Lung Cancer (2007) **56**, 35—42

available at www.sciencedirect.com

ScienceDirect

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

Genetic relationship among atypical adenomatous hyperplasia, bronchioloalveolar carcinoma and adenocarcinoma of the lung

Luca Morandi[a], **Sofia Asioli**[b], **Alberto Cavazza**[c], **Annalisa Pession**[a], **Stefania Damiani**[a,*]

[a] *Department of Oncology, Section of Anatomic Pathology ''M. Malpighi'', University of Bologna, Bellaria Hospital, Via Altura no. 3, 40139 Bologna, Italy*
[b] *Department of Biomedical Sciences and Human Oncology, University of Turin, Italy*
[c] *Service of Anatomic Pathology, Santa Maria Nuova Hospital, Reggio Emilia, Italy*

Received 27 July 2006; received in revised form 2 November 2006; accepted 27 November 2006

KEYWORDS
Atypical adenomatous hyperplasia;
Mitochondrial DNA;
LOH;
Lung cancer;
Genetic alterations

Summary Atypical adenomatous hyperplasia (AAH) has been recently defined by WHO as a small lesion, not exceeding 5 mm in major axis, composed of slightly enlarged alveolar septa lined by pneumocytes with plump, atypical nuclei. AAH is frequently found in tissue surrounding lung adenocarcinoma and is considered a precursor of this subtype of lung cancer by many Authors. However, the genetic relationship between adenocarcinoma and the associated foci of AAH is not well defined. In particular, it is not clear whether multiple foci of AAH and of adenocarcinoma in the same patients are clonally related to each other or represent independent neoplastic foci.

To clarify if AAH and the associated cancer are clonally related, we evaluated the genetic distance between these two lesions in 16 patients, using direct sequencing of mitochondrial DNA (D-loop region). Furthermore, LOH analysis for 7 microsatellites (D3S1478 at 3p21, D3S1300 at 3p14.2, D9S942 at 9p21, D5S346 at 5q21, D17S261 at 17p13.1, D18S46 at 18q21, D19S246 at 19q13.2) was also performed.

Our results indicate that, in at least 9 out of 13 informative cases (69.2%), AAH and the associated cancer were not clonally related as they showed a different mutation pattern in the mitochondrial D-loop region. These findings were also in agreement with the LOH data which showed losses in different loci in at least three cases. On the contrary an identical LOH pattern between BAC and AAH was found in one case. Similar but not identical LOH pattern between AAH and related tumors was found in other three cases.

* Corresponding author. Tel.: +39 051 6225523; fax: +39 051 6225759.
E-mail address: stefania.damiani@ausl.bo.it (S. Damiani).

0169-5002/$ — see front matter
doi:10.1016/j.lungcan.2006.11.022

Therefore, our results suggest that AAH and the associated cancer are genetically independent in agreement with the concept of cancerization field. Less frequently AAH foci could represent an early spread of cells from the main tumor, rather than a precursor lesion.

1. Introduction

Atypical adenomatous hyperplasia (AAH) has been proposed as a possible precursor of adenocarcinoma (AC) of the lung [1—4]. The main histological feature of AAH is a non-invasive spread of atypical epithelial cells in single rows along alveolar walls, within a lesion ''usually less than 5 mm in diameter'', as defined by the World Health Organization (WHO) [5]. The atypical cells may show features of Clara cells, type II pneumocytes, or a columnar cell phenotype (Fig. 1(a and b)). Cells and nuclei are smaller than those of bronchioloalveolar carcinoma (BAC). AAH is most commonly found in lung resected for primary lung cancer, although it has been found, less frequently, in lung resected for benign tumors or metastatic disease. Overall figures for AAH incidence quoted range from 9.3 to 21.4% of cases resected from primary lung cancer, whereas lungs removed for benign or metastatic disease yield AAH in between 4.4 and 9.6% of cases [6,7]. AAH may be present as a multi-focal lesion and coexists most often with BAC and/or AC, however less frequently it may also be associated with squamous cell carcinoma.

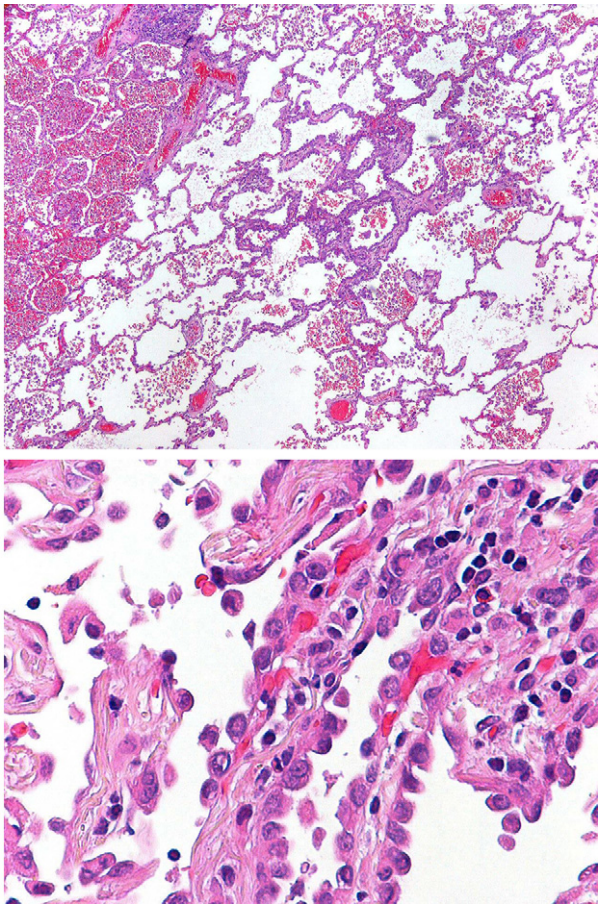

Fig. 1 (a) Atypical alveolar hyperplasia: a focal lesion, not exceeding 5 mm, showing slightly enlarged septa lined by plump pneumocytes. No papillary features or areas of parenchyma distortion are present. H&E ×100. (b) The pneumocytes in AAH have enlarged hyperchromatic nuclei sometimes showing intranuclear inclusions, similar to those seen in bronchioloalveolar carcinoma. H&E ×400.

Studies investigating cell proliferation, p53 protein overexpression, K-ras mutation, clonality and LOH analysis have suggested that AAH might be a putative precancerous lesion of lung adenocarcinoma [6—8]. One study indicated that high-grade AAH might not be only a precursor lesion, but rather an early spread of BAC or AC [9].

In the present study, we tried to evaluate the clonal relationship between AAH and corresponding carcinoma by sequencing the D-loop mitochondrial DNA (mtDNA) [10] and looking for LOH in seven different microsatellite loci.

2. Materials and methods

Twenty samples of AAH associated with fourteen adenocarcinoma or five non-mucinous BAC have been selected from 16 patients (see Table 1). The cases were retrieved from the files of the University of Bologna (patients 1—9) or of Anatomic Pathology of S. Maria Nuova Hospital, Reggio Emilia, Italy (patients 10—16).

Tissues were fixed in 10% buffered formalin and were paraffin embedded as routine. Selected blocks were serially cut and stained with Hematoxylin—Eosin (H&E).

Representative sections of each tumor were reviewed and classified according to WHO [5]. In order to avoid doubtful cases merging with true BAC, AAH foci were selected using stringent criteria, and only lesions not exceeding 5 mm in their major axis were included in the study.

2.1. Microdissection

Pertinent lesions were microdissected (Fig. 2) using the laser assisted SL μcut microtest (distributed by Nikon Firenze, Italy, http://www.mmi-micro.com). Five-μm thick sections were obtained and unstained sections were deparaffinated with Bio-Clear (Bio-Optica, Milano Italy), rinsed in ethanol 100—95% and stained with H&E. In each same patient normal lung epithelial tissue located far away the neoplastic lesions and patient normal lung epithelial tissue, located far away the neoplastic lesions, and lymphocytes from lymphnodes, were dissected for use as control reference DNA. Samples were similar in magnitude and ranged from 500 to 1000 cells in each case, as indicated by Sieben et al. [11].

The microdissected cells were placed in the SL μcut Transfer Film (Nikon, Firenze, Italy), and DNA was extracted as previously described [10]. An extraction control in which