Interstitial tumor-associated macrophages combined with tumor-derived colony-stimulating factor-1 and interleukin-6, a novel prognostic biomarker in non–small cell lung cancer

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Objectives: Recent experimental evidence has indicated that interstitial tumor-associated macrophages (TAMs), tumor-derived macrophage colony-stimulating factor (also known as CSF-1), and interleukin-6 (IL-6) interact in the pathogenesis of malignant epithelial tumors, including lung cancer. The present study aimed to explore their relationship and prognostic significance in surgically resected non–small cell lung cancer (NSCLC).

Methods: Tissue microarray and immunohistochemistry were used to detect the expression of CSF-1, IL-6, and CD68-positive TAMs in 417 patients with NSCLC undergoing complete pulmonary resection from 2003 to 2008. Their correlations and clinicopathologic data were analyzed using chi-square testing. Their prognostic values were evaluated by univariate Kaplan-Meier survival analysis and multivariate Cox proportional hazard model analysis.

Results: The expression of CSF-1 and IL-6 in NSCLC correlated positively with the infiltration degree of TAMs in the tumor stroma ($r = 0.184$ and $r = 0.196$, respectively; $P < .001$). The expression of both CSF-1 and IL-6 was statistically significant for survival ($P < .001$). Nevertheless, no such relationship was observed for CD68 in the tumor stroma ($P > .05$). When CSF-1 and/or IL-6 and CD68 were taken into consideration together, the result became statistically significant. Multivariate analysis showed that co-expression of CD68, CSF-1, and IL-6 remained the most significant and independent prognostic factor for survival ($P < .05$) but not the combinations of CSF-1 and IL-6, CD68 and CSF-1, or CD68 and IL-6 ($P > .05$). The 5-year survival rate in the CD68-negative and CSF-1– and IL-6–positive group was better than the rate in the CD68, CSF-1–, and IL-6–positive group ($P < .05$).

Conclusions: The combination of CD68 plus TAMs, CSF-1, and IL-6 is very likely to be a valuable independent predictor of survival in patients with NSCLC. Perhaps co-expression of CSF-1 and IL-6 induces interstitial TAMs to shift toward the tumor-promoting phenotype. (J Thorac Cardiovasc Surg 2014;148:1208-16)

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Lung cancer is the leading cause of cancer mortality in the world, with an overall 5-year relative survival rate of approximately 15% at diagnosis.[1,2] Even for patients with pathologic stage IA non–small cell lung cancer (NSCLC), the 5-year overall survival has been approximately 67%.[3] Approximately 40% of patients with stage I and those with 60% of stage II NSCLC will die within 5 years after curative pulmonary resection, mainly because of distant metastases and local recurrence.[4-6] Because of its prevalence and poor prognosis, the selection of appropriate biomarkers to evaluate severity, monitor progression, and estimate the efficacy of a specific therapy has become an unavoidable issue.

Solid tumors comprise not only cancer cells, but also many other nonmalignant stromal cells, producing a unique microenvironment to modify the neoplastic properties. One such stromal cell type that might promote cancer progression is tumor-associated macrophages (TAMs), which derive from circulating monocytic precursors.[7] They are recruited to the tumor site by cytokines and other tumor-derived factors

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Abbreviations and Acronyms

+	= positive
−	= negative
CSF-1	= colony-stimulating factor-1
DFS	= disease-free survival
IL-6	= interleukin-6
NSCLC	= non–small cell lung cancer
OS	= overall survival
TAM	= tumor-associated macrophage
TMA	= tissue microarray

and, once in situ, produce chemokines and growth and angiogenic factors that alter tumor growth, invasion, and metastasis.[8] Although an increasing body of preclinical and clinical evidence has associated TAMs with a poor prognosis, their prognostic significance in NSCLC has been contradictory.[9-12] Therefore, the cytokine profile of the tumor microenvironment is thought to educate TAMs toward a tumor-promoting or tumor-suppressing phenotype; however, the specific mechanisms remain not fully identified.

Several recent studies of ovarian cancer have demonstrated that the tumor-promoting TAMs were induced by interleukin-6 (IL-6), leukemia inhibitory factor, and colony-stimulating factor-1 (CSF-1) derived from cancer cells.[13-15] The potential relationship and biologic significance between TAMs and tumor-derived CSF-1 and IL-6 have never been clarified in NSCLC. Studies have shown that the pre- and postoperative serum levels of CSF-1 and IL-6 are important prognostic indicators in patients with NSCLC.[16,17] However, because the serum concentrations can be influenced by many factors, certain limitations exist. The present study intended to directly detect the expression of CSF-1, IL-6, and TAMs in the NSCLC microenvironment and explore their prognostic value. This could be of great significance to further clarify the biologic function of TAMs and thereby open up a new avenue for the comprehensive treatment of NSCLC.

METHODS

Tissue Samples

All patients with stage I to IIIA NSCLC undergoing complete pulmonary resection and systematic lymph node dissection at the Cancer Institute and Hospital of Tianjin Medical University (China) from 2003 to 2008 were considered eligible for retrospective analysis of the clinical prognostic factors. Patients were excluded from the present study if they had met any 1 of the following criteria: (1) the use of neoadjuvant therapy; (2) the presence of metastatic disease preoperatively; (3) complete clinicopathologic and follow-up data were not available; (4) death had occurred within 1 month after surgery. The histologic type for all NSCLC cases was re-assessed according to the modern classification system (World Health Organization 2011) by 2 dedicated pathologists. Tumor staging was done using the most recent International Association for the Study of Lung Cancer TNM classification system.[18] All patients were followed up until September 1, 2013. The research ethics committee of Tianjin Cancer Institute and Hospital provided ethical approval for the study of human subjects, and all patients provided written informed consent. The patients who were still alive after the last follow-up visit and those who had been lost to follow-up were censored in the present study.

Tissue Microarray and Immunohistochemistry

For tissue microarray (TMA) construction, 2 experienced pathologists reviewed hematoxylin and eosin–stained sections from each paraffin-embedded, formalin-fixed block. The most representative areas of the tumor region were carefully selected and sampled for the TMA collector blocks. To validate the concordance between the TMAs and whole tumor sections, we also detected CSF-1, IL-6, and CD68 expression for 40 cases randomly chosen from 417 patients.

Immunohistochemistry was performed with monoclonal anti-mouse CD68 antibody at a 1:5 dilution (clone KP1, Abcam, Cambridge, UK), anti-rabbit CSF-1 polyclonal antibody at a 1:100 dilution (code BA0750, Boster Biological Technology, Wuhan, China), and anti-IL6 rabbit polyclonal antibody at a 1:100 dilution (code BS0781R, Bioss Biological Technology, Beijing, China). After deparaffinization, rehydration, and heat-induced antigen retrieval, the tissues were immersed in methanol containing 3% hydrogen peroxide for 30 minutes to block the endogenous peroxidase activities. After incubated with normal goat serum (diluted 1:10) for 20 minutes to block the nonspecific antibody-binding sites, the sections were incubated with primary antibodies overnight at 4°C. After 30 minutes of incubation with the secondary antibody, the sections were developed in diaminobenzidine solution under microscopic observation and counterstained with hematoxylin to visualize the nuclei. Negative control slides with the primary antibodies omitted were included in all assays.

Two pathologists independently evaluated the staining of all anonymized samples. Five different areas at 400× magnification from each sample were systematically evaluated for the expression of CSF-1, IL-6, and CD68. The mean value of the 5 cores was considered representative of 1 tumor. For CSF-1 and IL-6 staining, we calculated the sum of both the staining intensity (0, negative; 1, weak; 2, intermediate; 3, strong) and the percentage of positive cells (0, none or <5%, 1, 5% to 25%; 2, 25% to 50%; 3, >50%). Scores of 0 to 2 were regarded as negative and scores of 3 to 6 as positive. For CD68 staining, the tissue samples were divided into 2 groups: negative, no infiltration; and positive, infiltration.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences, version 16.0 (SPSS, Chicago, Ill), statistical programs. The nonparametric Spearman test was used for correlation analysis; the association between the ranked data and the clinical parameter was analyzed using the chi-square test or Fisher's exact test. Overall survival (OS) was defined as the interval between surgery and death or the last observation. Disease-free survival (DFS) was measured from the date of resection to the detection of recurrent tumor or the last follow-up assessment. OS and DFS were analyzed using the Kaplan-Meier method and the log-rank test, and multivariate analyses were tested using the Cox proportional hazard model.

RESULTS

Patient Characteristics

Of the eligible patients, 3 were excluded because of the use of neoadjuvant therapy and 2 had died within 1 month after surgery, leaving 417 cases for analysis. The mean follow-up period was 43 months (range, 2 to 120). The clinical and histopathologic details of the 417 cases are listed in Tables 1 and 2.