Clinical relevance of neuroendocrine differentiation in lung adenocarcinoma

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Abstract Objective: The aim of our study was to investigate the prevalence and clinical relevance of neuroendocrine (NE) differentiation in lung adenocarcinoma. Methods: Eighty-six adenocarcinoma paraffin-embedded specimens and cases which were followed up completely for 3 years, were obtained from 86 patients (35 men and 51 women) who underwent surgical resection for pathologically supported adenocarcinoma in the Cancer Hospital of Tianjin Medical University, from June 2005 to December 2006. Immunohistochemical EnVision two-step method was used to detect the expression of neuron-specific enolase (NSE), synaptophysin (SYN) and chromogranin A (CGA). All data were analyzed using SPSS statistics software and Kaplan-Meier survival curves were constructed, meanwhile, we conducted a Log-rank test. Results: All patients with lung adenocarcinoma, 35 cases with NE differentiation (40.7%). The statistical analysis showed that the positive rate of NE differentiation in lung adenocarcinoma was significantly associated with cancer recurrence and histological differentiation. In addition, CGA, NSE and SYN positive rates were 27.9%, 50.0%, 43.0%, respectively. A statistically significant difference was found between positive expression of SYN and other clinicopathological parameters, such as pathological type, histological differentiation, lymph node metastasis, postoperative recurrence and 3-year survival rate (P = 0.001) and so on. Conclusion: NE differentiation can be used as a metastatic potentially indicator of biological behavior of lung adenocarcinoma, and combined detection of NSE and SYN markers may be recommended to examine NE differentiation of lung adenocarcinoma. Positive expression of SYN indicates poor prognosis.

Key words lung adenocarcinoma; neuroendocrine differentiation; biological behavior; prognosis

The presence of neuroendocrine differentiation has been demonstrated in many nonneuroendocrine tumors in previous studies [1]. They are referred to as tumors with neuroendocrine differentiation since less than 50% of tumor cells are identified as accompanied by neuroendocrine differentiation, and those cells are often showing individual cell cytomorphology or architectural feature of nesting [2]. Many types of non-small cell lung carcinoma (NSCLC) also share varying degrees of neuroendocrine features, such as squamous cell carcinoma, adenocarcinoma and large cell neuroendocrine carcinoma [3]. It is reported that neuroendocrine differentiation is seen more frequently in adenocarcinoma [4]. It is a pity that no further study regarding lung adenocarcinoma with neuroendocrine differentiation has been conducted. At present, adenocarcinoma has surpassed squamous cell carcinoma as the most common histologic subtype of lung cancer in many countries and the morbidity is still increasing, so it is necessary to research its clinical and biological properties, which is also of clinical significance. The purpose of the present study was to evaluate the prevalence and clinical relevance of neuroendocrine differentiation in a series of patients with lung adenocarcinoma by utilizing a combination of neuroendocrine markers, neuron-specific enolase (NSE), synaptophysin (SYN) and chromogranin A (CGA).

Materials and methods

Patients

Eighty-six lung adenocarcinoma paraffin specimens obtained from an equal number of patients who had undergone surgical resection and pathological confirmation at the Cancer Hospital of Tianjin Medical University in the period 2005–2006 inclusive, and all the patients were followed up completely for 3 years. The study group comprised 35 males and 51 females with a median age of 62 years (range, 36–78 years). Clinical staging as I, II, III phrases. According to the latest WHO histological classification of tumors of the lung, all cases were classified into four categories, bronchioloalveolar carcinoma (BAC) (23 cases), adenocarcinomas mixed subtype (30 cases), Acinar
adenocarcinoma (19 cases), other subtypes (14 cases).

**Methods**

Mouse anti-human NSE, CGA, SYN monoclonal antibodies and Envision two-step reagent kit were produced by DAKO Company in Denmark, purchased from Genetic Technology Company in Shanghai. Representative tissue blocks were selected from 3–4 ones every case. Further paraffin sections (4 μm) were cut from each block. Instructions on Envision kit were followed. DAB staining was applied. Sections were counterstained with hematoxylin. Positive controls (carcinoid paraffin sections) and negative controls (PBS) were used in all cases.

**Assessment**

According to criterion by Xu [5], definite brown-yellow granules in cytoplasm with clear background indicated positive tumor cells. Percentage of positive tumor cells in a random collection of 10 highpower fields (× 400) (0 = 0–5%, 1+ = 5%–10%, 2+ = 10%–20%, 3+ = 20%–50%). Diagnosis of neuroendocrine differentiation is based on one marker’s positive expressing (3+) or a combination of at least 2 markers’ positive expressing (1+–2+).

**Follow-up**

All patients were followed up by phone, case-tracking and correspondence. The length of follow-up was calculated as either the time between operation and death or the time from operation to the last follow-up. Maximum follow-up was 3 years.

**Statistical analysis**

All statistical analysis was taken by SPSS 13.0 software. Results using Kaplan-Meier survival curve model and Log-rank test to conduct univariate survival analysis. P < 0.05 is considered statistically significant.

**Results**

**Expressing of neuroendocrine markers in lung adenocarcinoma**

Positive cells were all showing clearly brown granules in cytoplasm. Universal cytoplasmic staining could be seen in NSE positive cells and SYN positive cells (Fig. 1). While CGA positive cells appeared to be comprised of cells with excessive and focal staining (Fig. 2).

Thirty-five cases (40.7%) showed neuroendocrine positive, 27.9% of cases were showing CGA positive expressing, NSE was positive in 50.0% of cases. While 43.0% were SYN positive.

**Neuroendocrine differentiation in different histological types of lung adenocarcinoma**

Neuroendocrine differentiation and the expressing of three markers were shown in Table 1. No correlation was found between neuroendocrine differentiation and histological types. The expressing of CGA and NSE also has nothing to do with histological types. In addition, the discrepancy of SYN positive expressing was statistically significant among different histological types, with the highest positive rate in Adenocarcinomas mixed subtype and the lowest in BAC (P < 0.05).

**Relationship between lymph node metastasis and neuroendocrine differentiation**

Statistical analysis suggested that there was no correlation between lymph node metastasis and neuroendocrine differentiation (Table 2). Among the three markers, SYN positivity was significantly higher in cases with lymph node metastasis compared to that in cases without lymph node metastasis (P < 0.01).

**Relationship between recurrence and neuroendocrine differentiation**

Table 3 showed comparison of neuroendocrine differentiation with cancer recurrence during 3 years. There was association between them. Cases with recurrence during 3 years also had a higher neuroendocrine positive rate (P < 0.01). Furthermore, SYN positivity was higher in cases with recurrence (P < 0.01).

**Relationship between neuroendocrine positivity and histological differentiation**

Table 4 suggested that cases with the lowest differentiation presented the highest neuroendocrine positive rate. Furthermore, the lower the histological differentiation, the higher the positive rate of CGA and SYN was.

**Relationship between neuroendocrine positivity and survival**

Among cases with neuroendocrine differentiation, the three-year survival rate was 40% (14 patients died). While among cases without neuroendocrine differentiation, the three-year survival rate was 37%. A Log-rank test was performed to examine the correlation between neuroendocrine differentiation and the survival rate. The results failed to show any correlation. Compared to CGA and NSE which were also showing no correlation with the survival rate (P > 0.05), obvious statistical discrepancy were obtained between SYN positivity and the survival rate (x² = 10.422, P = 0.001).

**Discussion**

WHO histological classification of lung and pleural tumors in 1999 added the concept of NSCLC with neuroendocrine differentiation [6–8]. That is NSCLC showing no histological features that suggest neuroendocrine (NE) differentiation, but can be demonstrated by immunohis-