them on the proper use of inhaled drugs. After 4 weeks of ICS treatment, we followed up patients who adhered to the medication and collected their induced sputum and peripheral blood again.

Cell Culture and Treatment

BEAS-2B cells (ATCC, Manassas, VA, USA) and 16HBE cells (EnoGene, Nanjing, China) were cultured in DMEM high glucose medium and RPMI 1640 medium (both from GIBCO, Grand Island, NY, USA), respectively, with 1% penicillin-streptomycin (HyClone, Logan, UT, USA) and 10% fetal bovine serum (Thermo Fisher, Waltham, MA, USA). Then, they were incubated with or without 20 and 50 ng/mL of IL-4/IL-13 (PeproTech, Rocky Hill, CT, USA) at 37°C in a humidified atmosphere containing 5% CO₂ for 48 h.

Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA from induced sputum cells and cultured cells was isolated by TRIzol (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into cDNA by using the PrimeScript RT reagent kit (Takara, Tokyo, Japan). The Roche LightCycler 480 II real-time system (Basel, Switzerland) and SYBR PreMix Ex Taq (Takara, Dalian, China) were used for qRT-PCR to quantify the mRNA level of CEACAM5 (forward: TCTTGGCTGATTGATGGGAAC, reverse: CACTGGCTGAGTTATTGGCCT). The reverse transcription parameters were as follows: 37°C for 15 min, followed by 85°C for 5 s and 4°C for 1 min. The qRT-PCR parameters were as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. GAPDH was used to normalize all samples (forward: ACCCAGAAGACTGTGGATGG, reverse: TTC-TAGACGGCAGGTCAGGT). The data were analyzed by the 2^{- $\Delta\Delta C_t$} method and reported as the relative variation (log2 transformed).

Statistical Analysis

All data in this study were analyzed by using GraphPad Prism 8.0 (GraphPad, San Diego, CA, USA). We used mean $\pm$ SD and paired or unpaired *t* test for normally distributed data, and the results were expressed as medians with interquartile ranges. We used nonparametric tests (Kruskal-Wallis test) for nonnormally distributed data. We used Fisher's exact test to analyze categorical data and used Spearman's rank-order correlation for correlation analysis. ROC was generated to determine the diagnostic value of CEACAM5. Statistical significance was considered at *p* < 0.05.

Results

Protein Expression of CEACAM5 in Induced Sputum Supernatant

The expression of CEACAM5 in induced sputum supernatant was detected by ELISA, and we found that the expression of CEACAM5 was upregulated in asthma patients (Fig. 1a). Then, we analyzed the correlation between the clinical data and the protein level of CEACAM5. The results showed that the protein expression of CEACAM5 in induced sputum supernatant was positively correlated with FeNO and IgE (shown in Fig. 1b–c). These data suggested that CEACAM5 may be related to eosinophilic inflammation in asthma.

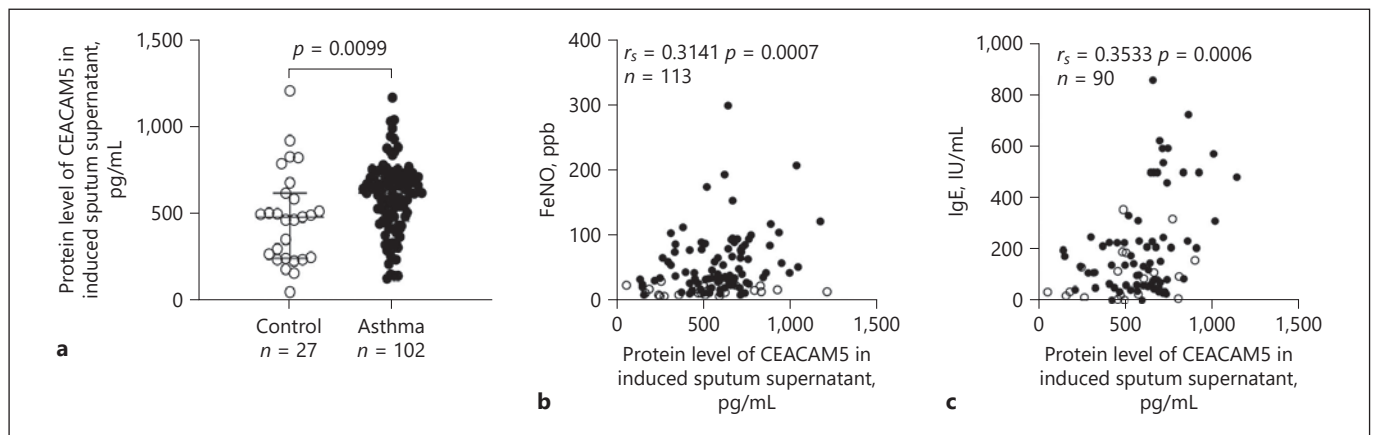


Fig. 1. CEACAM5 in induced sputum supernatant is increased and correlates with FeNO and IgE in asthma. **a** Protein level of CEACAM5 in induced sputum supernatant. Relationship between the protein level of CEACAM5 in induced sputum supernatant and FeNO (**b**) and IgE (**c**).

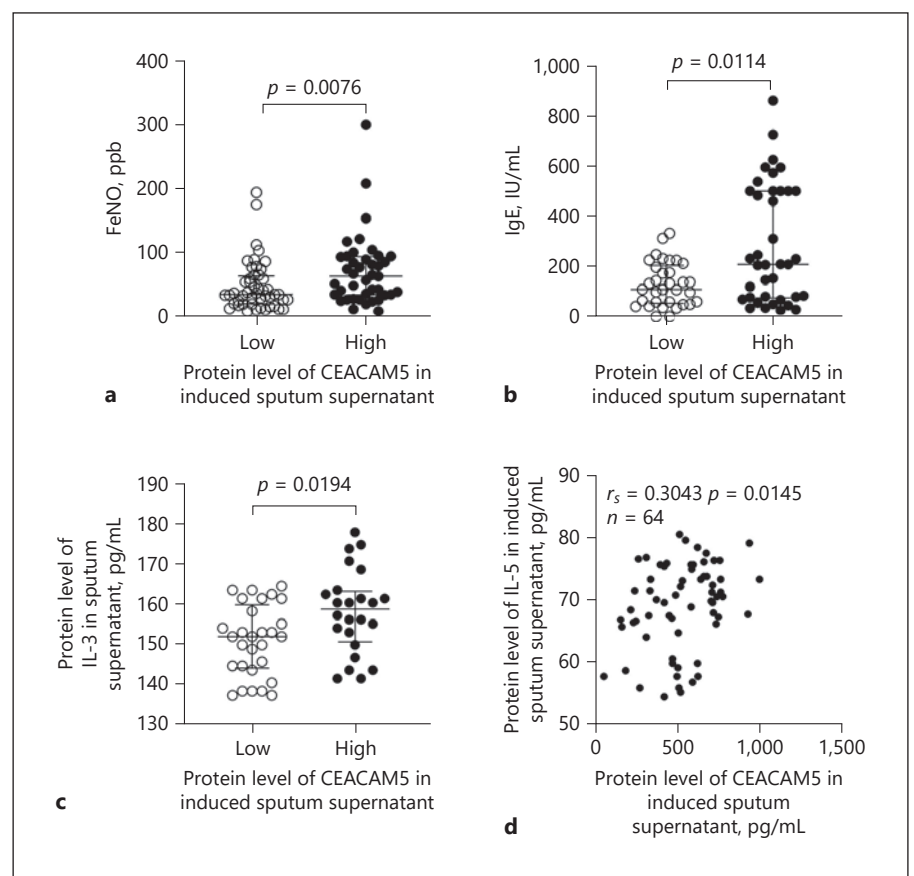


Fig. 2. Clinical characteristics of asthma are present in the CEACAM5-high and CEACAM5-low subgroups. The difference of FeNO (**a**), IgE (**b**), and IL-13 (**c**) between CEACAM5-high and CEACAM5-low subgroups in induced sputum supernatant. Correlation between CEACAM5 and IL-5 expression in asthma patients (**d**).

Clinical Characteristics of Asthma Patients with High CEACAM5 Expression

To further study the relationship between eosinophilic inflammation and CEACAM5, we divided patients with

asthma into CEACAM5-high and CEACAM5-low subgroups according to the upper quartile of CEACAM5 protein level in induced sputum supernatant in the healthy control group. Compared with the CEACAM5-low group,

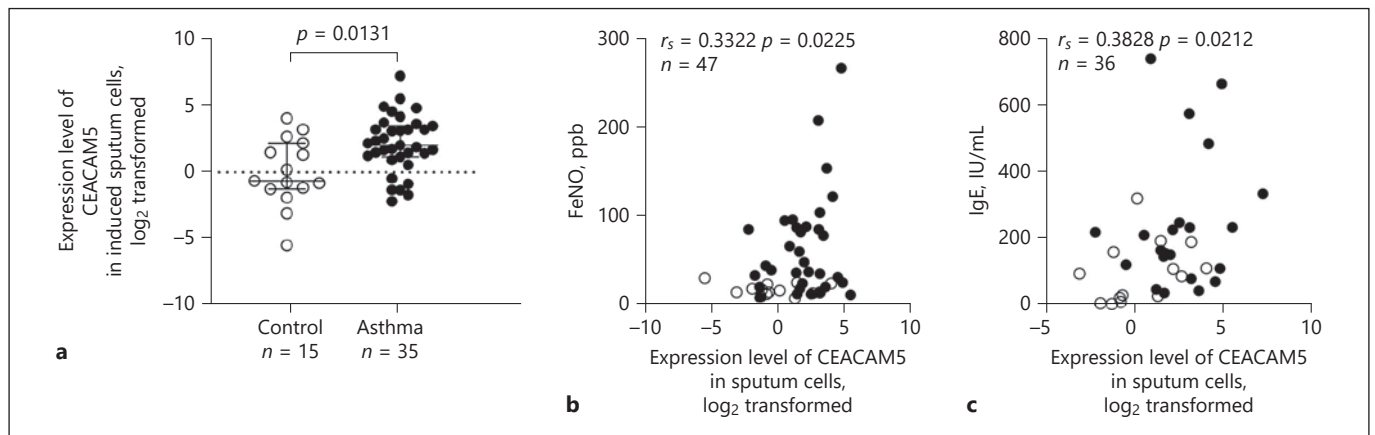


Fig. 3. Expression of CEACAM5 in induced sputum cells. **a** mRNA expression level of CEACAM5 in induced sputum cells (log₂ transformed). Relationship between the mRNA expression level of CEACAM5 in induced sputum cells and FeNO (**b**) and IgE (**c**).

the CEACAM5-high group had higher levels of FeNO, IgE, and IL-13 (shown in Fig. 2a–c). Moreover, the IL-5 expression in induced sputum supernatant of asthma patients showed a significant positive correlation with CEACAM5 (shown in Fig. 2d). These results indicate that CEACAM5 expression is associated with more severe eosinophilic airway inflammation in subjects with asthma.

Expression of CEACAM5 in Induced Sputum Cells and Serum

To verify the expression of CEACAM5 in asthma patients from multiple perspectives, we also detected CEACAM5 mRNA level in induced sputum cells and protein level in the serum. Similar to the previous results, the expression of CEACAM5 was higher in induced sputum cells and serum of asthma patients (shown in Fig. 3a, 4a). CEACAM5 is a well-known tumor marker. To understand the difference of CEACAM5 expression between asthma and NSCLC patients, we examined the expression of CEACAM5 in NSCLC and found that the expression of CEACAM5 was higher in NSCLC than in asthma patients (shown in Fig. 4b). The expression of CEACAM5 in induced sputum cells was also positively correlated with FeNO and IgE (shown in Fig. 3b–c). However, the expression of CEACAM5 in the serum was only positively correlated with FeNO (shown in Fig. 4c–d). Moreover, a correlation was observed between the protein level of CEACAM5 in induced sputum supernatant and serum (shown in Fig. 5). The mRNA level of CEACAM5 in induced sputum cells was not related to the protein level of CEACAM5 in induced sputum supernatant and serum

(shown in online suppl. Fig. S1a–b; for all online suppl. material, see www.karger.com/doi/10.1159/000521754). When we divided patients with asthma into CEACAM5-high and CEACAM5-low subgroups according to the upper quartile of CEACAM5 protein level in the serum in the healthy control group, we found no difference in FeNO, IgE, and IL-13 between the two groups of asthma patients (shown in online suppl. Fig. S2a–c). We also found that the protein level of IL-13 in induced sputum supernatant was not correlated with the expression of CEACAM5 in induced sputum supernatant, induced sputum cells, and serum (shown in online suppl. Fig. S3a–c).

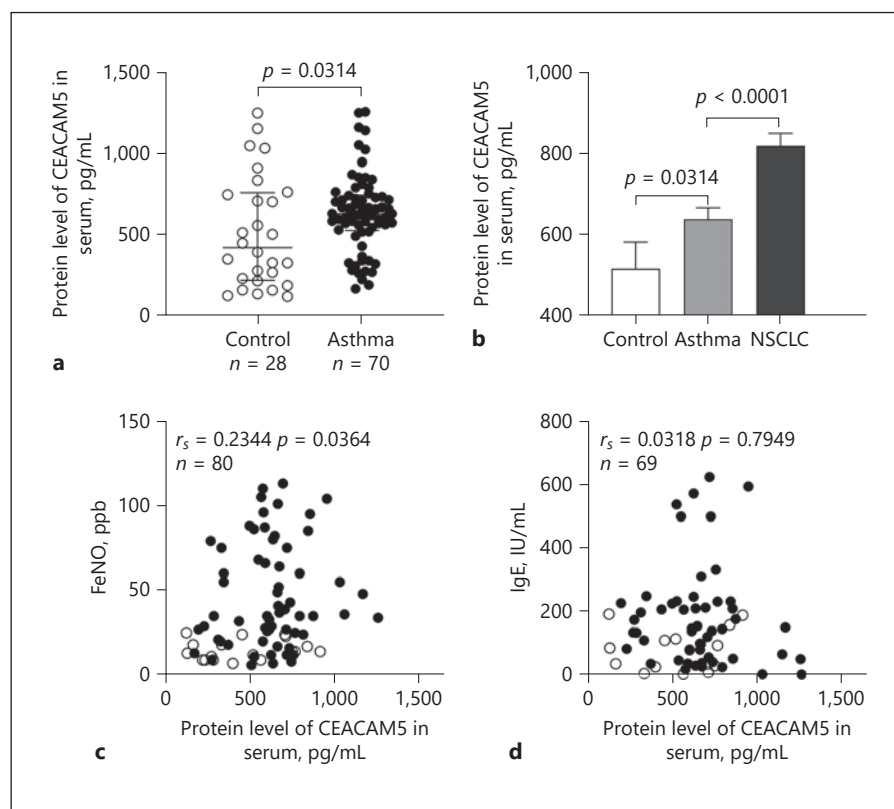
Diagnostic Value of CEACAM5 for Asthma

We next analyzed the diagnostic value of CEACAM5 in asthma using the ROC curve. The AUC values in induced sputum supernatant, induced sputum cells, and serum were 0.6607 ($p = 0.0104$), 0.7210 ($p = 0.0141$), and 0.6393 ($p = 0.0318$), respectively. This finding indicated that CEACAM5 presented satisfactory diagnostic capacity for asthma (shown in Fig. 6a–c). In addition, we found that the protein levels of CEACAM5 in the supernatant of sputum and serum decreased after 4 weeks of ICS treatment (shown in Fig. 7a–b). These data indicated that CEACAM5 was involved in the heterogeneity of ICS responsiveness in asthma.

Expression Level of CEACAM5 in BEAS-2B and 16HBE Cells

In addition to the clinical level, the expression levels of CEACAM5 in BEAS-2B cells and 16HBE cells after stim-

Fig. 4. Expression of CEACAM5 in the serum. **a** Protein level of CEACAM5 in the serum between healthy and asthma groups. **b** Differential expression of CEACAM5 between healthy, asthma, and NSCLC groups. Relationship between the protein level of CEACAM5 in the serum and FeNO (**c**) and IgE (**d**).



ulation by IL-4 (20 ng/mL) or IL-13 (50 ng/mL) for 48 h were detected by qRT-PCR. Figure 8 shows that the expression levels of CEACAM5 were upregulated in BEAS-2B and 16HBE cells after stimulation.

Discussion

In this study, we found that the expression levels of CEACAM5 in induced sputum supernatant, induced sputum cells, and serum were higher in patients with asthma compared with healthy controls and that the expression of CEACAM5 in induced sputum supernatant and serum decreased after regular ICS treatment. In addition, the expression levels of CEACAM5 in induced sputum cells and induced sputum supernatant were positively correlated with FeNO and IgE. Patients with high CEACAM5 expression in induced sputum supernatant had higher levels of FeNO, IgE, and IL-13, suggesting that CEACAM5 may be involved in the development of eosinophilic inflammation in patients with asthma. Consistent with this finding, the results of in vitro cell experiments also showed that the expression of CEACAM5 was

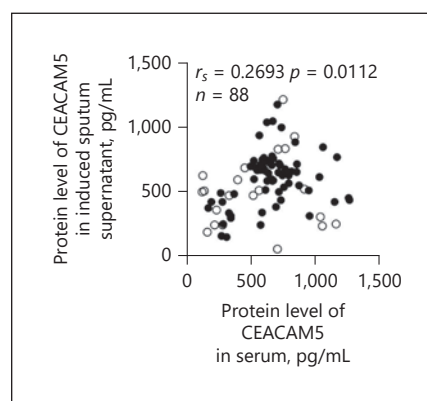


Fig. 5. Relationship between the protein level of CEACAM5 in the serum and induced sputum supernatant.

increased after IL-4 and IL-13 stimulation in cultured 16HBE and BEAS-2B cells.

CEACAM5 is known as a classic tumor marker and widely expressed on the surface of various cells. Previous studies have shown that CEACAM5 is also expressed in the respiratory tract and is strongly expressed in bron-

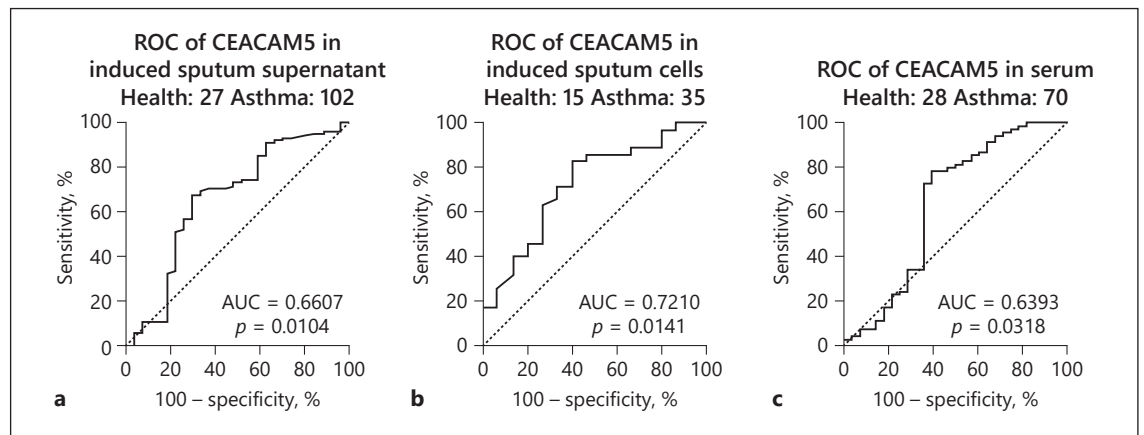


Fig. 6. Diagnostic value of CEACAM5. ROC curve of CEACAM5 in induced sputum cells (a), induced sputum supernatant (b), and serum (c).

Fig. 7. CEACAM5 level before and after ICS treatment for 4 weeks in asthma patients. Protein levels of CEACAM5 in induced sputum supernatant (a) and serum (b).

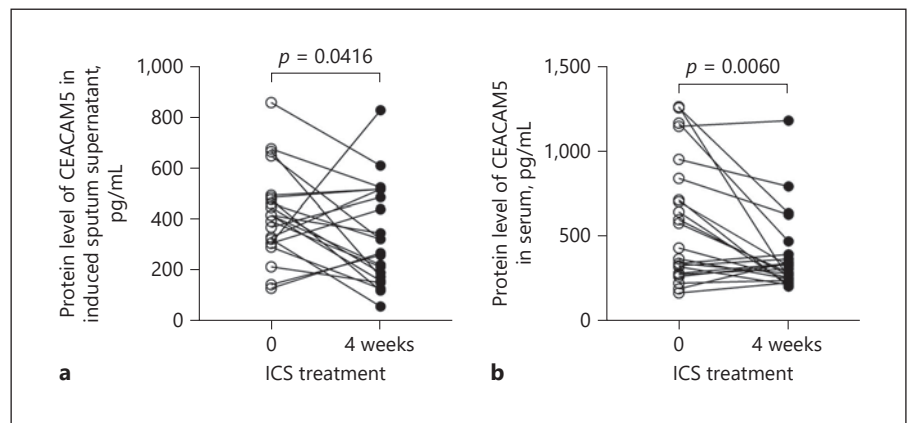
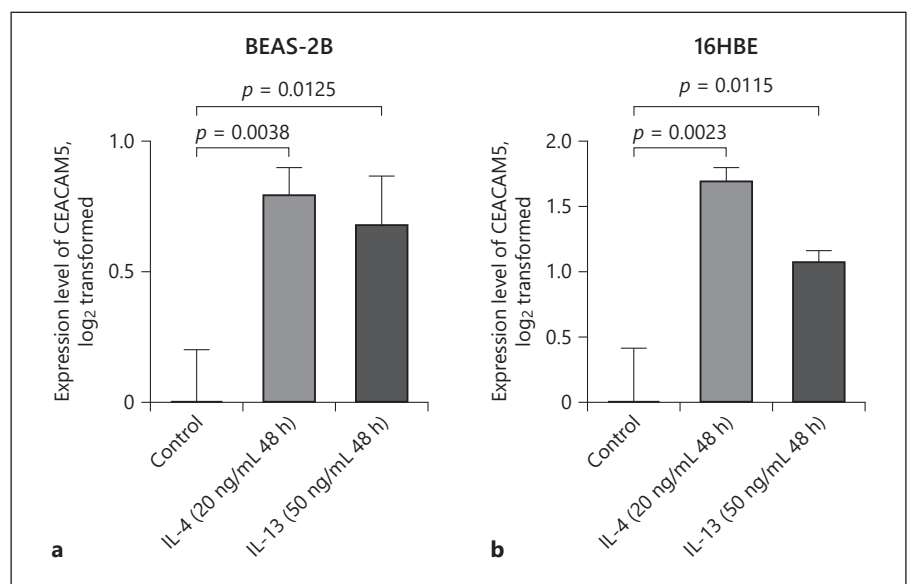


Fig. 8. Expression level of CEACAM5 in airway epithelial cells in vitro. Expression levels of CEACAM5 in BEAS-2B (a) and 16HBE (b) cells after stimulation with IL-4 and IL-13.



chial and alveolar epithelium [23]. We found that CEACAM5 in induced sputum supernatant was increased and correlated with FeNO and IgE in patients with asthma. This study is the first to report that CEACAM5 in induced sputum supernatant was increased in asthma patients. We found differential expression of FeNO, IgE, and IL-13 in CEACAM5-high and CEACAM5-low subgroups. This finding suggests that CEACAM5 may be a biomarker for Th2 asthma phenotype. Moreover, the expression of CEACAM5 in induced sputum cells and serum was higher in asthma patients compared with healthy controls. CEACAM5 expression in induced sputum cells was also positively correlated with FeNO and IgE. These data suggested that CEACAM5 may be related to eosinophilic inflammation in asthma. The ROC curve in induced sputum cells, induced sputum supernatant, and serum showed that CEACAM5 has a satisfactory diagnostic capacity in asthma.

A previous study found that the expression of CEACAM5 in the epithelium and submucosa of severe asthma patients was high [24]. We found that the expression levels of CEACAM5 were upregulated in BEAS-2B and 16HBE cells after IL-4 or IL-13 stimulation [25]. This part of the cell experiment was consistent with the airway results previously reported in asthma patients. Tanner et al. [24] also showed that regardless of smoking status, the expression of CEACAM5 was increased in patients with severe asthma, indicating that the expression of CEACAM5 was not related to smoking, which was consistent with our findings. In addition, a previous study found that the expression of CEACAM5 was upregulated in neutrophils in asthma [26]. However, Klaile et al. [17] showed that the expression of CEACAM5 was not related to neutrophil infiltration, and our results also showed that the expression of CEACAM5 had nothing to do with the percentage of neutrophils in peripheral blood. Instead, it was associated with eosinophilic inflammatory markers such as FeNO, IgE, IL-5, and IL-13.

The secretion of cytokines and the imbalance of T-cell differentiation and development play an important role in asthma. Some studies have shown that CEACAM5 plays a role in the regulation of T-cell activity and the secretion of cytokines. For example, Roda et al. [27] found that CEACAM5 can regulate the activity of T cells, and Shao et al. [28] further proved that CEACAM5 may regulate the activity of T cells by interacting with T-cell receptors. In addition, CEACAM5 binding to liver Kupffer cells can produce various cytokines. However, no studies have investigated these functions of CEACAM5 in asthma. Further studies are needed to determine whether

CEACAM5 plays such a role in regulating inflammatory markers in asthma.

Our study has some limitations. First, our sample size was very limited. A larger sample size with more regions and different ethnic groups is needed in future studies. Second, we only speculated that CEACAM5 was involved in eosinophilic airway inflammation in asthma patients, which was not verified by *in vitro* and *in vivo* experiments. Third, in this study, there were differences in age and BMI between the healthy control and the asthma group we included. Although we found no effect of age and BMI on the expression of CEACAM5, it would have been better if there were no differences in age and BMI between the healthy control and the asthma group.

Conclusions

The expression of CEACAM5 was upregulated in induced sputum and serum of patients with asthma. CEACAM5 may be involved in eosinophilic inflammation of asthma and may be used as a diagnostic biomarker and therapeutic target of asthma.

Acknowledgments

We thank the participants who volunteered for the study.

Statement of Ethics

All the subjects have given their written informed consent, and this study protocol was reviewed and approved by IEC for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen University, Approval No. (2021)071.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

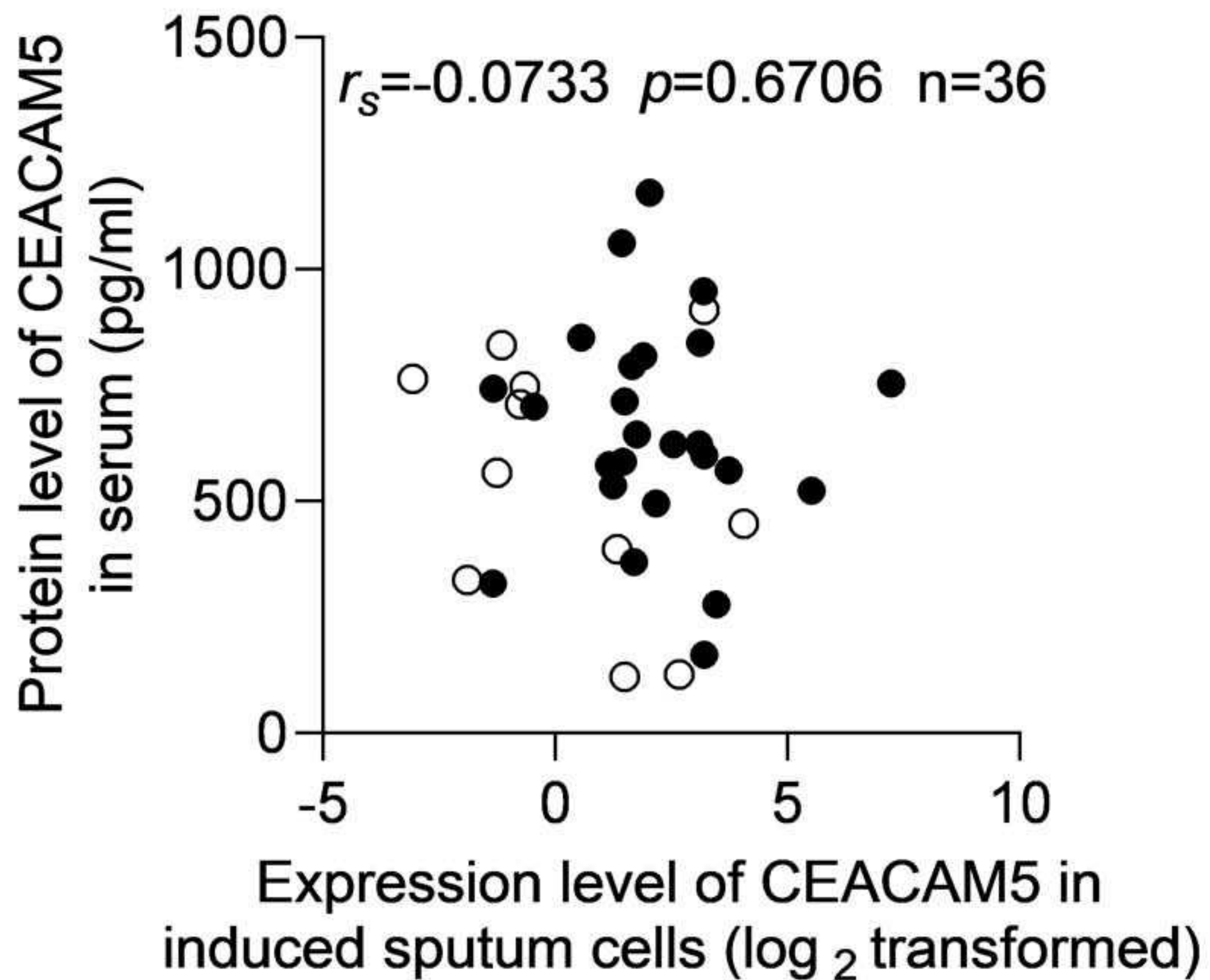
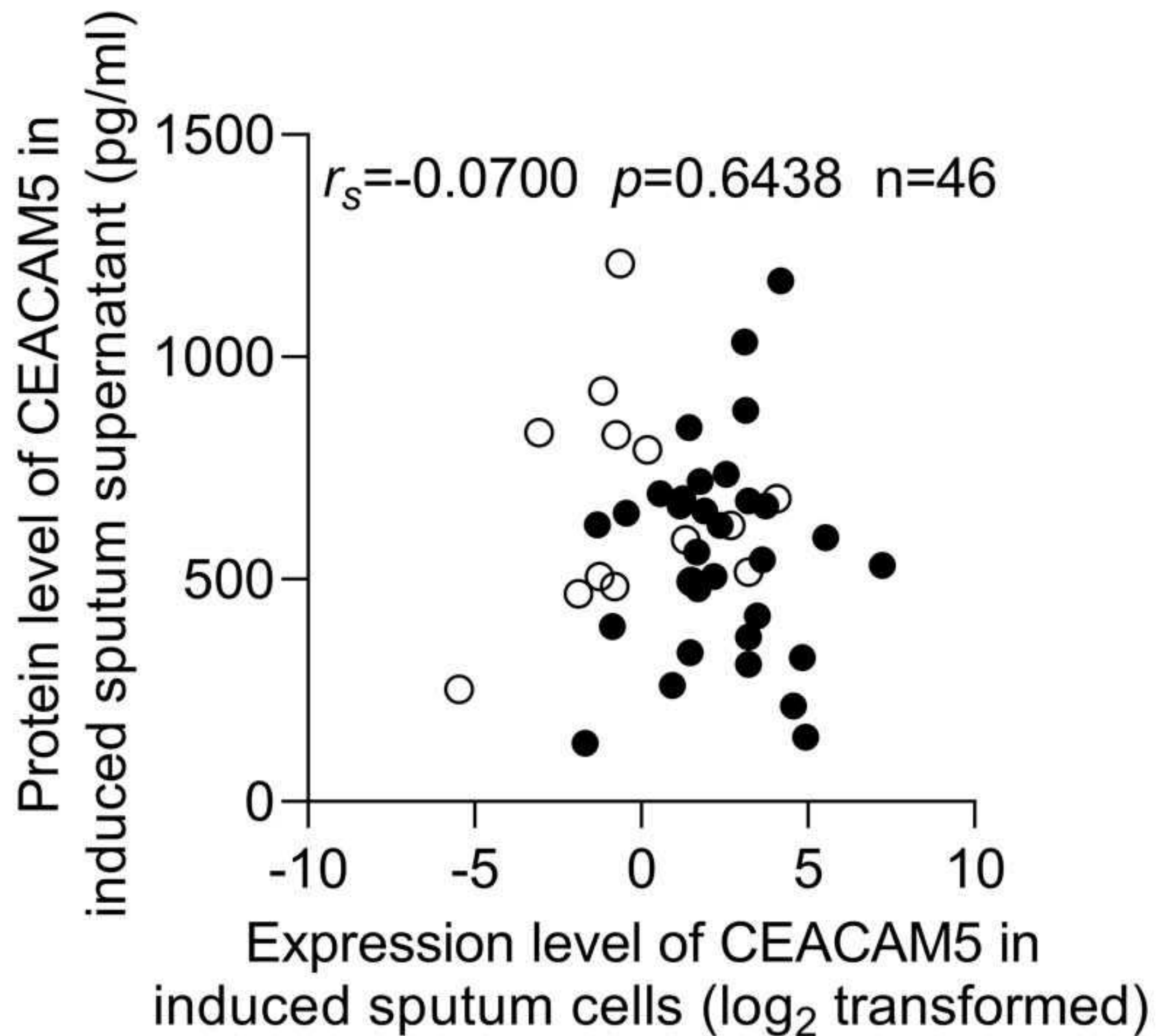
Y.G. and Y.L. designed the research and conceived of the manuscript; C.X., L.D., Z.Z., and F.C. implemented the experimental studies; C.X. and L.D. performed statistical analysis, interpreted results, and wrote the paper; Y.G. and Y.L. modified and approved the draft of the paper; all authors approved the final version of the manuscript.

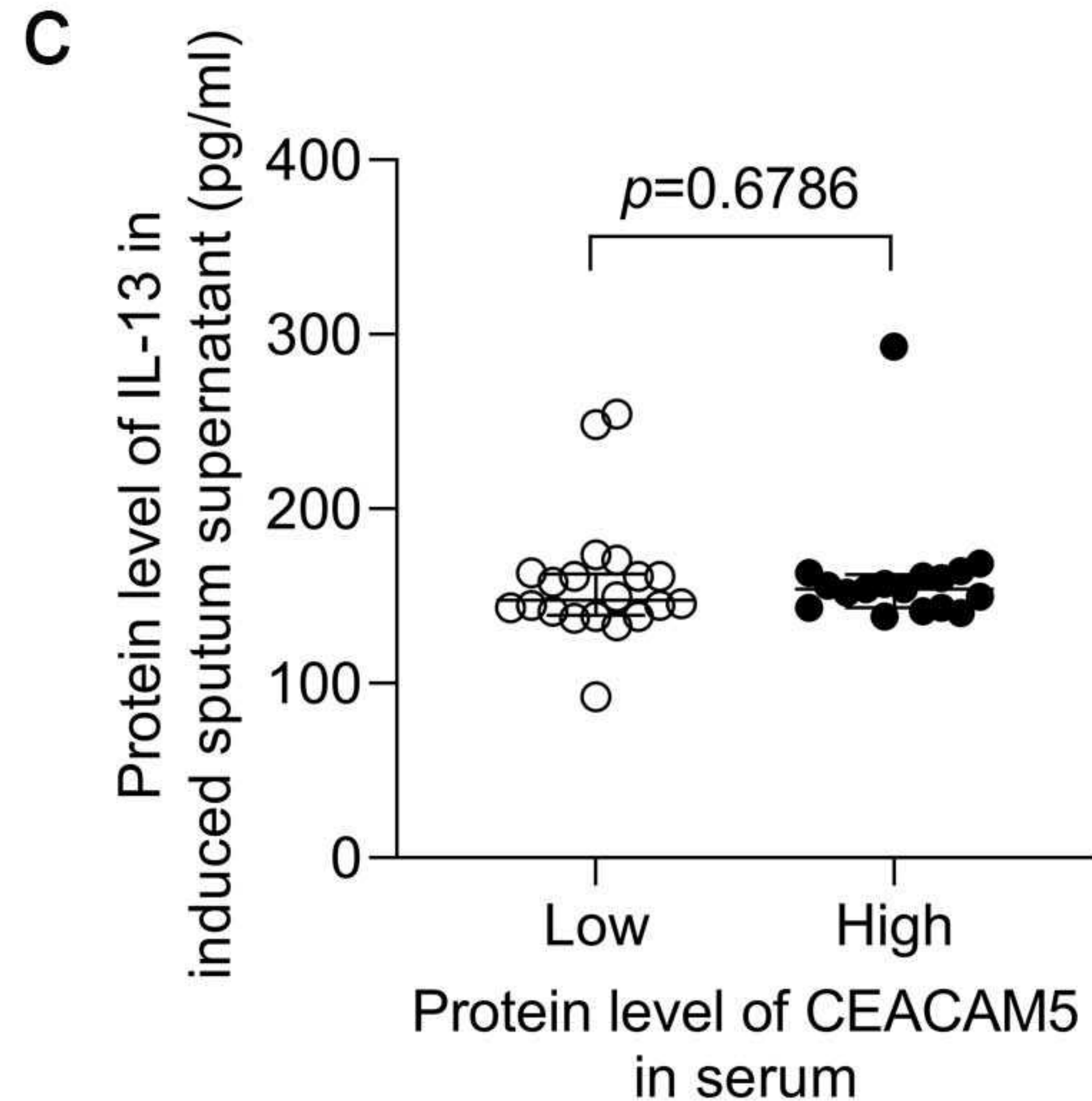
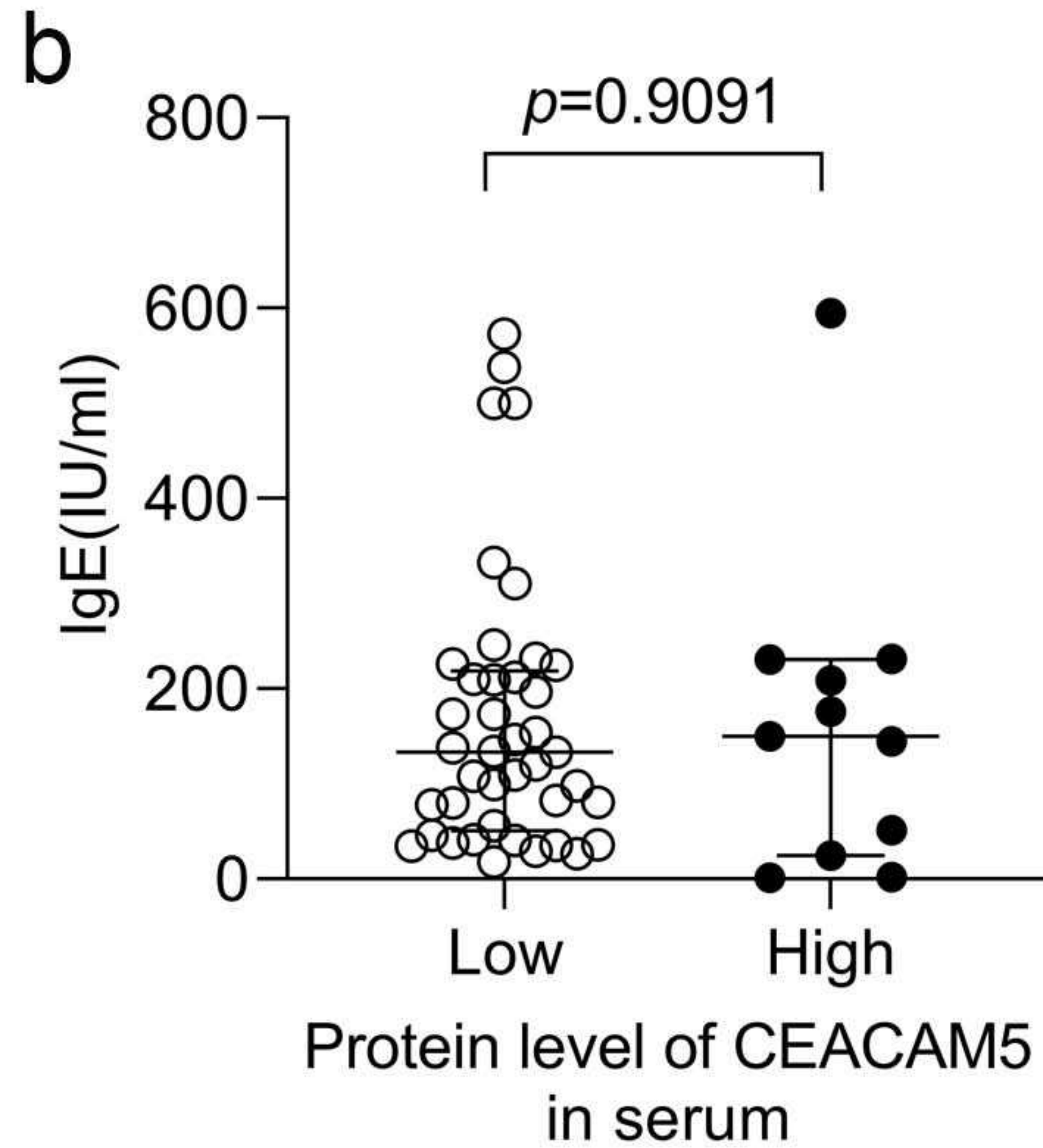
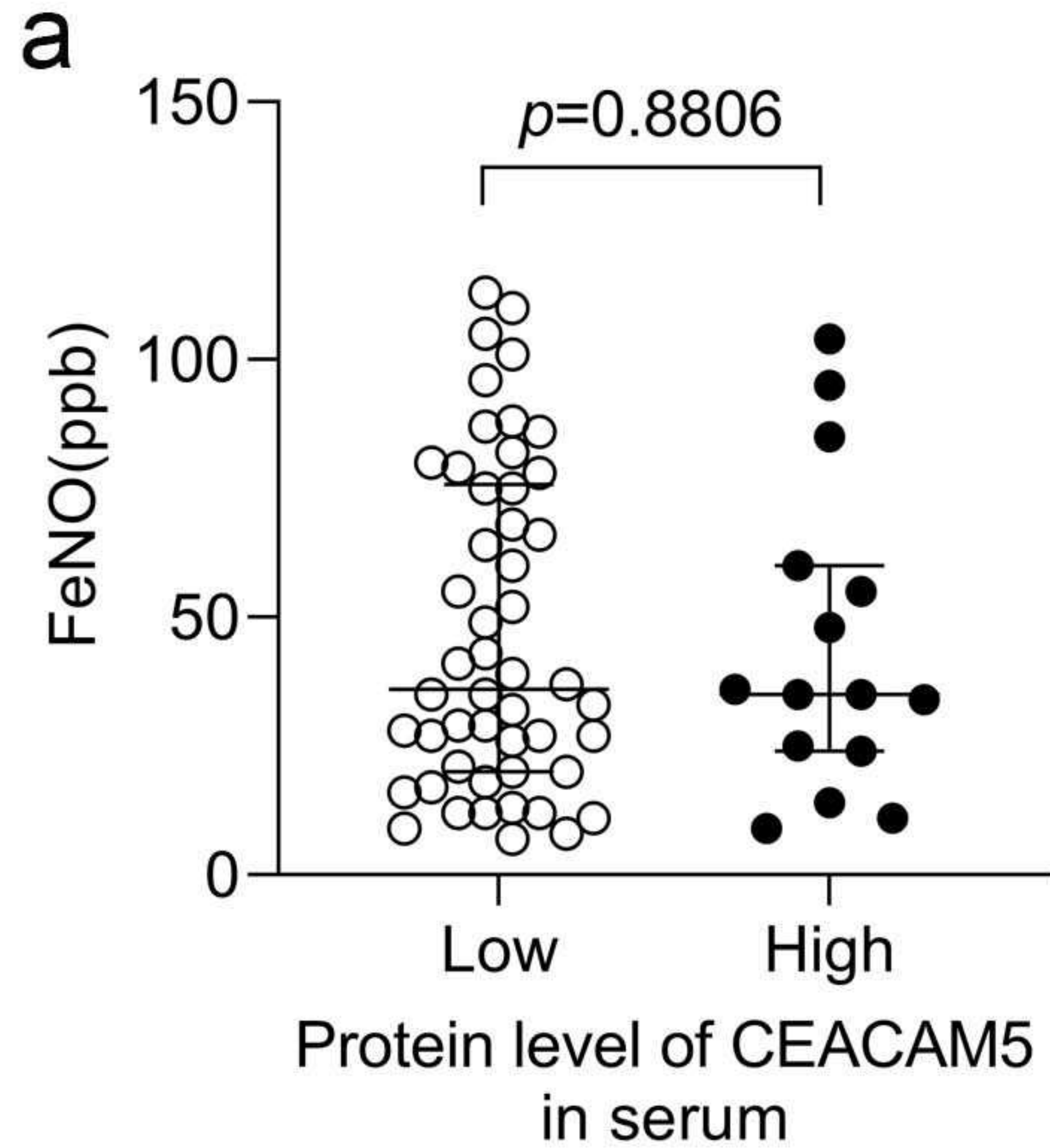
Data Availability Statement

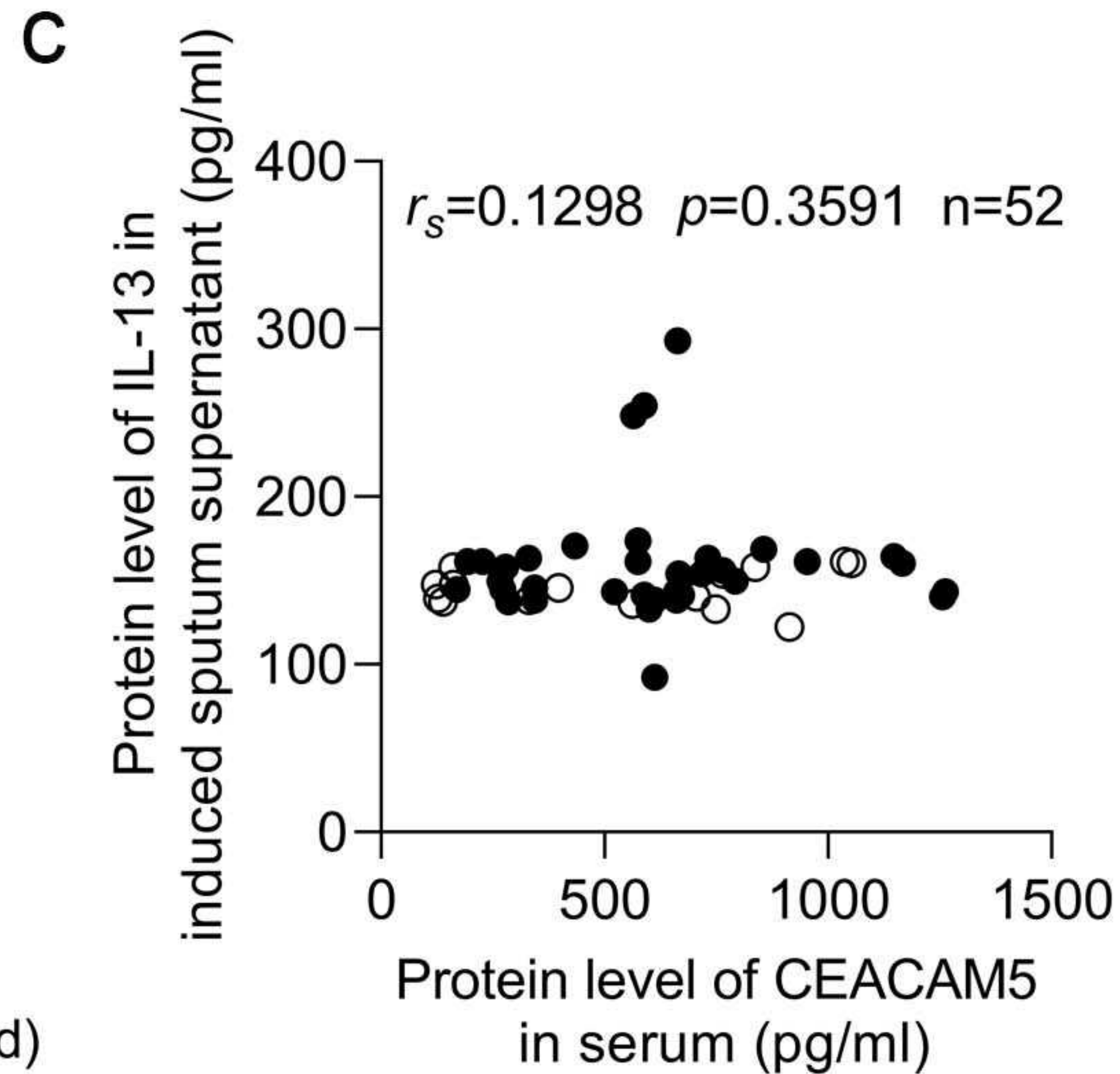
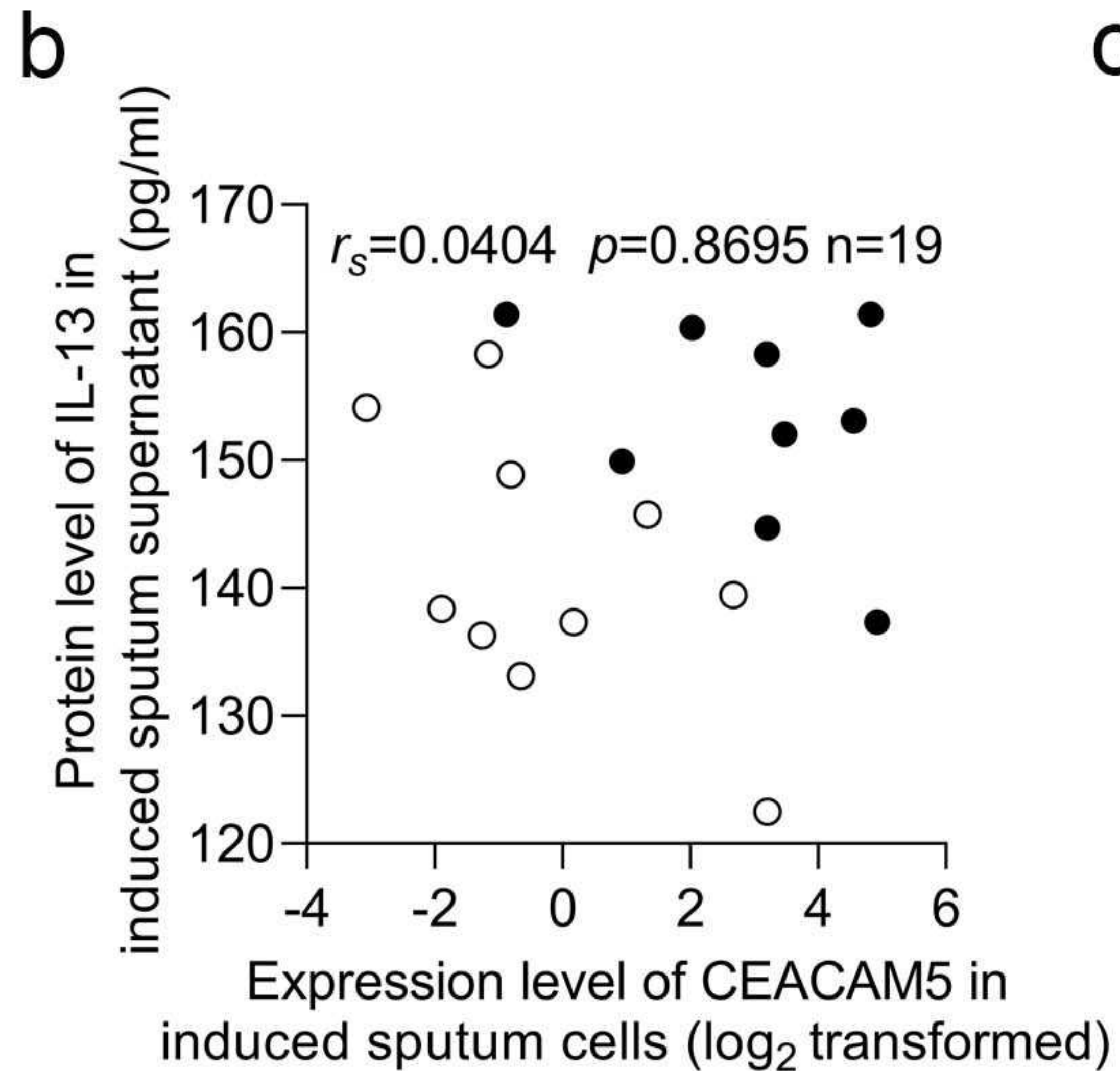
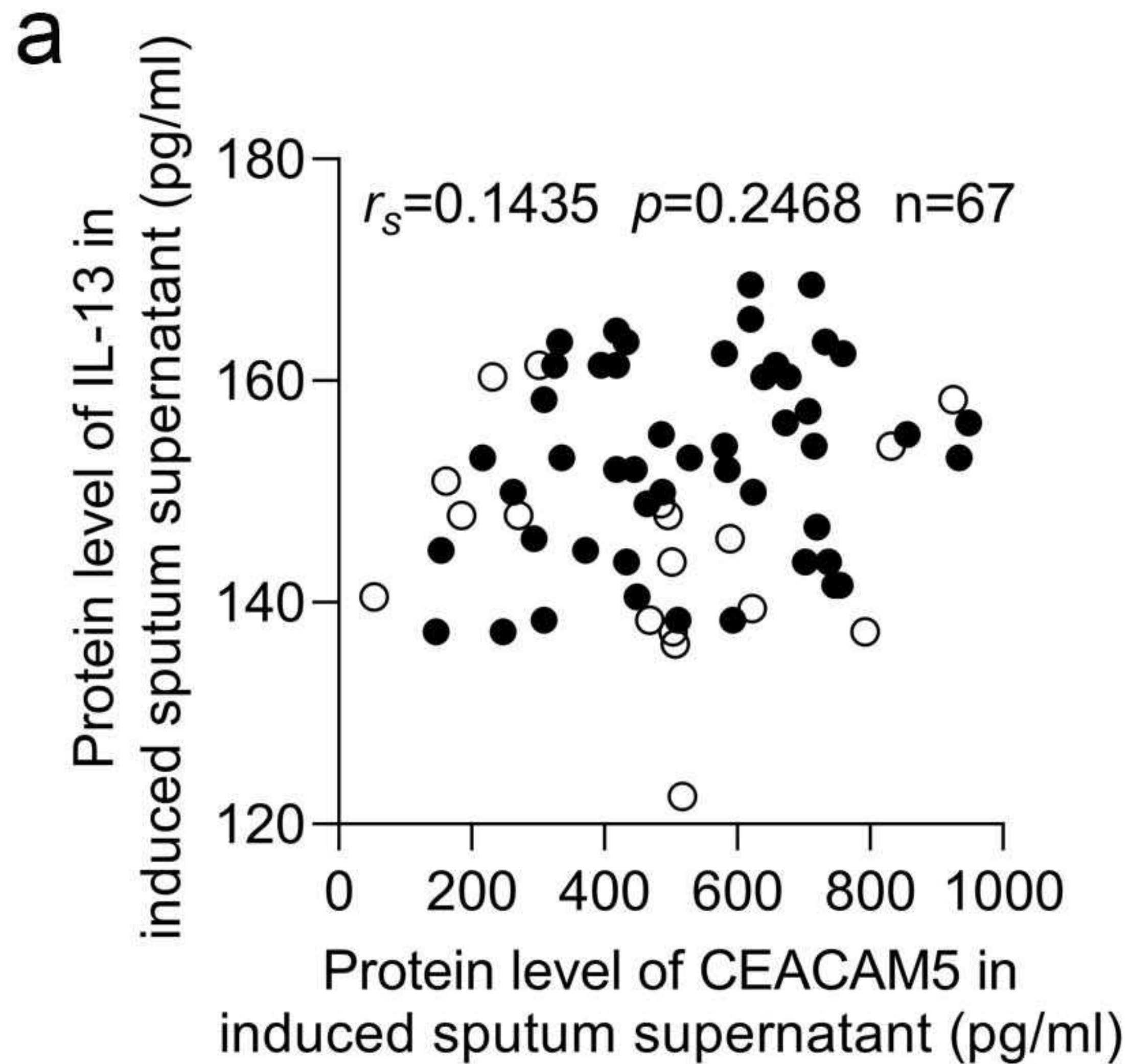
The data that support the findings of this study are openly available in the NCBI GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) No. GSE43696.

References

- Willis-Owen SAG, Cookson WOC, Moffatt MF. The genetics and genomics of asthma. *Annu Rev Genomics Hum Genet.* 2018;19:223–46.
- Global Initiative for Asthma (GINA). *Global Strategy for Asthma Management and Prevention updated 2016.* <http://www.ginasthma.org>.
- Huang K, Yang T, Xu J, Yang L, Zhao J, Zhang X, et al. Prevalence, risk factors, and management of asthma in China: a national cross-sectional study. *Lancet.* 2019;394:407–18.
- Bloom CI, de Preux L, Sheikh A, Quint JK. Health and cost impact of stepping down asthma medication for UK patients, 2001–2017: a population-based observational study. *PLoS Med.* 2020;17:e1003145.
- Boonpiyathad T, Sözen ZC, Satitsuksano P, Akdis CA. Immunologic mechanisms in asthma. *Semin Immunol.* 2019;46:101333.
- Burbank AJ, Sood AK, Kesic MJ, Peden DB, Hernandez ML. Environmental determinants of allergy and asthma in early life. *J Allergy Clin Immunol.* 2017;140:1–12.
- Choi Y, Sim S, Park HS. Distinct functions of eosinophils in severe asthma with type 2 phenotype: clinical implications. *Korean J Intern Med.* 2020;35:823–33.
- Hill DJ, Edwards AM, Rowe HA, Virji M. Carcinoembryonic antigen-related cell adhesion molecule (CEACAM)-binding recombinant polypeptide confers protection against infection by respiratory and urogenital pathogens. *Mol Microbiol.* 2005;55:1515–27.
- Kammerer R, Stober D, Singer BB, Obrink B, Reimann J. Carcinoembryonic antigen-related cell adhesion molecule 1 on murine dendritic cells is a potent regulator of T cell stimulation. *J Immunol.* 2001;166:6537–44.
- Gupta A, Rosato AJ, Cui F. Vaccine candidate designed against carcinoembryonic antigen-related cell adhesion molecules using immunoinformatics tools. *J Biomol Struct Dyn.* 2021 Oct;39(16):6084–98.
- Hammarström S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. *Semin Cancer Biol.* 1999;9:67–81.
- Horst AK, Wagener C. CEA-related CAMs. *Handb Exp Pharmacol.* 2004;(165):283–341.
- Beauchemin N, Arabzadeh A. Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) in cancer progression and metastasis. *Cancer Metastasis Rev.* 2013;32:643–71.
- Bramswig KH, Poettler M, Unseld M, Wrba F, Uhrin P, Zimmermann W, et al. Soluble carcinoembryonic antigen activates endothelial cells and tumor angiogenesis. *Cancer Res.* 2013;73:6584–96.
- MacSween JM, Warner NL, Bankhurst AD, Mackay IR. Carcinoembryonic antigen in whole serum. *Br J Cancer.* 1972;26:356–60.
- Sorokin JJ, Sugarbaker PH, Zamcheck N, Piskich M, Kupchik HZ, Moore FD. Serial carcinoembryonic antigen assays. Use in detection of cancer recurrence. *JAMA.* 1974;228:49–53.
- Klaile E, Klassert TE, Scheffrahn I, Müller MM, Heinrich A, Heyl KA, et al. Carcinoembryonic antigen (CEA)-related cell adhesion molecules are co-expressed in the human lung and their expression can be modulated in bronchial epithelial cells by non-typable *Haemophilus influenzae*, *Moraxella catarrhalis*, TLR3, and type I and II interferons. *Respir Res.* 2013;14:85.
- Nie X, Wei J, Hao Y, Tao J, Li Y, Liu M, et al. Consistent biomarkers and related pathogenesis underlying asthma revealed by systems biology approach. *Int J Mol Sci.* 2019;20:4037.
- Paplińska-Goryca M, Nejman-Gryz P, Proboyszcz M, Kwiecień I, Hermanowicz-Salamon J, Grabczak E, et al. Expression of TSLP and IL-33 receptors on sputum macrophages of asthma patients and healthy subjects. *J Asthma.* 2020;57:1–10.
- American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med.* 2005;171:912–30.
- Menzies D, Nair A, Lipworth BJ. Portable exhaled nitric oxide measurement: comparison with the “gold standard” technique. *Chest.* 2007;131:410–4.
- Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. *Eur Respir J.* 1997;10:1683–93.
- Kuespert K, Pils S, Hauck CR. CEACAMs: their role in physiology and pathophysiology. *Curr Opin Cell Biol.* 2006;18:565–71.
- Tanner A, Ward J, Wilson S, Howarth P. CEACAM5 (CD66e) mucosal immunoreactivity and its relationship to asthma. British Thoracic Society Winter Meeting 2018. 2018. p. 2018.
- Ordoñez C, Screaton RA, Ilantzis C, Stanners CP. Human carcinoembryonic antigen functions as a general inhibitor of anoikis. *Cancer Res.* 2000;60:3419–24.
- de Jong E, Bosco A. Dissecting asthma transcriptomics: does site matter? *Am J Respir Cell Mol Biol.* 2018;58:144–6.
- Roda G, Jianyu X, Park MS, DeMarte L, Hovhannisyan Z, Couri R, et al. Characterizing CEACAM5 interaction with CD8α and CD1d in intestinal homeostasis. *Mucosal Immunol.* 2014;7:615–24.
- Shao L, Allez M, Park MS, Mayer L. Immunomodulatory roles of the carcinoembryonic antigen family of glycoproteins. *Ann N Y Acad Sci.* 2006;1072:194–209.

a**b**





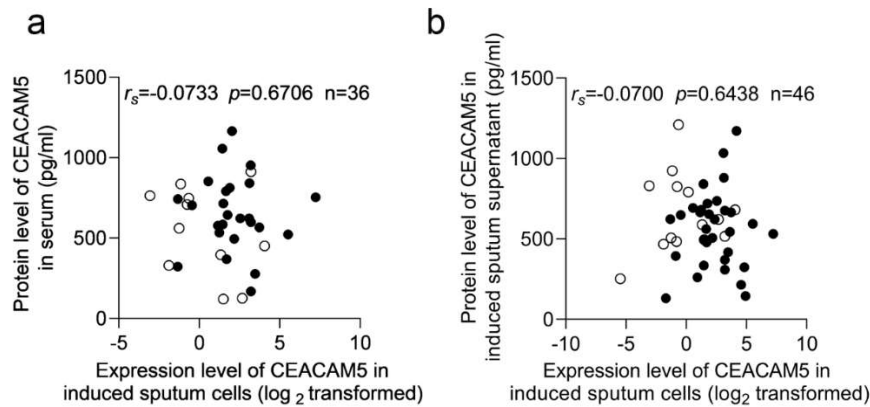


Fig. S1. Relationship between the expression level of CEACAM5 in induced sputum cells and the protein level of CEACAM5 in the (a) serum and (b) sputum supernatant.

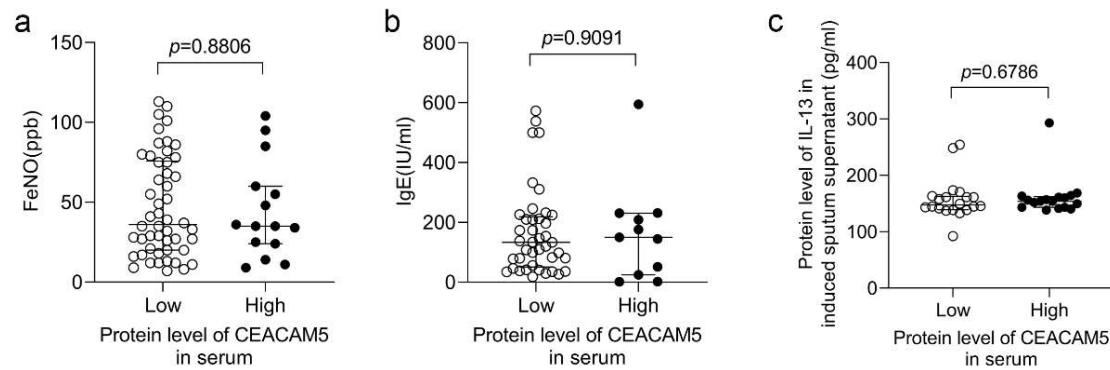


Fig. S2. The difference of (a) FeNO, (b) IgE, and (c) IL-13 between CEACAM5-high and CEACAM5-low subgroups in serum.

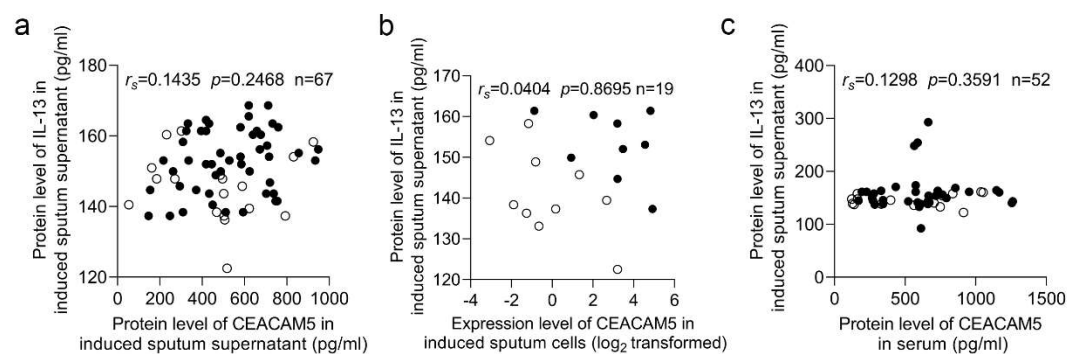


Fig. S3. Relationship between the protein level of IL-13 in induced sputum supernatant and (a) the protein level of CEACAM5 in induced sputum supernatant, (b) expression level of CEACAM5 in induced sputum cells ($\log_2$ transformed), and (c) protein level of CEACAM5 in the serum.

