Complement component 3 is a prognostic factor of non-small cell lung cancer

KAILONG LIN[1], SIYI HE[2], LUHANG HE[1], JUNYIN CHEN[1], XIAOMING CHENG[3], GUOQIANG ZHANG[4] and BO ZHU[1]

Institutes of [1]Cancer, [2]Cardiovascular Surgery and [3]Respiratory Diseases; [4]Department of Thoracic Surgery, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, P.R. China

Received October 2, 2013; Accepted March 27, 2014

DOI: 10.3892/mmr.2014.2230

Abstract. Lung cancer is the leading cause of cancer-related mortality worldwide. The complement component 3 (C3) is a central protein of the complement system, expressed in numerous cancer tissues and considered crucial for tumor progression. This study aimed to investigate the prognostic value of C3 in non-small cell lung cancer (NSCLC) and the underlying mechanisms. We used immunohistochemistry to observe the expression of C3 in malignant pulmonary lesion specimens from biopsy of 80 patients with NSCLC at stages I-III, who underwent lobectomy. We further assessed the correlation between C3 expression and a number of clinical features, as well as its prognostic value. To investigate the mechanism by which C3 exerts its effects, the correlation of C3 expression to T lymphocyte infiltration was also assessed. There was no significant correlation between the C3 level and clinical features such as gender, smoking status, degree of differentiation, histological type and malignant tumor stage based on the TNM classification system, while a significant correlation was found to age. However, analysis of overall survival (OS) and disease-free survival (DFS) rates showed that low C3 expression was related to poor prognosis. Univariate survival analysis revealed that C3 level and TNM stage are independent prognostic factors. Multivariate analysis demonstrated that the low level of C3 and TNM stage are also associated with poor prognosis. Furthermore, in tissues from biopsies, the C3 level positively correlated to the number of infiltrated $CD4^+$ and $CD8^+$ T lymphocytes ($P<0.01$). These findings indicate that C3 is a valuable marker for prognostic evaluation of NSCLC and may be considered as a therapeutic target for the treatment of lung cancer.

Correspondence to: Professor Bo Zhu, Institute of Cancer, Xinqiao Hospital, Third Military Medical University, 183 Xinqiao Street, Chongqing 400037, P.R. China
E-mail: b.davis.zhu@gmail.com

Key words: non-small cell lung cancer, cancer-related inflammation, complement component C3, lymphocyte infiltration, prognostic factor

Introduction

Lung cancer is the most common malignancy in the world and accounts for the majority of cancer-related mortality. Furthermore, non-small cell lung cancer (NSCLC) has the highest prevalence rate but only a 14% 5-year survival rate in patients subjected to surgery (1). So far, considerable progress has been made to identify the local environmental factors that promote tumor progression.

Solid tumors are infiltrated with numerous types of inflammatory cells, and a few of these types, such as tumor-associated macrophages and myeloid-derived suppressor cells (MDSCs) have been confirmed to significantly affect the biological activities related to tumor (2-6). Moreover, inflammatory factors also play important roles in the tumor microenvironment. For example TGF-β, IL-6 and IL-17, secreted by inflammatory and other stroma cells, are considered as messenger molecules, allowing the crosstalk between inflammatory and tumor cells mainly at the local level (2,7-10), while other inflammatory factors, including complements and C-reactive protein, function as an independent system widely distributed in the body fluid, which can mobilize a wider range of inflammatory response molecules, exerting profound effects on tumor.

The complement is one type of such an inflammatory factor system, containing >30 membrane-bound and soluble plasma proteins, and which plays an essential role in the innate immune response against pathogens or foreign cells. Components of the complement system can recognize each other upon activation and form complexes with proteases that cleave and activate other enzymes in a cascade-like manner; the resulting membrane attack complex (MAC) is thought to contribute to the immunoclearance of abnormal cells in our body via lysing the targets, including malignant tumors (11-15). In addition, complement fragments deposited on tumor cells can be recognized by complement receptors expressed in immune effector cells leading to direct cytotoxicity, phagocytosis or enhanced antibody-dependent tumor killing (15,16). However, some complement regulators such as the anaphylatoxin C5a, can recruit MDSCs into the tumor and lead to suppression of the anti-tumor $CD8^+$ T-cell-mediated response (17). These reports indicate that each complement component may have entirely distinct biological effects on tumor progression.

The complement component 3 (C3) is considered to be a central player of the complement system, since this protein has an important role in the three different complement activation pathways. Upon cleavage of C3 by a series of enzymes, the main complement components C3b and C5b are produced, which form MAC and other by-products, such as C3a and C5a. Previous studies have revealed a bilateral effect of C3 on tumor progression: on the one hand, C3-deficient mice showed significantly impaired tumor growth in the absence of C5a, indicating that C3 may exert a pro-tumor effect (17); on the other hand, in a photodynamic therapy of mouse glioma, C3 played crucial roles in mediating related immune responses against tumor (18), while another study reported that the enhanced C3 deposition was accompanied by an increase in tumor cell lysis in human renal tumor cell lines (19). Considering clinical results, it is notable that the level of C3 are increased in the sera of patients with cancer, e.g., lung, colorectal, esophageal, and gastric cancer, compared to healthy controls (20-22), indicating that C3 may be a suitable biomarker for the outcome of malignancies. However, there is limited information available on C3 expression in NSCLC tissues, and on the prognostic potential of C3 with regards to survival rate of NSCLC patients. Moreover, since C3 exerts numerous effects, it is difficult to interpret its adverse effects reported in the clinic.

The present study was a retrospective investigation of the prognostic value of C3 in cancer tissues of NSCLC patients. We further analyzed the correlation between the C3 level and that of the anti-tumor immune T cells $CD4^+$ and $CD8^+$.

Table I. Clinical features and their correlation with the complement component 3 (C3) level in non-small cell lung cancer.

	C3 level			
Features	Total	High	Low	P[a]
Patients	80	54	26	
Age (years)				**0.015**
<60	46	26	20	
≥60	34	28	6	
Gender				0.095
Male	64	46	18	
Female	16	8	8	
Smoking status				0.436
Smoker	48	34	14	
Non-smoker	32	20	12	
Differentiation				0.932
Normal-moderate	18	12	6	
Poor	62	42	20	
Histological type				0.074
Adenocarcinoma	22	14	8	
Squamous cancer	38	30	8	
Other	20	10	10	
TNM stage				0.267
I-II	50	36	14	
III	30	18	12	

[a]P, from Pearson's χ^2 test. Bold print denotes significant values.

Materials and methods

Patient population. A total of 80 NSCLC patients at stages I-III (50 diagnosed at stage I-II and 30 at stage III) who had been subjected to lobectomy at the Xinqiao Hospital, at the Third Military Medical University between January 2000 and December 2003, were included in this study. The clinical features of these patients were retrieved from the hospital records. The mean age of patients was 56 and none of them had undergone chemotherapy prior to surgery. The follow-up period was 60 months from the date of surgery and patients who died of causes irrelevant to lung cancer were excluded.

Immunohistochemistry. Tissues from malignant pulmonary lesions were fixed on glass slides (Shenying instrument Factory, Haimen, Jiangsu, China) using formalin and were embedded in paraffin. Tissues were obtained from the Department of Pathology, at the Xinqiao Hospital and were examined by hematoxylin and eosin staining. An approval from the Ethical Committee of Xinqiao Hospital (Chonqing, China) was received.

After deparaffinization in dimethylbenzene, slides were hydrated. To retrieve the antigen, the slides were treated with pepsin for 10 min for C3 detection, or heated at 95˚C for 20 min in citrate buffer for $CD4^+$ and $CD8^+$ detection, and then with 3% H_2O_2 for 20 min to quench the endogenous peroxidase activity. Nonspecific binding was blocked by incubating in normal goat serum for 10 min. Next, the slides were incubated overnight at 4˚C with primary antibodies: polyclonal rabbit anti-human anti-C3c diluted at 1:100 (RAB-0027; Maixin Biotechnology Co. Ltd., Fuzhou, Fujian, China), monoclonal mouse anti-human anti-$CD4^+$ diluted at 1:20 (M-0078; Changdao Biotechnology Co., Ltd., Shanghai, China), or monoclonal rabbit anti-human $CD8^+$ diluted at 1:100 (ZA-0508; Origene Biotechnology Co., Ltd., Beijing, China). Next, slides were incubated with polymer enhancer from the Elivision plus Polyer HRP IHC kit (Maixin Biotechnology Co., Ltd.) for 20 min for C3 detection or 1 h for $CD4^+$ and $CD8^+$ detection, at room temperature. The sections were washed with phosphate-buffered saline (PBS) and incubated with goat anti-rabbit or anti-mouse secondary antibody labeled with horseradish peroxidase from the Elivision plus Polyer HRP IHC kit (Kit-9902; Maixin Biotechnology Co.Ltd.) for C3 or the EnVision™ kit (Dako, Glostrup, Denmark) for $CD4^+$ and $CD8^+$. After incubation for 30 min, the sections were colored using 3,3'-diaminobenzidine and counterstained with hematoxylin and eosin. As negative controls, we used tissue sections incubated with PBS instead of the primary antibody.

The expression of C3 was evaluated based on the percentage of positively stained areas relative to the entire section, and the intensity of staining. i) Positive area scores: 0, ≤5; 1, 6-25; 2, 26-50; 3, 51-75; and 4, >75%. ii) Staining intensity scores: 1, yellow; 2, tan; and 3, dark brown. The final expression score was the product of these two scores, with 0-6 representing low and 7-12 high expression. $CD4^+$ and $CD8^+$ positive [(CD4/8(+)] or negative [(CD4/8(-)] expression was determined by the

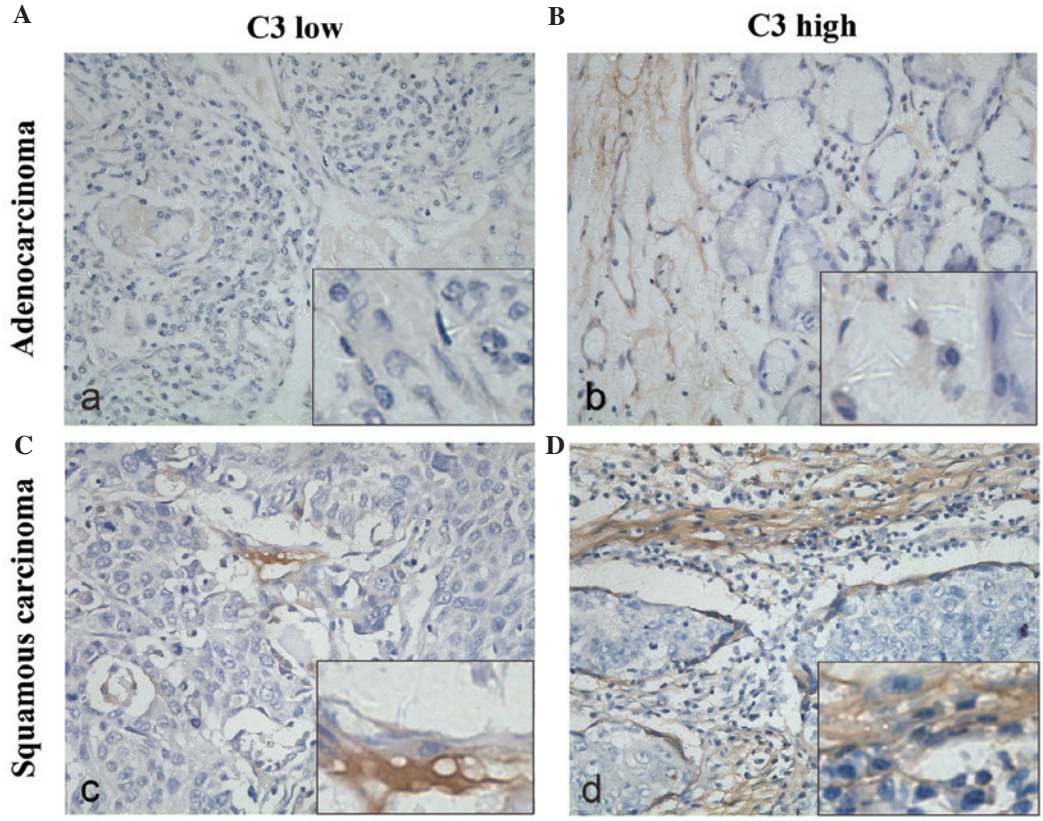

Figure 1. *In situ* expression of complement component 3 (C3) in non-small cell lung cancer. Representative pictures (magnification, x10) of resected specimens demonstrating (A and C) low; and (B and D) high expression of C3 in (A and B) adenocarcinoma; and (C and D) squamous carcinoma tissues of the lung. C3 is mainly expressed in stromal and peritumoral nest areas.

percentage of positively-stained cells relative to all cells, with ≥25% considered positive. All scores were independently evaluated by two experienced pathologists.

Statistical analysis. The correlation between C3 expression and clinical features as well as local lymphocyte infiltration in NSCLC patients was examined using the χ^2 or Fisher's exact tests. The Kaplan-Meier method was used to estimate the overall survival (OS) and disease-free survival (DFS) rate; the statistical significance of these data was evaluated with a log-rank test. Univariate and multivariate Cox proportional hazard regression models were used to assess the prognostic value of diverse factors alone or combined. $P<0.05$ were considered statistically significant. The hazard ratio (HR) describes the relative risk of the complication based on the comparison of event rates. It is the ratio between the predicted hazard for a member of one group and that for a member of the other group. All statistical analyses were performed using the SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA).

Results

The C3 level in malignant tissues does not correlate to most of the clinical characteristics of NSCLC patients. We observed the resected specimens of the 80 NSCLC patients using immunohistochemistry. Representative tissues showing positive staining for C3 are shown in Fig. 1. C3 was strongly expressed in the cytoplasm of positive cells, in stromal and peritumoral nest areas, and different degrees of expression were observed in adenocarcinoma (Fig. 1A and B) or squamous carcinoma cells (Fig. 1C and D). A total of 54 specimens (67.5%) expressed high levels of C3. Then, we analyzed the correlation between the C3 expression level and common clinical characteristics of these patients including age, gender, smoking status, degree of differentiation, histological type and TNM stage. As shown in Table I, no significant correlation was found, except for the factor age.

The C3 level correlates to prognosis of NSCLC. We performed a survival analysis based on OS and DFS rate data for the 80 NSCLC patients who had undergone lobectomy of the lung. Based on the immunohistochemical scoring of C3 expression described in Materials and methods, patients were divided into two groups: C3 low and C3 high. The Kaplan-Meier curve showed that the C3 low group had significantly shorter OS and DFS than the C3 high group (Fig. 2), which indicates that low C3 expression may correlate to poor prognosis. The median OS and DFS times were 17 and 8 months for the C3 low group, and 28 and 16 months, respectively, for the C3 high group.

Univariate survival analysis revealed that the C3 level, TNM stage and histological type are independent prognostic factors of OS; the estimated mean recurrence hazard ratio (HR) was 0.494 at 95% confidence interval (CI). Age, gender, smoking status and degree of tumor differentiation had no significant correlation with the prognostic value (Table II). In addition, multivariate survival analysis was

Table II. Univariate and multivariate analysis of clinical parameters for overall survival in non-small cell lung cancer patients.

	Univariate		Multivariate	
Variable	HR (95% CI)	P	HR (95% CI)	P
Gender (female vs. male)	1.334 (0.611-2.910)	0.469		
Age (<60 vs. ≥60 years)	0.958 (0.513-1.788)	0.893		
Smoking status (smoker vs. non-smoker)	1.009 (0.536-1.901)	0.978		
TNM stage (I, II vs. III)	0.300 (0.159-0.566)	**0.000**	0.259 (0.115-0.582)	**0.001**
Differentiation (well-moderate vs. poor)	0.857 (0.394-1.861)	0.696		
Histological type (Ad vs. non-Ad)	0.404 (0.169-0.965)	**0.041**	0.392 (0.151-1.016)	0.054
C3 (low vs. high)	0.494 (0.263-0.927)	**0.028**	0.397 (0.189-0.823)	0.015

Bold print denotes significant values. HR, hazard ratio; CI, confidence interval; Ad, adenocarcinoma.

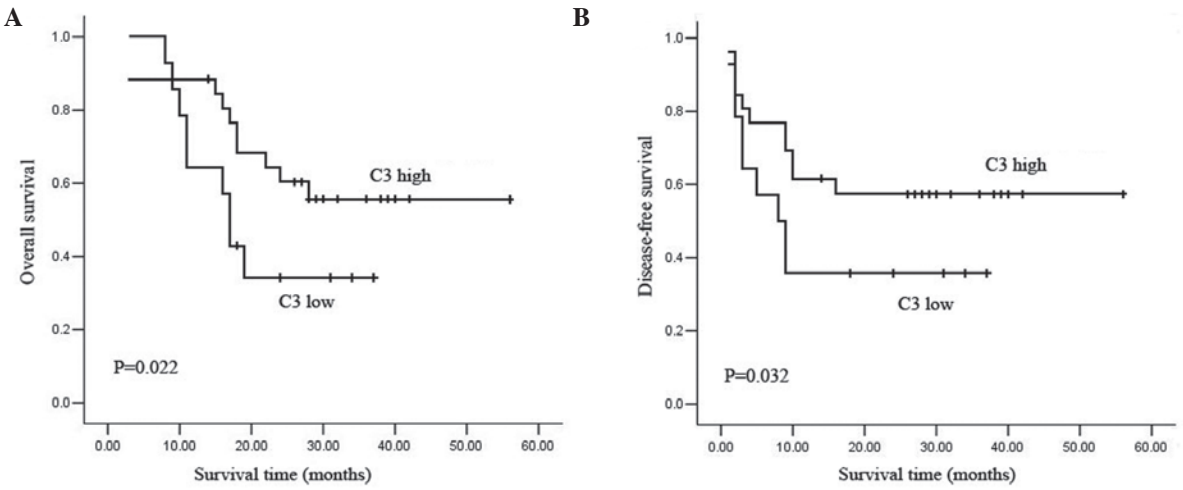

Figure 2. Kaplan-Meier curves of survival rate of 80 non-small cell lung cancer patients with different levels of expression of complement component 3 (C3). (A) Overall survival and (B) disease-free survival in the C3 high (n=54) and the C3 low groups (n=26).

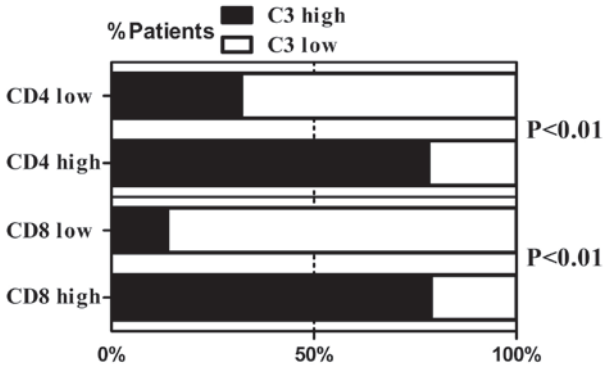

Figure 3. Association of complement component (C3) level with local CD4 and CD8 levels in non-small cell lung cancer. Histograms show the percentage of patients for each immune marker. Stromal C3 level is significantly associated with $CD4^+$/$CD8^+$ cell infiltration (P<0.01).

performed using the Cox proportional hazards model for OS. The result also indicated that the C3 level and TNM stage are independent prognostic factors (Table II). A similar result was obtained for DFS (Table III).

The C3 expression correlates to local T lymphocyte infiltration. To investigate the potential influence of C3 on lymphocytes, we performed a correlation analysis between the level of C3 expression and the degree of T-cell infiltration using Fisher's exact tests. Two groups of resected NSCLC specimens were immunohistochemically stained with anti-$CD4^+$ (51 specimens) and anti-$CD8^+$ (67 specimens) antibodies, respectively. Each specimen was paired to a C3-stained specimen. As shown in Fig. 3, both $CD4^+$ and $CD8^+$ T cells showed a high degree of infiltration when C3 was highly expressed. Fig. 4 shows the immunohistochemical staining of C3 and T-cells, which reflected the results of Fig. 3.

Discussion

In patients with NSCLC, several parameters, such as stage, serum albumin level and a number of blood biomarkers, including novel proteins and autoantibodies to tumor-associated antigens, have been used for disease detection at early or advanced stages (23-27). Since inflammation is widely considered a hallmark of cancer (28), an increasing amount of studies aim to find inflammation-associated biomarkers to provide

Table III. Univariate and multivariate analysis of clinical parameters for disease-free survival in non-small cell lung cancer patients.

	Univariate		Multivariate	
Variable	HR (95% CI)	P	HR (95% CI)	P
Gender (female vs. male)	0.876 (0.403-1.904)	0.738		
Age (<60 vs. ≥60 years)	0.918 (0.492-1.712)	0.788		
Smoking status (smoker vs. non-smoker)	1.147 (0.609-2.163)	0.671		
TNM stage (I, II vs. III)	0.256 (0.135-0.485)	**0.000**	0.229 (0.106-0.494)	**0.000**
Differentiation (well-moderate vs. poor)	0.777 (0.358-1.688)	0.525		
Histological type (Ad vs. non-Ad)	0.427 (0.179-1.020)	0.055		
C3 (low vs. high)	0.524 (0.280-0.980)	**0.043**	0.358(0.173-0.741)	0.006

Bold print denotes significant values. HR, hazard ratio; CI, confidence interval; Ad, adenocarcinoma.

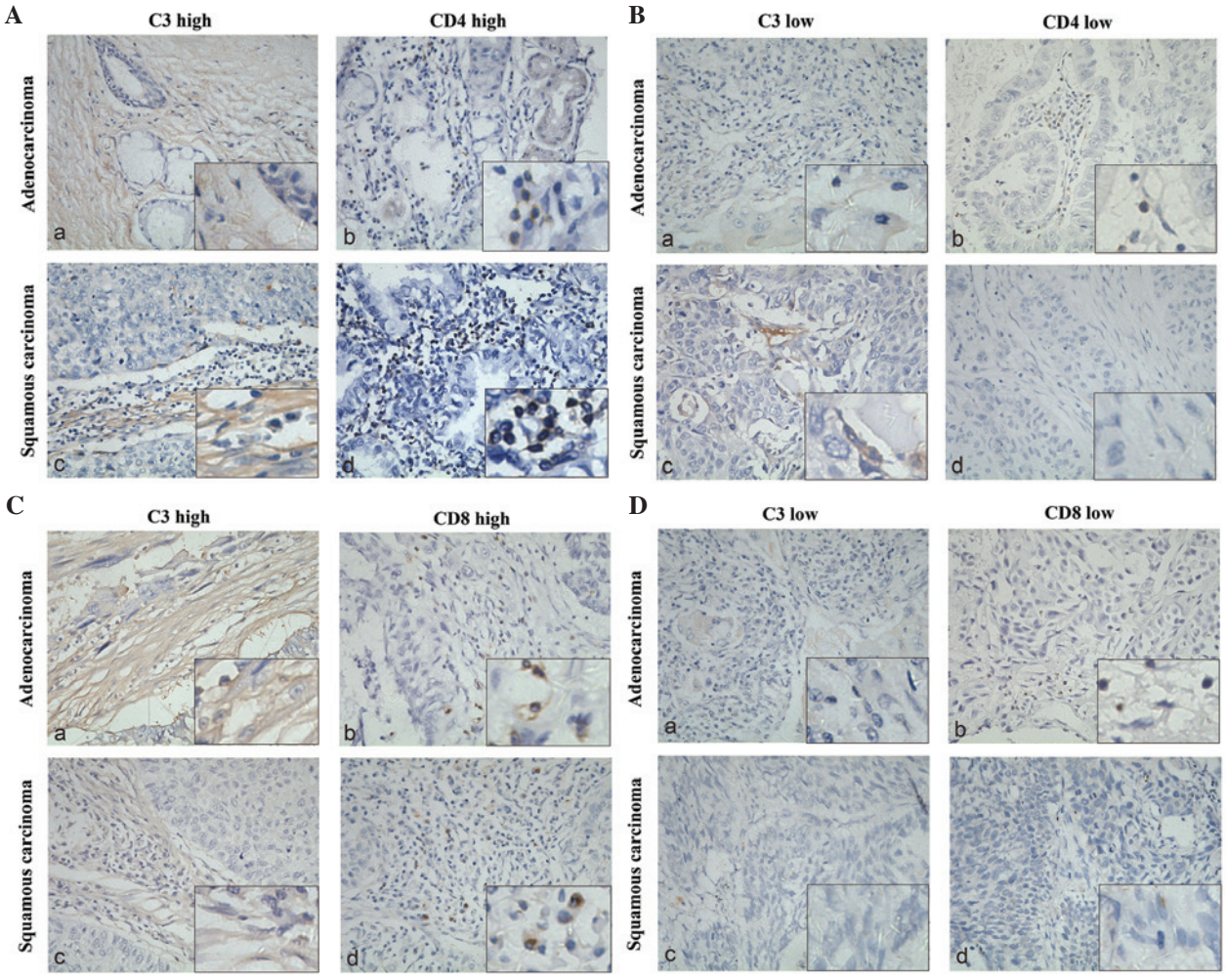

Figure 4. Representative pictures (magnification, x10) of resected specimens demonstrating positive correlation between the expression of complement component 3 (C3) and $CD4^+/CD8^+$ infiltration in different histological types of non-small cell lung cancer tissues. Specimens with (A) both high expression of C3 (a and c) and $CD4^+$ (b and d); (B) both low expression of C3 (a and c) and $CD4^+$ (b and d); (C) both high expression of C3 (a and c) and $CD8^+$ (b and d); and (D) both low expression of C3 (a and c) and $CD8^+$ (b and d).

better prognostic tools for cancer. The complement has evolved as a first-defense system against non-self cells or undesirable host elements. The spectrum of complement-mediated functions ranges from direct cell lysis to the control of humoral and adaptive immunity. As a system with important involvement in inflammatory responses, the complement is also assumed to be involved in cancer-related biological processes. In this study, we sought to elucidate the prognostic value of an

important complement component, C3, and its correlation to lymphocyte infiltration, in order to assess the prognostic value of this inflammatory factor in tumor progression.

Considering that the complement is involved in the recognition of non-self elements, it is logical to hypothesize that changes in the composition of tumor cell membranes render these cells a target for recognition by the complement. Consistent with this assumption, a number of clinical studies have reported an activation and subsequent deposition of complement in cancer patients (29-30). In the present study, using resected specimens from NSCLC patients who had undergone surgery, we found that nearly all cancer tissues express C3 in the stromal and peritumoral nest areas, indicating that the complement may be synthesized by the stromal and inflammatory cells in these areas in the NSCLC environment. However, the antigens responsible for complement activation and the relevant pathways are not yet known. Moreover, a high level of deposition of the complement component 5 (C5) protein was found in lung cancer cell lines, and its activated product C5a was increased in plasma from patients with NSCLC (17), thus tumor cells may also be able to form the complement, through the action of an extrinsic pathway. Therefore, a more systematic identification of active complement components and analysis of the pathways and mediators by which cancer cells may activate the complement is needed to interpret these results.

A series of studies have confirmed that the complement system contributes to mechanisms that affect the growth of tumors in mouse models (17,18), but there is still considerable controversy on the exact mechanisms and relevant conditions that promote them in the human body. Complement and its related proteins are elevated in the biological fluids of patients with numerous types of tumor, and their activity has been associated with the clinical outcome of these patients (20-22). For example in patients with chronic lymphocytic leukemia, a positive correlation was observed between survival time and the activity of the classical complement pathway (31). We performed a survival analysis to assess whether the C3 levels in cancer tissues positively correlate to OS and DFS of NSCLC patients; investigating the role of the complement system in neoplastic progression in NSCLC patients is a novel approach, allowing to directly assess the prognostic value of C3, thereby providing potentially alternative tools for cancer therapy.

It is well established that the downstream products of C3 activation, C3a and C5a, are important anaphylatoxins that recruit immune cells (neutrophils, phagocytic cells and more) to the site of inflammation (32). However, certain types of recruited inflammatory cells, such as MDSCs, can promote tumor progression, which is not consistent with clinical results. The present study provided evidence that higher numbers of $CD4^+$ and $CD8^+$ cells infiltrate tumor tissues where C3 is highly expressed. Therefore, our findings support that C3 may also contribute to tumor suppression by attracting anti-tumor immune cells in a MAC-independent manner, which may explain the fact that high C3 levels predict long survival times. We assume that C3 can recruit inflammatory cells in the human body, although in a mouse model of multistage epithelial carcinogenesis (HPV16 mice) C3 did not recruit inflammatory cells (33); the tumor environment may not be identical between the two species.

In conclusion, the level of the core component of the complement system, C3, has a significant prognostic value in NSCLC patients at all stages, and further correlates to local $CD4^+$ and $CD8^+$ T lymphocyte infiltration. This may represent an advisable mechanism to explain previous results.

Future studies will be performed to identify the relevant regulating factors and pathways that are involved in the roles played by C3 in tumor suppression. In addition, the effectiveness of C3 as a diagnostic and prognostic marker needs to be further assessed, both in tissue and serum samples, in the context of developing C3-based agents for future clinical application.

Acknowledgements

We thank the Department of Pathology, Xinqiao Hospital, Third Military Medical University, Chongqing, China for generously offering of the NSCLC specimens.

References

1. Martin J and Rusch V: Lung neoplasms. In: Surgery: Scientific Principles and Clinical Practice. Greenfield L, Mullholland M, Oldham K, Zelenock G and Lillemoe K (eds). Lippincott Williams and Wilkins, Philadelphia, PA, pp1373-1400, 2001.
2. Sica A, Allavena P and Mantovani A: Cancer related inflammation: the macrophage connection. Cancer Lett 267: 204-215, 2008.
3. Kuang DM, Zhao Q, Peng C, Xu J, Zhang JP, Wu C and Zheng L: Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. J Exp Med 206: 1327-1337, 2009.
4. Sica A and Bronte V: Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest 117: 1155-1166, 2007.
5. Marigo I, Dolcetti L, Serafini P, Zanovello P and Bronte V: Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. Immunol Rev 222: 162-179, 2008.
6. Haverkamp JM, Crist SA, Elzey BD, Cimen C and Ratliff TL: In vivo suppressive function of myeloid-derived suppressor cells is limited to the inflammatory site. Eur J Immunol 41: 749-759, 2011.
7. Ikushima H and Miyazono K: TGFβ signalling: a complex web in cancer progression. Nat Rev Cancer 10: 415-424, 2010.
8. Calon A, Espinet E, Palomo-Ponce S, *et al*: Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. Cancer Cell 22: 571-584, 2012.
9. Sansone P and Bromberg J: Targeting the interleukin-6/Jak/stat pathway in human malignancies. J Clin Oncol 30: 1005-1014, 2012.
10. Maniati E, Soper R and Hagemann T: Up for Mischief? IL-17/Th17 in the tumour microenvironment. Oncogene 29: 5653-5662, 2010.
11. Donin N, Jurianz K, Ziporen L, Schultz S, Kirschfink M and Fishelson Z: Complement resistance of human carcinoma cells depends on membrane regulatory proteins, protein kinases and sialic acid. Clin Exp Immunol 131: 254-263, 2003.
12. Bubeck D, Roversi P, Donev R, Morgan BP, Llorca O and Lea SM: Structure of human complement C8, a precursor to membrane attack. J Mol Biol 405: 325-330, 2011.
13. Walport MJ: Complement. First of two parts. N Engl J Med 344: 1058-1066, 2001.
14. Walport MJ: Complement. Second of two parts. N Engl J Med 344: 1140-1144, 2001.
15. Boross P and Leusen JH: Boosting antibody therapy with complement. Blood 119: 5945-5947, 2012.
16. Ross GD, Vetvicka V, Yan J, Xia Y and Vetvicková J: Therapeutic intervention with complement and beta-glucan in cancer. Immunopharmacology 42: 61-74, 1999.
17. Markiewski MM, DeAngelis RA, Benencia F, *et al*: Modulation of the antitumor immune response by complement. Nat Immunol 9: 1225-1235, 2008.
18. Li F, Cheng Y, Lu J, Hu R, Wan Q and Feng H: Photodynamic therapy boosts anti-glioma immunity in mice: a dependence on the activities of T cells and complement C3. J Cell Biochem 112: 3035-3043, 2011.

19. Blok VT, Daha MR, Tijsma O, *et al*: A bispecific monoclonal antibody directed against both the membrane-bound complement regulator CD55 and the renal tumor-associated antigen G250 enhances C3 deposition and tumor cell lysis by complement. J Immunol 160: 3437-3443, 1998.
20. Oner F, Savaş I and Numanoğlu N: Immunoglobulins and complement components in patients with lung cancer. Tuberk Toraks 52: 19-23, 2004.
21. Zimmermann-Nielsen E, Iversen LH, Svehag SE, Thorlacius-Ussing O and Baatrup G: Activation capacity of the alternative and classic complement pathways in patients operated on for colorectal cancer. Dis Colon Rectum 45: 544-553, 2002.
22. Saito T, Kuwahara A, Kinoshita T, *et al*: Increases in immunoglobulin and complement in patients with esophageal or gastric cancer. Surg Today 22: 537-542, 1992.
23. Espinosa E, Feliu J, Zamora P, *et al*: Serum albumin and other prognostic factors related to response and survival in patients with advanced non-small cell lung cancer. Lung Cancer 12: 67-76, 1995.
24. Takigawa N, Segawa Y, Okahara M, *et al*: Prognostic factors for patients with advanced non-small cell lung cancer: univariate and multivariate analyses including recursive partitioning and amalgamation. Lung Cancer 15: 67-77, 1996.
25. Yee J, Sadar MD, Sin DD, *et al*: Connective tissue-activating peptide III: a novel blood biomarker for early lung cancer detection. J Clin Oncol 27: 2787-2792, 2009.
26. Zhong L, Coe SP, Stromberg AJ, Khattar NH, Jett JR and Hirschowitz EA: Profiling tumor-associated antibodies for early detection of non-small cell lung cancer. J Thorac Oncol 1: 513-519, 2006.
27. Qiu J, Choi G, Li L, *et al*: Occurrence of autoantibodies to annexin I, 14-3-3 theta and LAMR1 in prediagnostic lung cancer sera. J Clin Oncol 26: 5060-5066, 2008.
28. Trinchieri G: Cancer and inflammation: an old intuition with rapidly evolving new concepts. Annu Rev Immunol 30: 677-706, 2012.
29. Lucas SD, Karlsson-Parra A, Nilsson B, *et al*: Tumor-specific deposition of immunoglobulin G and complement in papillary thyroid carcinoma. Hum Pathol 27: 1329-1335, 1996.
30. Blok VT, Daha MR, Tijsma OM, Weissglas MG, van den Broek LJ and Gorter A: A possible role of CD46 for the protection in vivo of human renal tumor cells from complement-mediated damage. Lab Invest 80: 335-344, 2000.
31. Varga L, Czink E, Miszlai Z, *et al*: Low activity of the classical complement pathway predicts short survival of patients with chronic lymphocytic leukaemia. Clin Exp Immunol 99: 112-116, 1995.
32. Klos A, Tenner AJ, Johswich KO, Ager RR, Reis ES and Köhl J: The role of the anaphylatoxins in health and disease. Mol Immunol 46: 2753-2766, 2009.
33. de Visser KE, Korets LV and Coussens LM: Early neoplastic progression is complement independent. Neoplasia 6: 768-776, 2004.