Pathology – Research and Practice 213 (2017) 502–508

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Immunocytochemical expression of a panel of markers in pleural effusions from patients with primary lung adenocarcinoma

Eleftheria Chaini[a], Anna-Maria Athanassiadou[c], Antonis Vassias[b], Angelos Tsipis[c,*], Maria Gonidi[d], Kyriakos D. Hainis[a], Andrianni Charpidou[b], Pauline Athanassiadou[c]

[a] Pulmonary Department, Corfu General Hospital, Greece
[b] Oncology Unit, 3rd Dpt. of Internal Medicine, "Sotiria" General Hospital, Medical School, National & Kapodistrian University of Athens, Greece
[c] 1st Pathology Laboratory, Cytology Unit, Medical School, National & Kapodistrian University of Athens, Greece
[d] Cytology Laboratory, Corfu General Hospital, Greece

ARTICLE INFO

Article history:
Received 18 July 2016
Received in revised form 2 February 2017
Accepted 3 February 2017

Keywords:
Pleural effusions
Immunocytochemistry
Lung adenocarcinoma

ABSTRACT

Introduction: This study's principal objective was to evaluate the critical role of the application of immunocytochemistry to a novel panel of diagnostic markers for the accurate detection of the source of malignancies in pleural effusions of lung adenocarcinoma.
Materials and methods: In 40 effusion smears from lung adenocarcinoma, the expression of the E-cadherin, a-catenin, Thyroid Transcription Factor (TTF-1), Epidermal Growth Factor Receptor (EGFR), p53, caspase 9 and 3, Bax and Bcl-2 was examined by immunocytochemistry.
Results: All cases showed positive immunoreactivity of tumour cells to caspase 3 (42,5%), caspase 9 (40%), Bcl-2 (30%), Bax (40%), p53 (55%), E-cadherin (82,5%), a-catenin (80%), TTF-1 (87,5%) and EGFR (62,5%). The Pearson's x^2 analysis demonstrated a highly significant correlation to each of the other marker when analysed separately. Caspase 3 expression was correlated significantly with caspase 9 ($p < 0.0001$), Bax ($p = 0.002$), Bcl-2($p = 0.014$) and p53 ($p = 0.011$). Caspase 9 was correlated with Bax ($p = 0.005$) and p53 ($p = 0.047$), p53 correlated with E-cadherin ($p = 0.011$), a-catenin($p = 0.011$), EGFR ($p < 0.0001$) and Bax ($p = 0.032$). Correlation was also observed between Bcl-2 and Bax expression ($p < 0.0001$), E-cadherin and a-catenin expression ($p < 0.0001$) and a-catenin and TTF-1 expression ($p = 0.002$).
Conclusions: The use of a panel of biomarkers can be of great value in determining effusion immunoprofile in patients with lung adenocarcinoma for clinical application.

1. Introduction

The primary lung carcinoma leading to pleural effusions is adenocarcinoma because of the peripheral location. It is often very difficult to differentiate a reactive mesothelial cell with atypia from a malignant adenocarcinoma cell because of significant morphologic overlap [1,2].

Immunocytochemistry with the use of biological markers can help in distinguishing between reactive and neoplastic mesothelial cells and cells of metastatic neoplasm and understanding the underlying oncogenic mechanisms in advanced stages of the disease [3,4].

Thyroid Transcription Factor (TTF-1) is a tissue-specific homeodomain- containing transcription factor, which plays an important role in the early differentiation and morphogenesis in the developing lung and thyroid gland. The expression of TTF-1 has been observed in 71%–76% of adenocarcinomas and 81%–92% of small cell carcinomas [5,6].

Epidermal Growth Factor Receptor (EGFR) is a transmembrane protein with tyrosine kinase activity. Over expression and/or mutations of EGFR lead to uncontrolled cell division and have been implicated in the development of non-small cell lung cancer (NSCLC) and especially adenocarcinoma [7–9].

Abbreviations: EGFR, epidermal growth factor receptor; IAPs, inhibitor of apoptosis proteins; NSCLC, non-small cell lung cancer; TTF-1, thyroid transcription factor; XIAP, X-linked inhibitor of apoptosis.

* Corresponding author at: 1st Pathology Laboratory, Cytology Unit, Medical School, National & Kapodistrian University of Athens, paparseni 70 tk 11524, Greece.
E-mail addresses: elhaini@otenet.gr (E. Chaini), annamariaathens@aim.com (A.-M. Athanassiadou), a_bassias@yahoo.com (A. Vassias), angelostsipis@gmail.com (A. Tsipis), mgonidi@yahoo.gr (M. Gonidi), hainisdk@otenet.gr (K.D. Hainis), dcharpidou@yahoo.gr (A. Charpidou), linaathens@aol.com (P. Athanassiadou).

http://dx.doi.org/10.1016/j.prp.2017.02.002
0344-0338/

Apoptotic caspases 3 and 9 members of a family of proteases called caspases (cysteine-dependent aspartate-directed proteases) play a central role in cell apoptosis [10]. The action of caspases is suppressed in NSCLC tumours, which results in decreased cell apoptosis, contributing to tumour development and resistance to therapy [11]. The mitochondrial pathway of caspases-dependent apoptosis is under the regulation of proteins of the Bcl-2 family, such as Bax and Bcl-2. Bax, a proapoptotic protein, induces the release of cytochrome c from the mitochondria, leading to the activation of caspases and execution of apoptosis. In contrast, Bcl-2 competes the action of Bax by blocking the release of cytochrome *c*, inhibiting apoptosis [12]. The function of Bax and Bcl-2 proteins is regulated by the tumour suppressor protein p53, which participates in the regulation of cell cycle, DNA repair, apoptosis, angiogenesis and ageing. Increased expression of protein p53 is often present in cancer, including NSCLC [13,14].

E-Cadherin is a transmembrane protein and plays an important role in the formation and architectural maintenance of epithelial tissues, as well as in signalling pathways. The intracellular domain binds to the cytoskeleton of actin through a-catenin, which interacts with E-cadherin via a-catenin [15–17].

This study's aim was to determine which of the above oncogenic mechanism acts in the progression of lung adenocarcinoma. Therefore, in considering the critical role of immunocytochemistry, the application of a novel panel of diagnostic markers for accurate detection of the source of malignancies in effusions was used.

2. Material and methods

The material consisted of malignant pleural effusions from 40 patients with newly diagnosed lung adenocarcinoma, who had not undergone therapy. Thirty-four patients were men, and six were women. The mean age of patients was 66 years, ranging from 53 to 78 years. Thirty-six patients were smokers, and four had never smoked. All pleural effusions were exudates according to Light's criteria [18].

Cytologic effusion smears were fixed immediately in 95% alcohol, subsequently stained by the standard Papanicolaou method and routinely processed for microscopic examination. Additionally, air-dried smears were stained by Hemacolor. The cytologic diagnosis was made based on cytomorphologic studies and ancillary studies. Immunocytochemical and/or cytochemical studies were also used for the differentiation between adenocarcinoma cells and atypical mesothelial cells.

2.1. Immunocytochemistry

Specimens were tested for the expression of 10 primary antibodies by using Bond Polymer Refine Detection Kit (Leica Biosystems, Newcastle, U.K.). The detection system avoids the use of streptavidin and biotin and therefore eliminates nonspecific staining as a result of endogenous biotin. All steps were performed on the Leica Band III automated system (Leica Microsystems)

Smears were rehydrated and treated with hydrogen peroxide block for 10 min, then washed and incubated with the primary antibodies for 30 min. The antibodies used in this study are summarized in Table 1. Subsequently, specimens were incubated with polymer reagent for 10 min and finally were treated with a 3, 3-diaminobenzidine chromogenic substrate for 10 min, counterstained with Mayer's haematoxylin, dehydrated and coverslipped. Known positive controls were also stained simultaneously. Negative controls were stained by omitting the primary antibody incubation.

Immunocytochemical stains were evaluated and scored independently and in a blind manner by two investigators. Five hundred tumour cells were counted in randomly selected fields in each case, and the number of cells with positive staining was divided by the total number of cells counted. The number of immunopositive cells was semiquantitavely estimated negative (−) or positive (+). For p53 and TTF-1 immunoreactivity cells with distinct brown staining confined to the nuclei were regarded as immunoreactive. P53 and TTF-1 were classified as negative when <10% of the nuclei were reactive and positive when >10% of the nuclei were reactive. EGFR, Caspase 3 and 9, Bcl-2, Bax, E-Cadherin and a-catenin-stained slides were assessed for cytoplasmic and membrane reactivity. Expression <10% was considered negative, and >10% was recorded as positive (mild, moderate and strong reactivity) (Figs. 1–9) .

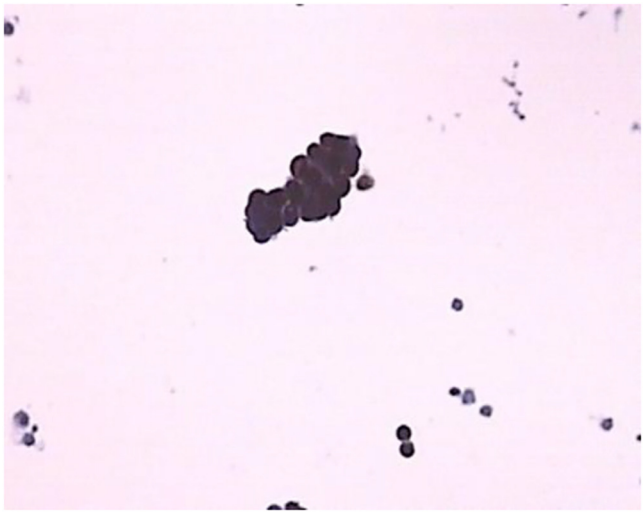

Fig. 1. Pleural effusion smears. A cluster of adenocarcinoma cells with positive nuclear reaction for p53 (x 400).

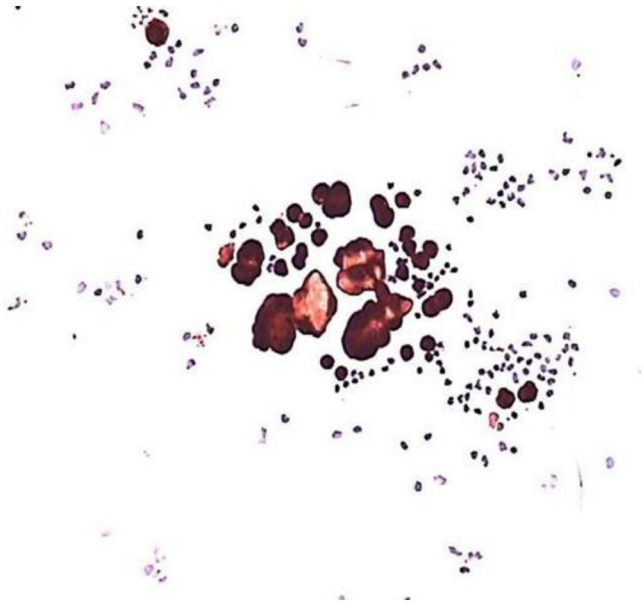

Fig. 2. Pleural effusion smears. Clusters of adenocarcinomas cells with positive nuclear immunoreactivity for TTF-1 (X 400).

2.2. Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics v20. The level of statistical significance was set at 5%. The relationship between examined markers (caspase 3, caspase 9, Bcl-2, Bax, p53,