A Study on the expression of erbB4/HER4 in non-small cell lung cancer*

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Abstract  Objective: To test the expression of HER4 in non-small cell lung cancer (NSCLC) and elucidate the relationship between its over-expression and the clinical pathology of NSCLC.  Methods: 70 cases of paraffin-embedded tissues from informative NSCLC were tested for the expression of HER4 by means of immunohistochemical assay.  Results: HER4 were overexpressed in NSCLC in 91.4%. The overexpression of HER4 correlated only with the lymph node metastasis, TNM staging and survival after operation.  Conclusion: ErbB4 is one of the genes to regulate the growth of NSCLC in advanced stages and artificial interference of the overexpression of HER4 in NSCLC might be a good way for the treatment of NSCLC in advanced stages.

Key words  erbB4 gene; HER4 protein; non-small cell lung cancer

ErbB4 gene is an oncogene which encodes the forth member of human epidermal-growth-factor receptor family (HER4). After binding the ligand, self-phosphorylated HER4 mediates the signal transduction, and finally regulates the growth and division of normal and transformed cells. In order to research the relationship between overexpression of HER4 and clinicopathology of NSCLC and reveal the function of erbB4 gene in NSCLC, the expression of HER4 in NSCLC tissues was detected in this study.

Materials and methods

Patients

Seventy patients included in this study all from the chest surgery of two hospitals were required to have been histologically or cytologically proven NSCLC. 55 patients were males and 15 were females, the mean age was 59.74 years (ranged from 34 to 77 years). These patients were followed-up until May 2000.

Fresh tumor tissues were obtained from these patients at initial surgery, then fixed by formalin and embedded in paraffin. Tumor histology was assessed on paraffin-embedded sections and classified according to WHO criteria. Tumor histologies were classified as: squamous carcinoma (43 tumors), adenocarcinoma (27 tumors). 20 cases of control group were the lung tissues 5 cm away from the NSCLC and histologically proven normal lung tissues.

Reagents

HER4 status was evaluated by immunocytochemistry carried out by using the PAb SC-283 (Santa Cruz Biotechnology, USA) and revealed by the EnVision detection system (Dako, Glostrup, Denmark).

Immunohistochemistry ABC method

Sections (5 μm) were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by incubating sections in 3% H2O2-methanal for 5 min. Sections were washed in PBS (pH 7.2), then immersed in citric acid buffer (pH 6.0) and microwaved for 5 min. Sections were washed in PBS again. Primary antibodies were added for 1 h at 37 °C. After primary antibody incubation, sections were stained with secondary antibody (30 min at 37 °C), followed by horseradish peroxidase-labeled streptavidin complex (15 min). Diaminobenzidine tetrachloride was used as chromagen and applied for 5 min. Sections were lightly counterstained in hematoxylin, dehydrated, and mounted.

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Assessment of HER4 immunoreactivity
The percentage of neoplastic cells showing cell nucleus immunoreactivity was semiquantitatively evaluated (range 0%–100%). –, no immunoreactive cells; +, less than 25% cells were immunoreactive; ++, 25%–50% cells were immunoreactive; ++++, more than 50% cells were immunoreactive \[1\]. Cases were considered HER4 overexpression only when the difference between the tumor cell staining score and the non-neoplastic epithelial cell staining score was ≥ ++ \[2\].

Statistical method
Descriptive analysis comparing the differences used the χ² test (for categorical variables). Differences in postoperative survival between the two groups (with and without HER4 overexpression) were tested using the Log-rank test. Statistical analysis has been computed using SPSS 10.0 software.

Results
The location and expression of HER4 in normal lung cells and in NSCLC cells
HER4 resulted positive (+) in nonneoplastic lung tissue (Fig. 1, Fig. 2), 65% in epithelial cells of tunica mucosa bronchiorum and 30% in alveolar epithelial cells. The stain was lightly in membrane and cytoplasm of less than 25% cells, and disjunct in the membrane. The location of HER4 in cells of NSCLC was different with nonneoplastic lung tissue (Fig. 3, 4). The stain of HER4 was located in cell nucleus mainly, and in cytoplasm partly. The overexpression of HER4 was in 91.4% NSCLC (64/70), and nonoverexpression in nonneoplastic lung tissue (Tables 1 and 2).

The relationship between overexpression of HER4 and clinico-pathology of NSCLC
The overexpression of HER4 was uncorrelated with the histopathologic type and the level of differentiation of NSCLC (\(P>0.05\)), but correlated with lymph node metastasis and TNM staging (\(P<0.05\); Table 3)

The relationship between overexpression of HER4 and postoperative survival rate
In Log Rank Test (Fig. 5), the survival rate of the HER4 overexpression group was lower than the survival rate of the HER4 nonoverexpression group, the difference was significant (\(P=0.0258\)). This indicated that the prognosis was worse in NSCLC which overexpressed HER4.