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Phenotype and genotype associations of lung carcinoma with atypical adenomatoid hyperplasia, squamous cell dysplasia, and chromosome alterations in non-neoplastic bronchial mucosa

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Abstract

The frequency of preneoplastic lesions of the lung and bronchial mucosa as well as potential genotype alterations in spatial relationship to pulmonary malignancies still need intensive investigations in order to understand the occurrence and manifestation of lung cancer in detail. To investigate the contemporary manifestation of lung cancer precursor lesions, peripheral (non-neoplastic) lung parenchyma and bronchial mucosa of operated lung carcinomas were analyzed at distinct distances (1, 2, 3, and 4 cm) from the tumor boundary for pre-neoplastic lesions – atypical adenomatoid hyperplasia (AAH) and squamous cell dysplasia (SCD), in 150 surgical specimens. Short-term tissue cultures of additional 55 primary and secondary lung tumors and their surrounding non-neoplastic bronchial mucosa were performed at the same distances in order to search for chromosome alterations, *i.e.* genotype aberrations. In phenotype observations, atypical adenomatoid hyperplasia was noted in 19/150 (13%) cases, and squamous cell dysplasia in 46/150 (31%) cases. The degree of cellular atypia decreased with increasing distance from the tumor boundary in both AAH and SCM. AAH was observed more frequently in adenocarcinomas, SCQ more frequently in squamous cell carcinomas. In genotype observations, the average number of abnormal metaphases measured 4.5/10 high power fields (HPF) in primary lung carcinomas, and only 2/10 in metastases. Data indicate that the so-called preneoplastic lesions in the lung are not completely tumor-precursor lesions, but, in addition, induced by the tumor itself.

Keywords: lung, atypical adenomatoid hyperlasia, squamous cell dysplasia, phenotype, genotype.

Introduction

Morphology changes, which precede the occurrence of cancer, are called preneoplasia. These lesions are well known in many organs, such as skin, stomach, urogenital tract, liver, or upper respiratory tract. From the morphology point of view they can be divided into two different groups, namely changes in the cellular sociology with and without adenomatous growth. Characteristic alterations are the structuration into squamous cells and into adenomatoid lesions, or adenomas. Chronic inflammatory effects can induce both lesions, and, in addition, by specific internal and external substances, which induce cancer in mammals and humans [1, 2]. In addition, the lung is an organ, which is most heavily exposed to external potential hazardous carcinogenic substances, especially smoking, asbestos, or radiation [3]. Thus, preneoplastic lesions of the lung have been described in detail by several authors; however, most of the investigations are limited to changes of the conducting airways, *i.e.*, investigations on squamous dysplasia [2, 4, 5]. This was in congruence with the most frequent cell type in the past, *i.e.*, the squamous cell carcinoma [2]. With increasing incidence of adenocarcinomas, especially in women, the interest and search for preneoplastic lesions of the peripheral lung has lead to observations of cellular atypias of pneumocytes and epithelial cells of the bronchioli terminals and respirators.

These lesions can be observed in direct connection to a primary lung cancer, or at various distances, and seldom measure more than 5 mm in maximum diameter. They display either adenomatoid growth patterns still bound to the interalveolar septula, or desquamative features, which remind to some "maturation" into the airspaces [6–8]. In this review article, which is mainly based upon own investigations we will analyze and demonstrate the phenotype and genotype features of air-conducting and air-exchange structures of human lung. We will focus on their spatial and chromosome relations to primary lung cancer, and their similarities in between.

Characteristics of squamous cell dysplasia

Squamous cell dysplasia of bronchial mucosa (SCD) is well known, and a common finding in patients with chronic inflammatory lesions of the conducting air systems, such as heavy smokers, chronic obstructive lung diseases, exposure to silicates, diesel exhaust, or asthma [2, 9]. SCD has been reported to be reversible even at the stage of carcinoma *in situ* [2]. It is characterized by a replacement of the normal cellular sociology of bronchial mucosa, which includes among others basal cells, ciliated cells, or goblet cells, by proliferating squamous cells only.

These squamous cells do not exist in healthy bronchial mucosa. They proliferate from the basal membrane into the inner lumen of the bronchi, and display various degrees of cellular atypia. According to the degree (or loss) of maturation, the thickness of the epithelial bronchial layer, and to their mitotic activity these atypias are graded into mild, moderate, and severe lesions (grade I, II, III). The most advanced lesion in terms of malignancy possesses all characteristics of malignant growth pattern with still intact basal membrane, and is called carcinoma *in situ* [2]. The grading characteristics are listed in Table 1.

Table 1 – ***Criteria for grading SCD, based upon HE staining***

Thickness of epithelial layer (increasing with -> dysplasia, no criterion for carcinoma *in situ*)
Cell size (increasing with -> dysplasia, no criterion for carcinoma *in situ* / severe dysplasia)
Maturation / orientation (decreasing from mild dysplasia -> carcinoma *in situ*
Nuclei (variation of N/C ratio, chromatin nucleoli, mitoses, spatial distribution in epithelial layers, increasing from mild dysplasia -> carcinoma *in situ*)

The thickness of the squamous cell layer increases with increasing grade; however, it is no criterion for a carcinoma *in situ*. The same observation holds true for the cellular size in contrast to the orientation of the cellular growth within the layer. The important diagnosis of a carcinoma *in situ* should be based upon the cellular growth pattern into "all directions", the marked variance of the nuclear/cytoplasm ratio (NIC) and the mitotic figures, which occupy all compartments of the layer. The corresponding scheme illustrates the findings in Figure 1.

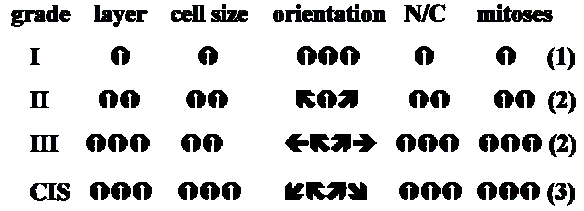

Figure 1 – ***Relationship between grading SCD and the contributing features (arrows indicate the severeness / frequency of alterations)***

Most criteria permit a clear distinction between moderate and severe dysplasia in contrast to the distinction between severe dysplasia versus carcinoma *in situ*. The analysis of growth orientation remains the most specific criterion in order to distinguish between these two advanced entities. An example of such a carcinoma *in situ* is presented in Figure 2. The "whirl-like proliferation" indicating a cellular growth "in all directions" is a characteristic finding in these cases. There is no doubt, that SCD is a "real" preneoplastic lesion; however, the proof still is based upon experimental findings, toxicological observations, and genetic instability [9–11]. In humans, the final proof of this theory remains difficult, although several tasks for confirmation of these considerations exist. One of the tasks is the analysis of the spatial relationship between primary lung cancer and contemporary SCDs; another contributing factor with clinical importance is the analysis of the SCD frequency in one lung, *i.e.*, whether SCD remains a singular lesion or can be considered to be a multiple one. If SCD is indeed a pre-neoplastic lesion, it should be associated with already existent lung carcinomas in terms of cell type and spatial relationship. To our knowledge only one detailed study has been published which analyzed the frequency of SCDs in relation to their distances from the boundary of lung carcinomas [12]. This singular study confirmed an association of SCD with squamous cell carcinomas and a decreasing frequency of this lesion with increasing distance from tumor boundary. A new study confirmed these findings. This detailed analysis revealed a total of 19 SCDs in 9 cases out of 25 cases with squamous cell carcinomas, and a total of 5 SCDs in 4 cases out of 13 cases with adenocarcinomas. SCDs were seen less frequent in pulmonary metastases; however, again as multiple lesions. With the exception of metastases, all SCDs displayed a close spatial association with their distance from the tumor boundary, and were noted most frequently at a 3 cm distance from the tumor boundary [13]. These data demonstrate that SCD is associated with the cell type (squamous cell carcinoma), and with the distance from the tumor boundary. In addition, it is a multiple lesion, a finding of clinical significance. After detection of a SCD the bronchial system should be carefully searched for additional SCDs in order to mark areas with increased risk for lung cancer, and, if possible, their destruction by laser therapy or similar methods. The analysis of the genotype of SCDs needs a remark. Basically, genotype and phenotype are structures, *i.e.*, formations of identical nature, and the distinction into two different terms is somewhat arbitrary. Similar to morphological investigations, the analysis of genes is founded on techniques, which either analyze numerical aberrations in terms of missing or additional chromosomes, genes, or gene compartments, or abnormalities in their function (expression). A simple and robust technique in analyzing chromosomes is the so-called karyotyping with Giemsa (G)-banding performed after short-term tissue culture. This technique permits the detection of chromosomes and genes in dividing cells by chromosome classification. The results in analyzing numerical chromosome properties of bronchial mucosa in relation to the distance from the tumor boundary are presented in Figure 3.

Numerical chromosome aberrations are frequent in primary lung cancer. However, it is striking, that even the non-neoplastic bronchial epithelium presents with such genetic disturbances of cellular growth. Thiberville *et al.* (1995) reported on cumulative gene losses in premalignant lesions of the bronchi, findings that were related to those of Sozzi *et al.* (1992) who found deletions of 17p and p53 mutations in SCD [14, 15]. The most frequent findings were losses of chromosomes in contrast to insertions, which were seen less frequent, although still in quite a high percentage of dividing cells (10–20%).

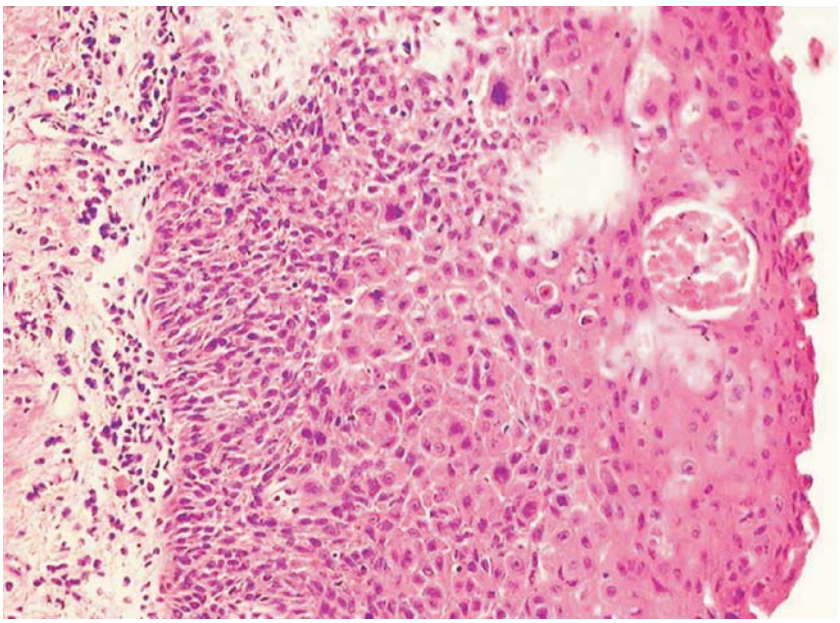

Figure 2 – ***Carcinoma "in situ" of a left main bronchus (HE, ×200). Note the "whirl-like" patterns of the epithelial layer indicating a cellular growth "in all directions"***

Losses of y-chromosome

distance to tumor boundary	number
0 cm	14 70%
1 cm	13 65%
2 cm	13 65%
3 cm	11 55%
4 cm	2 10%

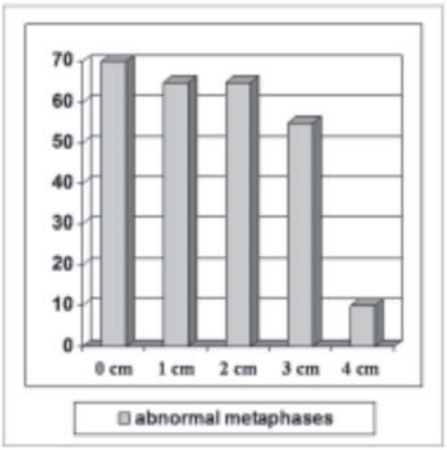

Figure 3 – ***Cases with abnormal metaphases (n = 10, loss of y-chromosome) in non-tumorous bronchial epithelium, associated to the distance from tumor boundary. Note that at a distance of 3 cm from tumor boundary 55% of cases are still present with a loss of the y-chromosome (lung cancer cytogenetics, n = 20, squamous cell carcinoma only)***

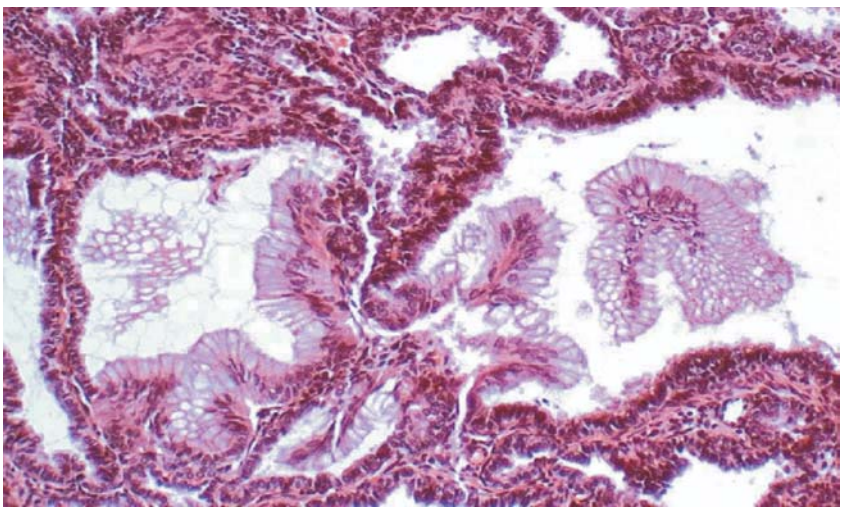

Figure 4 – ***Histomorphological findings in CAM (HE, ×200). Note the similarities between BAC and AAH***

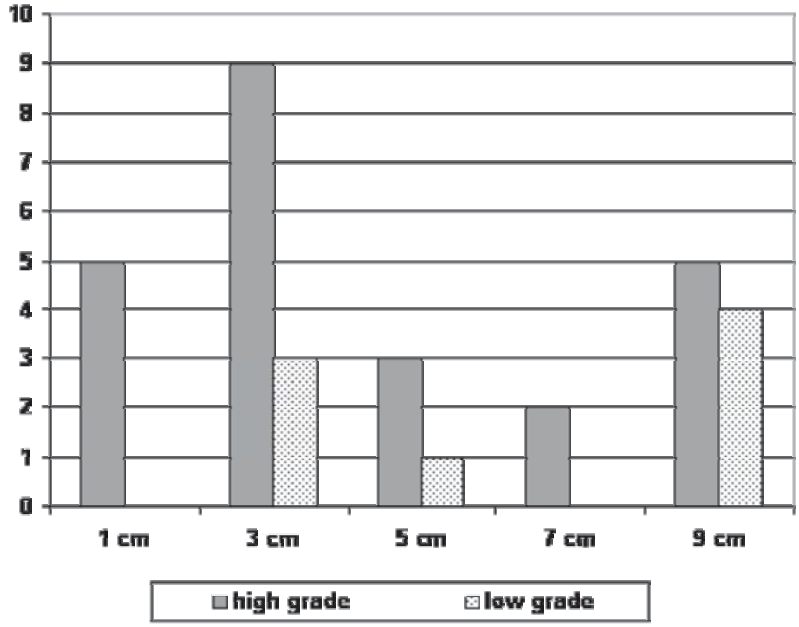

Figure 5 – ***Absolute number of AAHs detected in surgical lung / lobe specimens (n = 150) in relation to their distance from the tumor boundary and their cellular atypia (grade)***

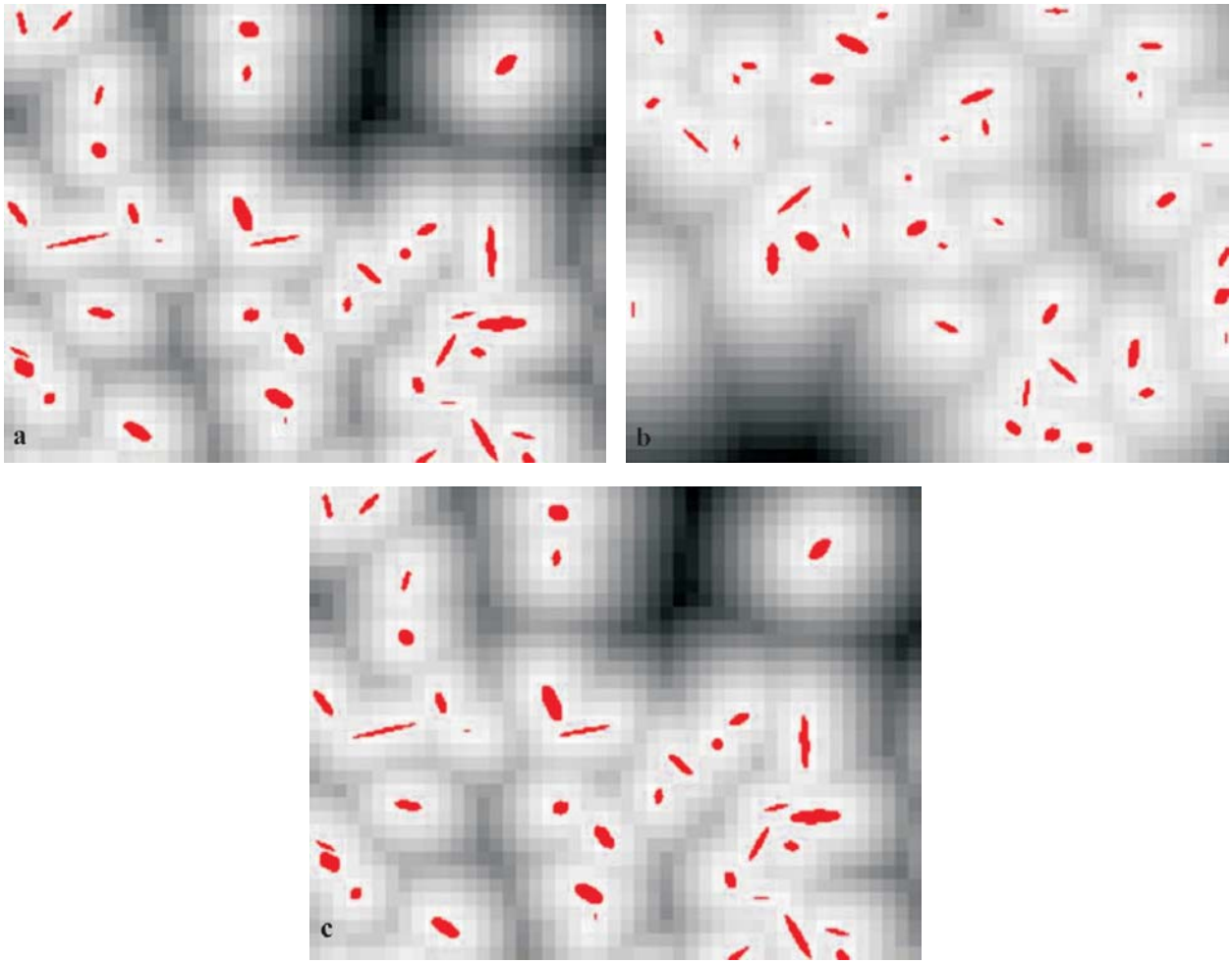

Figure 6 – ***Changes of vascularization in the development of peripheral lung adenocarcinoma (pale areas indicate a good, dark areas a poor blood supply). Note the clustering of vessels in AAH and adenocarcinoma;*** **a)** ***normal lung,*** **b)** ***AAH, and*** **c)** ***adenocarcinoma***

The number of such lesions decreases with increasing distance from the tumor boundary. A closer analysis revealed that metastases present with only few aberrations, and similar findings in non-neoplastic bronchial mucosa. The comparison of losses/insertions of distinct chromosomes in lung cancer tissue with those of non-neoplastic epithelium resulted in distinct chromosome aberrations, which are tumor-related, and those, which are not (Table 2).

Table 2 – ***Losses of chromosomes in non-neoplastic bronchial mucosa in relation to the tumor cell type***

Cell type	Related	Non-related
Squamous	y, 21, 22	19
Large cell	y, 21	15
Adeno	y, 19, 22	15, 21
Metastases	y	x, 21
Others	y	8, 21

Losses of the y chromosome are always tumor-related, in contrast to those of chromosome 15, which are not. Losses of chromosome 19 are tumor related in adenocarcinomas, and tumor independent in squamous cell carcinomas. Those of chromosome 21 are associated in cases of squamous cell carcinomas, and tumor independent in adenocarcinomas. Thus, the detection of SCD with losses of chromosome 21 indicates (co-)existence or high risk of squamous cell carcinoma, those of chromosome 19 favors an adenocarcinoma. The alteration of specific chromosomes in non-neoplastic bronchial mucosa supports the theory that preneoplastic lesions of the conducting airways might not only be induced by external inflammatory or carcinogenic agents, but, in addition by the tumor itself. The morphology of a preneoplastic lesion does not permit the distinction between tumor induction or tumor precursor nature. Chromosome analysis is of support to answer this important theoretical and clinical question.

Characteristics of atypical adenomatous hyperplasia

Atypical adenomatous hyperplasia (AAH) is a less known small lesion of peripheral lung epithelial cells (pneumocytes), measuring about 5 mm in maximum diameter. It can be observed in close connection to primary lung cancer, especially to peripherally located adenocarcinomas. Its incidence is increasing. The reasons are not known [2, 16, 17].

Morphologically is characterized by sheets of proliferating alveolar lining and cuboidal cells with dense nuclear chromatin [2, 8, 18, 19]. The size of the focal lesion in the peripheral lung permits its detection by high-resolution computerized tomography (CT) techniques [20]. Frequently, AAH has striking similarities with well-differentiated adenocarcinomas of the bronchiolo-alveolar type. It possesses well-defined boundaries, slightly thickened inter-alveolar septa, infrequently bronchiolar metaplasia, and is often observed in emphysematous lung [7, 8, 21]. The frequency of these lesions has been reported to range from 5–25% in resected lung specimens [7, 19, 21–23]. Cytometric studies supported by profiling of growth-related markers showed that AAH is often characterized by non-diploid cellular proliferation, which can even, be of monoclonal origin [24, 25].

At present, it is of specific interest to pathologists and epidemiologists as the incidence of peripheral adenocarcinomas of the lung is increasing, and AAH is considered to be closely associated with the development of peripheral adenocarcinomas. Although no detailed study has been reported to demonstrate the association of AAH with the bronchiolo-alveolar subtype of adenocarcinomas (BAC) of the lung, the morphologic similarities between an inborn error lung disease (congenital adenomatoid malformation (CAM), AAH and BAC are striking, as shown in Figure 4. Not knowing the history and age of the patient, the diagnosis of a BAC with contemporary AAH would be unavoidable.

Similar to SCD, AAH is not infrequently a multiple lesion with an overall frequency of about 15% in surgically excised lungs and lobes [7, 21, 26]. It is twice as frequent in adenocarcinomas compared to squamous cell carcinomas, and, in close association with the findings in SCD, it is also associated to its distance from the tumor boundary [13]. High grade AAHs are closer located to the tumor boundary than low grade AAHs as shown in Figure 5. In addition to the AAHs described, Mori *et al.* (2001) defined a peripheral preneoplastic lesion of the lung, which is characterized by agglutinations of goblet cells [8]. The authors called this lesion bronchiolar columnar cell dysplasia.

By use of comparative genomic hybridization (CGH) they found genetic aberrations in 5/6 cases, mainly losses of chromosomes 3, 9, 10, 13, 14, and gains of chromosomes 1, 17, 19, 20 (*i.e.*, chromosome alterations in peripheral lung parenchyma are not only associated to "common" AAH) [11]. In addition, AAH is characterized by specific alterations of carbohydrate binding capacities, expression of galectins, and of calcyclin, a protein of the S100 family [16, 17]. Patients with potentially curative operated lung carcinomas displayed a non-favorable survival if an AAH could be detected in their resection specimens [17].

Irreversible alterations of vascularization form additional characteristics of AAH, which are exemplarily demonstrated in Figure 6. The vessels tend to "agglutinate" and to form clusters of areas with increased and those with decreased blood supply (Figure 6). There are continuous changes in vascularization starting from normal lung, passing AAH, and finally ending up in adenocarcinomas. These include the increase in size and circumference of vessels as well as in volume and circumference fractions.

Conclusions

Preneoplastic lesions of the lung can be divided into those of the air conducting and those of the air-exchange structures – SCD and AAH. Despite their different morphology, both entities posses striking

similarities in terms of multiple lesions, genotype aberrations and spatial association to the distance from the tumor boundary.

The data indicate that SCD and AAH might not only develop in terms of pre-cancerous events with high risk of cell-type associated lung cancer. In addition, there are strong indications, that the cancer itself might be able too to induce a SCD or AAH. This theory is supported by genotype analysis, which results in tumor-associated, and tumor-independent chromosome aberrations, a result, which opens a new insight in the tumor – environment interaction scene.

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