

Original Article

Aberrant LRP16 protein expression in primary neuroendocrine lung tumors

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Abstract: Background: The Leukemia related protein 16 gene (LRP16) localized on chromosome 11q12.1, is an important estrogen-responsive gene and a crucial regulator for NF- κ B activation. LRP16 is frequently expressed in human cancers; however, the LRP16 gene remains unexplored in lung neuroendocrine tumors. The aim of this study was to investigate the role of LRP16 expression in primary lung neuroendocrine tumors. Methods: lung neuroendocrine tumors were analyzed for LRP16 gene expression by two-step non-biotin immunohistochemical method. Results: Fifty of ninety (55.6%) cases of neuroendocrine lung tumors tested were positive for LRP16 protein by immunohistochemistry. The expression of LRP16 was mainly located in cytoplasm and nucleus of tumor cells. LRP16 protein was corresponding to tumor type and clinical stage, as well as survival time. Conclusions: The results indicate that abnormal LRP16 expression is noted in neuroendocrine lung tumors and the expression can give insight into the pathogenesis of the disease. The LRP16 protein may serve as a potential marker in predicting prognosis of neuroendocrine lung tumors.

Keywords: Neuroendocrine lung tumors, LRP16, immunohistochemistry, prognosis

Introduction

Neuroendocrine lung tumors form a family of tumors with shared neuroendocrine differentiation features, but diverse clinical behaviors. According to the World Health Organization (WHO) classification of lung tumors [1], carcinoid tumors are low-grade neuroendocrine lung tumors consisting of typical carcinoid (TC) tumors and atypical carcinoid tumors (ATs), whereas small cell lung carcinomas (SCLCs) and large cell neuroendocrine carcinomas (LCNECs) form high-grade neuroendocrine subtype. High-grade neuroendocrine histology uniformly indicated poor prognosis regardless of its histology type [2]. Accurate discrimination of high-grade neuroendocrine carcinoma from other types of lung cancer is important for multimodal treatment [3, 4]. However, histopathologic recognition of neuroendocrine differentiation, especially on small specimens, is not always reliable, as these tumors should be discriminated from undifferentiated nonsmall cell lung carcinomas, such as poorly differentiated

lung adenocarcinoma or poorly differentiated squamous cell carcinoma, or other large cell carcinomas [5]. In fact, there is no immunohistochemical marker of high-grade neuroendocrine carcinoma. Thus, objective markers are needed to help in the pathologic diagnosis of those tumors [2]. It is worth searching for other factors involved in the pathogenesis of neuroendocrine lung tumors.

A growing body of evidence supports the concept that Leukemia related protein 16 gene (LRP16) has roles to play in cancer [6]. LRP16 localized on chromosome 11q12.1, is an important estrogen-responsive gene [7-11]. It has been found expressed at a high level in testicles, ovaries and mucosa of colon, at a moderate level in prostate, small intestine, spleen and thymus, and at a low level in peripheral blood leucocytes [8, 9]. LRP16 is also a coactivator [12] of estrogen receptor alpha (ER α), and androgen receptor (AR), as well as being their target gene [6, 11, 12], and it may play an important role in ER signaling pathways.

LRP16 in neuroendocrine lung tumors

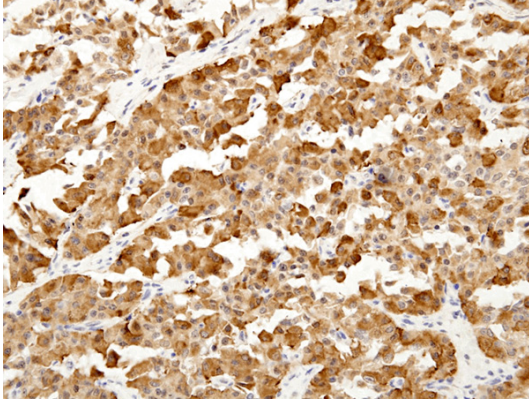


Figure 1. LRP16 expression in typical carcinoid tumors (TC). LRP16 protein is mainly localized in cytoplasm of tumor cells (LRP16 \times 200).

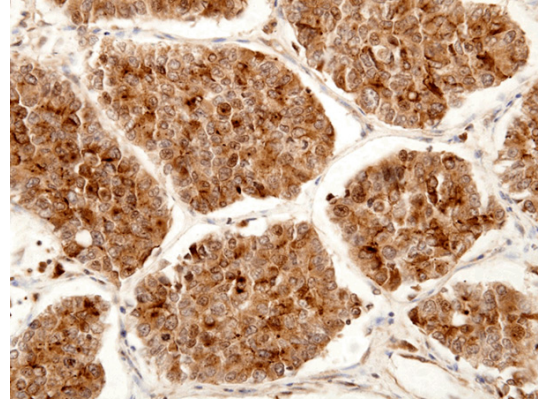


Figure 3. LRP16 expression in large cell neuroendocrine carcinomas (LCNECs). LRP16 was stained positive in the cytoplasm and nucleus of cancer cells. (LRP16 \times 400).

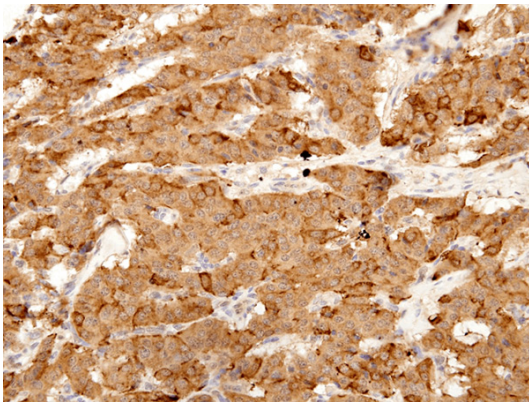


Figure 2. LRP16 expression in atypical carcinoid (ATs) tumors. LRP16 was stained positive in the cytoplasm of tumor cells. (LRP16 \times 400).

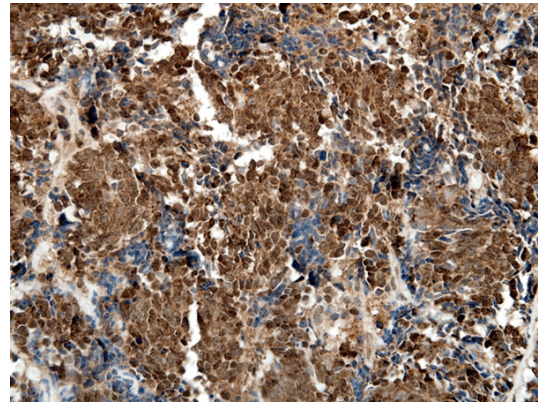


Figure 4. LRP16 expression in small cell lung carcinomas (SCLCs). LRP16 was stained positive mainly in the nucleus of cancer cells. (LRP16 \times 400).

Nuclear factor κ B (NF- κ B)-mediated pathways have been widely implicated in cell survival, development and tumor progression. Recent evidence also indicates that LRP16 is not only a crucial regulator for NF- κ B activation, but also suggest that LRP16 may be an important contributor to the aberrant activation of NF- κ B in tumors [13]. LRP16 is altered in many kinds of primary or cultured carcinoma in the form of aberrant expression. Alterations of the LRP16 gene are common and have been found in primary tumors and cell lines of breast, stomach, colon and rectum, pancreas, kidney, ovary, endometrium and prostate. However, there is no research in neuroendocrine lung tumors. The role of LRP16 in neuroendocrine lung tumors has not been reported in the literature so far. In this study, we investigate the pathogenetic role of the expression of LRP16 protein in

neuroendocrine lung tumors. It is hoped that this study will give information on the pathogenesis of the neuroendocrine lung tumors.

Materials and methods

Biopsy specimens

Paraffin embedded sections of ninety patients (71 men, 19 women) with histologically confirmed neuroendocrine lung tumors were obtained from the Department of Pathology, Chinese People's Liberation Army (PLA) General Hospital (Beijing, China) from 2003 to 2013. The neuroendocrine lung tumors were classified as typical carcinoid (TC) tumors (n = 14) and atypical carcinoid tumors (ATs) (n = 20), whereas large cell neuroendocrine carcinomas (LCNECs) (n = 18) and small cell lung carcino-

LRP16 in neuroendocrine lung tumors

Table 1. The relationship between expression of LRP16 and clinico-pathological features

	LRP16+ neuroendocrine tumors	LRP16- neuroendocrine tumors	<i>P</i>
Age			
< 60	24	20	0.612
≥ 60	26	20	
Sex			
Female	7	12	0.666
Male	43	28	
Tumor size (cm)			
< 5	29	23	0.962
≥ 5	21	17	
Tumor type*			
TC	3	11	0.001
ATs	8	12	
LCNECs	12	6	
SCLCs	27	11	
Tumor stage			
I	5	14	0.008
II	14	10	
III	28	15	
IV	3	1	
Metastases			
Yes	45	26	0.004
No	5	14	
Survival			
Median (months)	20	42	0.0001

*TC: typical carcinoid tumors; ATs: atypical carcinoid tumors; LCNECs: large cell neuroendocrine carcinomas; SCLCs: small cell lung carcinomas.

mas (SCLCs) (n = 38) types according to the World Health Organization recommendations. Ethical approval for this study was not required by our institution as the experiments carried out did not relate to patient's privacy, impairment or treatment.

Immunohistochemistry

A formalin-fixed, paraffin-embedded block was selected from each patient with neuroendocrine lung tumors for immunohistochemical study. Paraffin sections 4 µm thick were cut, dewaxed in xylene and rehydrated in a graded ethanol series. The sections were immersed in 3% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity and rinsed in running water. They were then immersed in boiling 0.01 M citrate buffer (pH 6.0) in a pressure cooker. The pressure cooker

was sealed and brought to full pressure. The heating time was 2 min. After that, the pressure cooker was de-pressured and cooled under running water. The lid was then removed, and the hot buffer was flushed out with cold water from a running tap. The cooled sections were washed twice in phosphate buffered saline (PBS) before immunocytochemical staining. The primary antibodies were then added to the sections. The monoclonal rabbit antibody against human LRP16 protein was diluted 1:100 for 1 h. After exposure to primary antibody overnight, the sections were allowed to react with the polyperoxidase-anti-mouse/rabbit IgG for 20 min using the standard PV-6000 Polymer Detection System (Zymed). This is a non-biotin detection system; thus, non-specific staining due to endogenous biotin was avoided. The

sections were then washed in water, counterstained with Mayer's haematoxylin for 1 min at room temperature, dehydrated, cleaned and mounted. Paraffin blocks of human breast invasive ductal carcinoma tissues were used as the positive controls. Negative controls were sections treated the same as above but with omission of the primary antibody and replaced by 0.01M PBS.

Results

LRP16 protein was positive in 55.6% (50/90) of the neuroendocrine lung tumors by immunohistochemistry (**Figures 1-4**). The LRP16 protein was noted mainly in the cytoplasm and nucleus of neuroendocrine lung tumor cells (**Figures 1, 2**). There was a trend that LRP16 protein was mainly located in nucleus of cancer cells of high grade neuroendocrine lung tumors (**Figures 3,**

LRP16 in neuroendocrine lung tumors

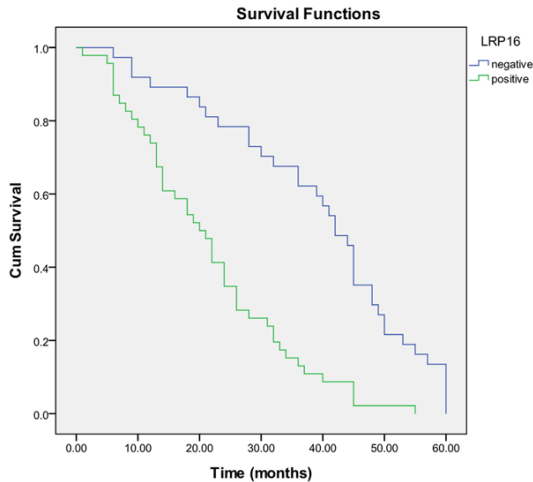


Figure 5. Kaplan-Meier survival analysis by LRP16 status (n = 90). The y-axis represents the percentage of patients; the x-axis, their survival in months. The green line represents LRP16-positive patients with a trend of worse survival than the blue line representing LRP16-negative patients (Log rank = 25.799; P = 0.0001). Overall median survival (OS) time was 20.0 months for the LRP16- positive group and 42.0 months for the LRP16-negative group.

4). Adjacent normal or inflammatory bronchial and alveolar epithelial cells were negative for LRP16.

Relationships between LRP16 expression and histological grade, clinical stage and prognosis

LRP16 was positive in 50/90 (55.6%) cases of neuroendocrine lung tumors, in which, TC was 3 (21.4%), AC 8 (40.0%), LCNEC 12 (66.7%) and SCLC 27 (71.1%). There was a significant difference among the tumor types (P = 0.001, **Table 1**). In group of TNM stage, LRP16 was positive in 5/19 (26.3%) of stage I, 14/24 (58.3%) of stage II, 28/43 (65.1%) of stage III and 3/4 (75.0%) of stage IV, respectively. There was a significant difference among the TNM stages (P = 0.008, **Table 1**). In group of metastases, LRP16 was positive in 45/71 (63.4%) of tumors with metastasis and in 5/19 (20.8%) of those without. There was also a significant difference between tumors with and without metastasis (P = 0.004, **Table 1**). No significant difference in expression of LRP16 protein was found in groups of sex, age and tumor size (**Table 1**). Follow-up data showed that there was a significant difference in overall median survival time between the tumors with LRP16 positive expression (20.0 months) and those with negative expression (42.0 months) (Log rank =

25.799; P = 0.0001) (**Figure 5**). In the result of multivariate analysis by Cox Regression, LRP16 expression was an independent prognostic factor (P = 0.001).

Discussion

Expression of LRP16 has been found in different tissues in varying degree, including ovary, testicle, prostate, small intestine, spleen, thymus and stomach [7, 8, 11]. Some studies demonstrated that LRP16 gene was overexpressed as an ER α or AR coactivator and played an important role in the carcinogenesis and progression of hormone-dependent cancer in breast, endometrial and prostate [10, 11]. In cancers of hormone-independent, overexpression of LRP16 was also detected in gastric and colorectal adenocarcinomas, compared with their matched normal tissues [14, 15]. Recently it has been found LRP16 is as a crucial regulator for NF- κ B activation indicating that LRP16 may be an important contributor to the aberrant activation of NF- κ B in tumors [13]. The present study investigated the expression of LRP16 protein in neuroendocrine lung tumors by immunohistochemistry and the results showed that LRP16 was noted mainly in the cytoplasm and nucleus of tumor cells. LRP16 protein was overexpressed in 55.6% of the neuroendocrine lung tumors samples. Thus, LRP16 can be used an adjuvant marker to identify tumor cells in cases when the diagnosis of neuroendocrine lung tumors is in doubt. Although the detailed molecular mechanism involved in this phenomenon or process is unclear, the present study has potential clinical benefits. In this group of 90 neuroendocrine lung tumors, our results indicated that the LRP16 expression was corresponding to the histological type (P = 0.001), the clinical TNM stage (P = 0.008) and tumor metastasis (P = 0.004). It is suggested that LRP16 may play an important role in the evolution and development of neuroendocrine lung tumors. Follow-up data showed that there was a significant difference in overall median survival time between the tumors with LRP16 positive expression (20.0 months) and those with negative expression (42.0 months) (Log rank = 25.799; P = 0.0001) (**Figure 3**). In the result of multivariate analysis by Cox Regression, PHLDA1 expression was an independent prognostic factor (P = 0.001). The expression of Leukemia-related protein 16 (LRP16) could be detected by immunohisto-

chemistry and it might be a useful molecular marker to predict the prognosis of neuroendocrine lung tumors.

Leukemia-related protein 16 (LRP16) was originally isolated from lymphocytes in order to identify a leukemia relapse-related gene, but there was no difference in patients with acute myeloid leukemia (AML) between the primarily diagnosed and the relapsed tumors [6], suggesting its function might be involved in carcinogenesis of tumors in hematopoietic and lymphoid tissues. A central role for Nuclear Factor- κ B (NF- κ B) in the pathogenesis of neuroendocrine lung tumors has been established and it is now clear that NF- κ B controls a signaling network in neuroendocrine lung tumor cells that may stimulate tumor cell proliferation and confers resistance to apoptosis [16-20]. Our results in this study may imply LRP16 is involved in pathogenesis, or occurrence of neuroendocrine lung tumors. Although the mechanism is not clear to date, it is suggested that LRP16 may stimulate proliferation of neuroendocrine tumor cells by NF- κ B pathway [13]. However, it still needs further basic research evidence.

Conclusions

Our study has suggested that expression of the LRP16 protein could be considered as a potential marker for diagnosis and prognosis of the neuroendocrine lung tumors.

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Disclosure of conflict of interest

None.

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LRP16 in neuroendocrine lung tumors

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