Expressions and clinical significance of a disintegrin and metalloprotease 8 (ADAM8) and epidermal growth factor receptor (EGFR) in non-small cell lung cancer

Guo-Cheng Wu,* Hua-Cheng Hu and Min-Hua Shi

Department of Respiratory Medicine; The Second Affiliated Hospital; Soochow University; Suzhou, Jiangsu, P.R. China

Key words: a disintegrin and metalloprotease 8, epidermal growth factor receptor, lung neoplasm, non-small cell lung cancer

Background and Objective: Upregulation of a disintegrin and metalloprotease 8 (ADAM8) is correlated with genesis, progression, invasion and metastasis of tumors. However, the expression of ADAM8, especially its correlation to epithelial growth factor receptor (EGFR), has seldom been reported in non-small cell lung cancer (NSCLC). This study was to investigate expressions of ADAM8 and EGFR in NSCLC, and to analyze their correlations.

Methods: Expressions of ADAM8 and EGFR in 49 specimens of NSCLC, 28 specimens of paracancerous lung tissues and 13 specimens of benign lung lesions were detected using tissue microarray (TMA) and immunohistochemistry (IHC). The interrelationship between the two factors and their correlations to clinicopathologic features of NSCLC were analyzed.

Results: ADAM8 and EGFR were mainly expressed in the cytoplasm and on the cell membrane. The positive rates of ADAM8 and EGFR were significantly higher in NSCLC than in paracancerous lung tissues and benign lung lesions (73.5% vs. 10.7% and 15.4%; 69.4% vs. 14.2% and 23.1%, p < 0.01). The positive rates of ADAM8 and EGFR were slightly lower in squamous cell carcinoma than in adenocarcinoma (73.1% vs. 80.0%; 65.4% vs. 75.0%, p > 0.05), while they were significantly higher in stage I–N3 NSCLC than in stage N0 (85.7% vs. 42.8%; 82.8 vs. 35.7%, p < 0.01), and significantly higher in stage III–IV NSCLC than in stage I–II (90.0% vs. 62.1%; 90.0% vs. 65.5%, p < 0.05). The expression of ADAM8 was positively correlated to EGFR (r = 0.589, p < 0.01), with a kappa value of 0.522.

Conclusion: ADAM8 and EGFR are overexpressed in NSCLC, and their expressions are consistent.

Researchers have revealed that multiple cellular factors, receptors and ligands, which are involved in the processes of genesis, progression and metastasis of tumors, may exert their effects only after their extracellular domains are shed by protease.1 A disintegrin and metalloprotease 8 (ADAM8) has the function of cell adhesion and protease. It participates in shedding of the extracellular domain of the epidermal growth factor receptor (EGFR) ligand. Overexpression of ADAM8 is associated with progression and prognosis of solid tumors.2,3 Using tissue microarray (TMA) combined with immunohistochemistry (IHC), we investigated different expressions of ADAM8 and EGFR in 49 specimens of non-small cell lung cancer (NSCLC), 28 specimens of paracancerous lung tissues and 13 specimens of lung tissues with benign lesions, and analyzed their correlations to clinicopathologic characters of patients.

Data and Methods

General information. Sixty-two specimens were extracted from patients who underwent operation in the Department of Thoracic Surgery of the Second and the First Hospitals Affiliated Suzhou University from January 2006 to November 2006. There were 49 cases of NSCLC and 13 cases with benign lung lesions. Among 49 NSCLC cases, there were 30 males and 19 females, ranged between 46–77 years, with a median age of 62 years. None of the NSCLC patients received radiotherapy or chemotherapy before operation, including 26 cases of squamous cell carcinoma, 20 cases of adenocarcinoma and three cases of large cell cancer. All NSCLC patients were subjected to chest CT, abdominal B-mode ultrasonography or CT, bone scanning, and cranial CT or MRI. TNM staging was performed according to the UICC staging standard 2003. Four cases were found at stage I a, seven at stage I b, four at stage II a, 14 at stage II b, 10 at III a, four at stage III b, and six at stage IV. Twenty-eight specimens of paracancerous lung tissues extracted 10 cm away from the cancer focus of stage I and II patients were regarded as normal lung tissue. Among 13 cases with benign lung lesions, there were seven males and six females, ranged from 20–71 years with a median age of 54 years, including four cases of pulmonary tuberculosis, one case of pulmonary cyst, and eight cases of inflammatory pseudotumor. All these cases were confirmed by pathological biopsy after operation. The distribution of age and sex of NSCLC group and benign lesion group was uniform and had no statistical significance.
Expressions and clinical significance of a disintegrin and metalloprotease 8 (ADAM8) and epidermal growth factor receptor (EGFR) in non-small cell lung cancer

Major reagents. Sheep anti-human ADAM8 (AF 1031) polyclonal antibody was purchased from R&D Co., Ltd. (USA), and the immunohistochemical kit containing murine anti-human EGFR (MAB-0196) monoclonal antibody was bought from Fujian Maxim Biotechnology Co., Ltd.

Methods. Preparation of tissue microarray. Tissue microarray was prepared by the laboratory of Shanghai Biochip Co., Ltd. A 1.5 cm diameter hole was bored on the blank wax cube (recipient cube) using a tissue array apparatus. A representative block of cancer tissues 1.5 cm in diameter was retrieved by puncturing the wax cube and inserted into the hole of the recipient cube until all specimens of tissues were implanted in the recipient cube. Consecutive slices of 4 μm thick were cut from the recipient cube for further use.

Immunohistochemistry (IHC). IHC was performed strictly according to the specifications provided by the manufacturer. The concentration of the primary antibody against ADAM8 was diluted to 50 μg/mL. The antibody against EGFR is an instant antibody. A known positive slice was taken as the positive control. For a negative control, the primary antibody was replaced by PBS.

Judgment of the results. Cells with light yellow, brown-yellow or dark brown circular or granular staining on the membrane and/or in plasma were regarded as ADAM8 or EGFR positive. Two hundred cells were observed in each of the four fields of view (FOV) under high magnification to count positive cells. Stainings were classified according to the percentage of positive cells: (-), the number of positive cells <20%; (+), the number of positive cells between 20–50%; (++), the number of positive cells between 51–75%; (+++), the number of positive cells >75%. Results of IHC were judged independently by two pathologists. When they had divided opinion, they exchanged their views to reach an agreement.

Statistics. Rates were compared using χ² test; the coexpression of ADAM8 and EGFR was analyzed using Kappa test; the correlation was analyzed using Spearman rank correlation test. All results were analyzed using SAS 6.12 software, and p < 0.05 was regarded as significantly different.

Results

Tissue microarray. The tissue microarray contained 90 arrays. These arrays were intact in appearance. Their distributions were uniform without remarkable displacement. The arrangement, spacing and density of these arrays were proper. No missing, displaced, packed or overlapped arrays were observed (Fig. 1).

The expression of ADAM8 and EGFR in the tissue of NSCLC, paracancerous lung tissues and tissues with benign lesions. Positive expressions of ADAM8 and EGFR proteins were mainly located in plasma and on the cell membrane (Fig. 2). The positivity of ADAM8 and EGFR in NSCLC tissues, paracancerous lung tissues, and tissues with benign lesions were 73.5% (36/49) and 69.4% (34/49), 10.7% (3/28) and 14.3% (4/28), 15.4% (2/13) and 23.1% (3/13), respectively. The positive rates of the two proteins were significantly higher in NSCLC tissues than in paracancerous lung tissues and tissues with benign lesions (p < 0.01), whereas the rates were not significantly different in paracancerous lung tissues and tissue with benign lesions (p > 0.05).

Correlation of expressions of ADAM8 and EGFR to pathological classification, clinical staging and lymphatic metastasis. In all 49 NSCLC specimens, the difference in the positive rates of ADAM8 and EGFR was not significant in respect to the pathological type (p > 0.05), whereas it was significant in respect to lymphatic metastasis and clinical TNM staging (p < 0.05). The positivity of EGFR was 65.5% for patients at stage I–II, and 90.0% for patients at stage III–IV, with significant difference (p < 0.01) (Table 1).

Coexpression of ADAM8 and EGFR in NSCLC specimens. The value of Kappa test for the consistency of ADAM8 with EGFR was 0.522, the probability of 95% was 0.341–0.702. Spearman rank correlation test revealed a positive correlation between the two proteins (r = 0.589, p < 0.01) (Table 2).

Discussion

ADAM8 is named as CD156 according to the naming of leukocyte differentiation antigens. Its gene is located at the chromosome 10q26.3, and it encodes 824 amino acids, including eight structural domains from the N-terminal to C-terminal in sequence of signal peptide, pro-domain, catalytic domain (metalloprotease domain), and receptor domain (inhibitor domain) [1]. Its expression is related to the occurrence and development of various tumors, such as gastric cancer, esophageal cancer and melanoma [2]. A study found that ADAM8 expression was significantly higher in the invasion and metastasis areas of human breast cancer tissues than in the non-invasion and non-metastasis areas [3]. The expression of ADAM8 in NSCLC tissues was significantly higher than in paracancerous lung tissues and tissues with benign lesions (p < 0.01), whereas the rates were not significantly different in paracancerous lung tissues and tissue with benign lesions (p > 0.05). The positive rates of the two proteins were significantly higher in NSCLC tissues than in paracancerous lung tissues and tissues with benign lesions (p < 0.01), whereas the rates were not significantly different in paracancerous lung tissues and tissue with benign lesions (p > 0.05). The positive rates of the two proteins were significantly higher in NSCLC tissues than in paracancerous lung tissues and tissues with benign lesions (p < 0.01), whereas the rates were not significantly different in paracancerous lung tissues and tissue with benign lesions (p > 0.05).
The configuration of dimerized receptors is changed. Subsequently, tyrosine kinase of cells is activated, and then the specific residues of tyrosine on the carboxyl end are phosphorylated. The phosphorylated tyrosine becomes the binding site for signal proteins with a SH2 or PTB structural domain, such as Grb2, PI-3K or PLC. Thus, multiple signal transduction pathways (Ras/Raf/MAPK, PI-3K/Akt and so on) are further activated, which would exert influence on biological effects, including gene expression, cell proliferation and differentiation. ADAMs participate in shedding of extracellular domains of seven known ligands of EGFR. Release of extracellular domains of some ligands of EGFR via hydrolysis may act as the regulation switch of EGFR signal transduction, suggesting that ADAMs may play an important role in the regulation of EGFR dependent signal transduction pathway.

This study revealed that the expression of ADAM8 was higher in tissues of NSCLC than in normal lung tissues or lung tissues with benign lesions, implying a promoting role of ADAM8 in carcinogenesis of lung cancer. No significant difference exists between squamous cell carcinoma and adenocarcinoma in respect to the expression of ADAM8, suggesting that ADAM8 does not relate to the pathological type of NSCLC. Lymphatic metastasis and TNM staging are correlated with the expression of ADAM8, which is, the higher the staging of NSCLC and the more remarkable the lymphatic metastasis, the stronger ADAM8 expression. We propose that ADAM8 is related to progression and metastasis of tumors to a certain extent. The above results were consistent with the studies reported by other groups.

The underlying mechanism of such a correlation remains unclear, which may be through the protease pathway and integrin pathway to affect genesis, progression and metastasis of tumors. The distribution and expression regulation of EGFR have been largely published. Moreover, we observed a positive correlation between overexpression of ADAM8 and EGFR in the tissue of NSCLC, which offers both theoretical and practical basis for their application value in targeting therapy of lung cancer.

At present, EGFR is the most investigated and effective target for the treatment of lung cancer. There are two major EGFR-targeting drugs, the anti-EGFR monoclonal antibody and inhibitors of the tyrosine kinase. The latter one is more frequently used in clinic and research. The sensitivity of the tyrosine kinase inhibitor is found to be related to the mutation of EGFR. Mutated EGFR loses its ligand-binding domain, but it may be self-phosphorylated to activate the tyrosine kinase activity, leading to cell proliferation. For most non-mutated EGFR, the therapeutic effect of the tyrosine kinase inhibitor is poor. We expect that the signal transduction pathway of EGFR could be inhibited by reducing the activity of its ligands. ADAMs play an important role in ErbB signaling pathway through hydrolyzing the ligand of ErbB. Activation of ErbB ligands, which is, the detachment of the extracellular domain, has become the target of exploring new drugs targeting the ErbB-pathway. The strategy of aiming at ADAMs has become an important supplement to the therapy through the anti-ErbB pathway. Currently, the phase I clinical trials on Prinomastat, an inhibitor of metalloprotease with a broad spectrum, and on BMS-561392, a selective inhibitor of ADAM17, have been terminated due to their side effects, such as muscular pain and skeletal pain. No reports concerning the basic and clinical researches on the selective inhibitor of ADAM8 have been presented. Further research on the inhibitor of ADAM8 may be...
carried out using the antisense oligonucleotide of ADAM8, the small molecular inhibitor of the functional/structural domain of ADAM8 and anti-ADAM8 antibodies.

Acknowledgements

Research Foundation for Young Teachers of Soochow University (No. Q3123640).

References


