

Evaluation of ALK, EGFR and PD-L1 Mutations in Pulmonary Carcinomas through Immunohistochemistry.

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Abstract

Introduction: Lung cancer, with about 2.2 million new cases and 1.8 million deaths, is the second most commonly diagnosed cancer and the leading cause of cancer death in 2020. Immunohistochemistry (IHC) now is used not only to diagnose and classify lung tumors into subtypes, but also to determine the eligibility of patients for different molecular-targeted therapies.

Objectives: Study of ALK, EGFR and PD-L1 mutations in Pulmonary Carcinomas through immunohistochemistry examinations to help determine the prognosis and cases that may benefit from target therapy. Detection of possible links between ALK, EGFR, PD-L1 and other variables such as age, sex, histological entity and degree of tumor differentiation.

Materials and Methodology: The study is retrospective and includes 266 patients diagnosed with lung cancer who underwent biopsy at the American Hospital in the period 2016-2020. Tissues obtained were subjected to IHC examination using antibodies against factors EGFR, ALK, PD-L1, etc.

Results: The study showed that out of 266 patients, 24% of lung cancer cases are females and 76% are males. The average age was 61.8 years. No statistically significant relationship was found between ALK, PD-L1 and EGFR with variables such as age, gender and degree of differentiation of adenocarcinomas. No significant link was found between ALK and PD-L1 and the histological entity, but a significant link was found between EGFR and histological type of pulmonary carcinomas.

Conclusions: Lung cancer is one of the most common cancers, found mainly in men, but also in women. Nowadays, IHC helps not only to diagnose lung cancer, but also to determine patients who can respond to target therapy and their prognosis. Therefore, the use of IHC to detect ALK, EGFR, PD-L1 mutations and their links to patient characteristics is becoming increasingly necessary.

Keywords: Lung cancer, Immunohistochemistry (IHC), ALK, PD-L1, EGFR

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Introduction

Lung cancer, with about 2.2 million new cases and 1.8 million deaths, is the second most commonly diagnosed cancer and the leading cause of cancer death in 2020. [1, 2]

IHC now is used not only to diagnose and classify lung tumors into subtypes, but also to determine the eligibility of patients for different molecular-targeted therapies. Anaplastic lymphoma kinase (ALK) is a transmembrane protein, member of the insulin receptor tyrosine kinase family (RTK).

ALK rearrangements occur in 3–7% of NSCLC (Non-Small Cell Lung Cancer) with EML4 (echinoderm microtubule-associated protein-like 4) as the most common fusion partner. [3, 4, 5]

They are more common in young people, patients who have never smoked or light smokers, and patients with adenocarcinoma. ALK inhibitors available for the treatment of ALK-rearranged lung tumors, include crizotinib (first generation ALK inhibitors), ceritinib and alectinib (Second-generation ALK inhibitors in the post-crizotinib setting). [6, 7] Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor which belongs to the HER family. It has an extracellular ligand binding domain, a transmembrane domain, and an intracellular domain (tyrosine kinase). The two most common mutations that account for greater than 85% of all EGFR gene mutations are in-frame deletions in exon 19 (LREA deletions) and substitution in exon 21 (L858R). [8, 9]

EGFR mutations predict the benefits of anti-EGFR therapies, especially tyrosine kinase inhibitors (TKIs). Erlotinib and gefitinib are orally active, selective EGFR-TKIs that produce objective response rates in about 10% of advanced non-small cell lung cancer (NSCLC). [10, 11]

Programmed death-ligand 1 (PD-L1) is a type 1 transmembrane protein that belongs to the B7 ligands family and may be expressed both on hematopoietic cells (dendritic cells, macrophages, mast cells, T-cells and B-lymphocytes) and nonhematopoietic cells (endothelial, epithelial and tumor cells). [12, 13]

PD-L1 expression is used as a biomarker that predicts which patients are most likely to respond to anti-PD-1/PD-L1 therapies. Some of these therapies which are approved in treatment of lung cancer are pembrolizumab (anti-PD-1), Nivolumab (anti-PD-1) and atezolizumab (anti-PD-L1). [14, 15]

Materials and Methodology

1. Immunohistochemical analysis

We examined paraffin-embedded tissue sections using the following primary antibodies: TTF-1, CK-7, CK-20, Ki67, etc. According to the above indicators and other immunohistochemical markers, we classified lung cancer into adenocarcinoma, squamous carcinoma, adenosquamous carcinoma, small cell carcinomas (SCLC), etc. ALK, EGFR, PD-L1 mutation analysis were made following guidelines of International Association for the Study of Lung Cancer (IASLC). [16, 17, 18]

ALK positivity was confirmed using Ventana IHC, which is based on a specific monoclonal antibody (D5F3) and was combined with a signal amplification system. ALK positivity was defined as strong granular cytoplasmic staining (any percentage of positive tumor cells) in the tumor cells; otherwise, the sample was deemed ALK negative. EGFR mutation analysis was made using the following major antibodies: DEL specific monoclonal antibody (pre-diluted, clone SP111, Ventana) specific monoclonal antibody L858R (pre-diluted, SP125 clone, Ventana) and the tEGFR antibody (1: 100, clone SP9, Spring Bioscience, Pleasanton, CA). EGFR positivity was based on membrane

and / or cytoplasmic staining, as follows: 0, without staining or pale staining in <10% of tumor cells; 1+, weak staining in $\geq 10\%$ of tumor cells; 2+, moderate staining in $\geq 10\%$ of tumor cells; 3+, strong staining in $\geq 10\%$ of tumor cells. For PD-L1 mutation analysis PharmDx PD-L1 IHC 22C3 was used. A minimum number of 100 tumor cells are required to consider a valid example for its evaluation in formalin-fixed paraffin-hardened tissues (FFPE) using the mouse 22C3 monoclonal clone. PD-L1 was considered positive when a membrane staining (partial or complete) of $\geq 1\%$ tumor cells was seen.

2. Statistical analysis

Our study is retrospective type and includes 266 patients diagnosed with lung cancer that underwent biopsy at the American Hospital in the period 2016-2020. The obtained data are presented in the form of graphs and tables. Statistical analysis of data is performed in the statistical program Chi-Square and Crosstab. Significance is denoted by P. Significant in our study was considered value of $P < 0.05$, while value of $P > 0.05$ was considered not significant.

Results

The study involves 266 patients of whom 203 (76.3%) are male and 63 (23.7%) females. Of the 266 patients surveyed, only in 201 of them age is known. Referring to these 201 patients it resulted that 5% of them were ≤ 40 years old, 33% were between 41 and 60 years old and 62% were over 60 years old. The average age was 61.85 with a standard deviation (Sd) = 10.145.

Age	No. of cases	% of cases
≤ 40 years old	10	5
41-60 years old	67	33
>60 years old	124	62
Total	201	100

Table 1: Age of the participating sample.

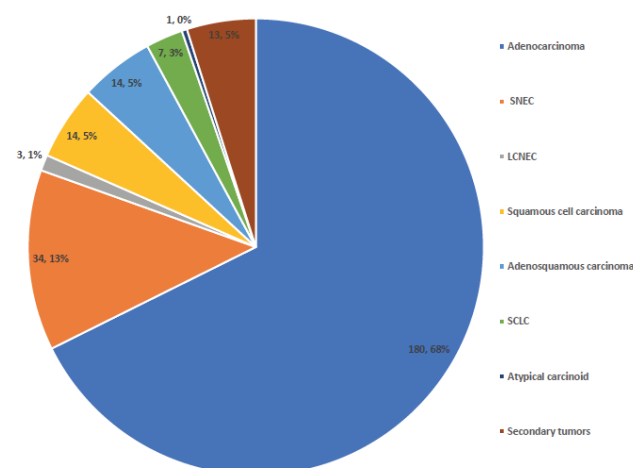


Table 2: Frequency of histological type

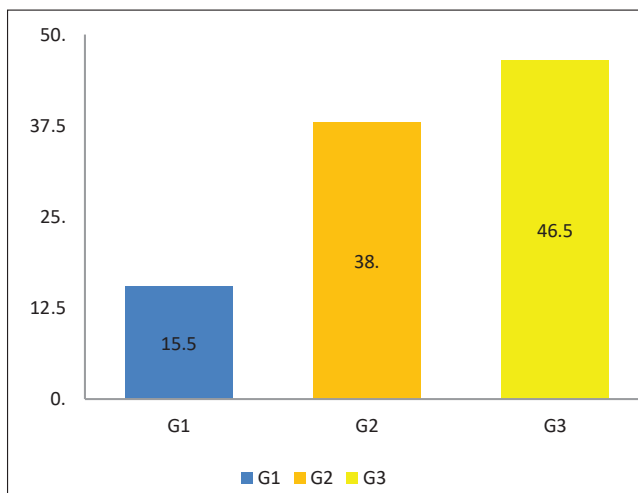


Table3: Grades (%) of adenocarcinoma

From the data obtained according to the histological type of carcinomas, it resulted that out of 266 patients 67.7% were adenocarcinomas, 12.7% were small cell neuroendocrine carcinomas, 1.1% were large cell neuroendocrine carcinomas, 5.3% were squamous cell carcinomas, 5.3% are adenosquamous carcinomas, 2.6% were small cell carcinomas (SCLC), 0.4% were atypical carcinoids and 4.9% were secondary tumors. From the 180 patients in our study diagnosed with Adenocarcinoma, only 155 of them had the grade of differentiation confirmed. It turned out that in these 155 patients, 15.5% were Grade 1, 38% were Grade 2 and 46.5% were Grade 3

Frequency according to ALK: In 178 cases studied 6 patients (3.4%) are positive and 172 patients (96.6%) are negative. Frequency according to EGFR: In 266 cases studied 50 patients (18.8%) are positive and 216 patients (81.2%) are negative. Frequency according to PD-L1: In 112 cases studied 39 patients (34.8%) are positive and 73 patients (65.2%) are negative.

Limitations of the study

Our study is retrospective and does not follow the dynamics of cases, to see the prognosis of patients and to extract data on the survival of patients according to the stage of the disease at the time of diagnosis and benefits from the treatment scheme chosen. Data were lacking in some patients. There were also cases when immunohistochemistry examinations for ALK and PDL were missing due to the absence of Antibodies. Our study includes a small number of cases and we must consider that the Chi-square test is very sensitive to the size of sample. Thus, in a small sample we may not have detected a significant relationship even if it actually exists.

Discussion

The results of our study showed that ALK is not related to gender, but is related to age. Positive cases belonged mainly to middle age, while in old age there were far fewer positive cases. Also, in our study the only histological type, ALK positive, were Adenocarcinomas. No significant relationship was found between ALK and the degree of differentiation of adenocarcinomas. In all three degrees of adenocarcinomas predominated a negative ALK. These data are consistent with the existing literature. The study failed to find statistically significant links between EGFR and gender and age, with EGFR negativity predominating in both variables. A statistical correlation was found between EGFR and histological type where positive cases with adenocarcinoma and adenosquamous carcinoma predominate. No significant association was found between EGFR and adenocarcinoma grade, but it turned out that grade II and III had more EGFR positive cases, unlike another study done in California where EGFR positive resulted more in first and second grade [19]. This incompatibility can be explained by the small number of patients with grade I adenocarcinoma included in our study, with different population characteristics or differences in technique used. Our study found no significant association between PD-L1 and gender, with both male and female predominant negative cases. PD-L1 had no significant relationship with age in our study, but it turned out that positive cases predominated in the age group over 60 years. Histological types were mostly negative. No significant association was found between PD-L1 and the degree of Adenocarcinoma. It should be taken in consideration that in addition to the small number of patients included in the study, knowledge about the expression of PD-L1 in lung cancer is still deficient, so further studies should be performed to determine whether there is a link between PD-L1 and these variables.[20]

Conclusions

Lung cancer is one of the most common cancers, found mainly in men, but also in women. Nowadays, IHC helps not only to diagnose lung cancer, but also to determine patients who can respond to target therapy and their prognosis. Therefore, the use of IHC to detect ALK, EGFR, PDL-1 mutations and their links to patient characteristics is becoming increasingly necessary.

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