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Accumulation of genomic alterations in 2p16, 9q33.1 and 19p13 in lung tumours of asbestos-exposed patients

Penny Nymark[a,d], *Mervi Aavikko*[a], *Jussi Mäkilä*[a], *Salla Ruosaari*[a], *Tuija Hienonen-Kempas*[a], *Harriet Wikman*[e], *Kaisa Salmenkivi*[d], *Risto Pirinen*[f], *Antti Karjalainen*[b], *Esa Vanhala*[c], *Eeva Kuosma*[b], *Sisko Anttila*[a,d], *Eeva Kettunen*[a,*]

[a]*Health and Work Ability, Finnish Institute of Occupational Health, Topeliuksenkatu 41 aA, 00250 Helsinki, Finland*
[b]*Creating Solutions, Finnish Institute of Occupational Health, Topeliuksenkatu 41 aA, 00250 Helsinki, Finland*
[c]*Work Environment Development, Finnish Institute of Occupational Health, Topeliuksenkatu 41 aA, 00250 Helsinki, Finland*
[d]*Department of Pathology, Haartman Institute and HUSLAB, University of Helsinki and Helsinki University Central Hospital, 00029 HUS, Finland*
[e]*Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Martinistr 52, 20246 Hamburg, Germany*
[f]*Institute of Clinical Medicine, Department of Pathology & Forensic Medicine, University of Kuopio and Kuopio University Hospital, P.O. Box 1627, 70210 Kuopio, Finland*

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ABSTRACT

We have previously demonstrated an association between genomic alterations in 19p13, 2p16, and 9q33.1 and asbestos exposure in patients' lung tumours. This study detected allelic imbalance (AI) in these regions in asbestos-exposed lung cancer (LC) patients' histologically normal pulmonary epithelium. We extended the analyses of tumour tissue to cover a large LC patient cohort and studied DNA copy number alteration (CNA) and AI in 19p13, 2p16, and 9q33.1 for the first time in combination. We found both CNA and AI in ≥2/3 of the regions to be significantly and dose-dependently ($P < 0.001$) associated with pulmonary asbestos fibre count. Twenty percent of the exposed patients' LC showed CNA in ≥2/3 of the regions, whereas none of the non-exposed patients' LC showed CNA in more than one region. AI was evident in 89% of the exposed and in only 26% of the non-exposed patients' LC. The genomic alterations in 19p13, 2p16, and 9q33.1 in compilation identified asbestos-exposed patients' lung tumours better than each of the regions alone.

Abbreviations: LC, lung cancer; AI, allelic imbalance; CNA, copy number alteration; LOH, loss of heterozygosity; FF, fresh frozen; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence *in situ* hybridization; TMA, tissue microarray; BAC, bacterial artificial chromosome; MS, microsatellite; AC, adenocarcinoma of the lung; SCC, squamous cell lung cancer; SCLC, small-cell lung cancer; NSCLC, non-small cell lung cancer; LCLC, large-cell lung cancer; TSG, tumour suppressor gene; *DBC1*, deleted in bladder cancer 1.

* *Corresponding author*. Tel.: +358 30 4742211; fax: +358 30 4742021.
E-mail addresses: penny.nymark@ttl.fi (P. Nymark), mervi.aavikko@helsinki.fi (M. Aavikko), jussma@utu.fi (J. Mäkilä), salla.ruosaari@gmail.com (S. Ruosaari), tuija.hienonen-kempas@roche.com (T. Hienonen-Kempas), h.wikman@uke.uni-hamburg.de (H. Wikman), Kaisa.Salmenkivi@hus.fi (K. Salmenkivi), risto.pirinen@pkssk.fi (R. Pirinen), antti.karjalainen@echa.europa.eu (A. Karjalainen), esa.vanhala@ttl.fi (E. Vanhala), eeva.kuosma@ttl.fi (E. Kuosma), Sisko.L.Anttila@hus.fi (S. Anttila), eeva.kettunen@ttl.fi (E. Kettunen).

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Allelic imbalance
DNA copy number alteration

These alterations form the basis for the development of a combinatorial molecular assay that could be used to identify asbestos-related LC.

1. Introduction

Lung tumours cause the most cancer-related deaths worldwide. In addition to tobacco smoking, exposure to asbestos is a well-known risk factor to which a significant fraction (5–7%) of lung cancers (LC) are attributable (LaDou, 2004). Asbestos refers to a group of naturally occurring fibrous silicate minerals that have been used for instance in the building industry. However, the identification of lung tumours associated with patients' earlier asbestos exposure has been problematic. Epidemiological studies have used the evaluation of an individual's work history and possibly their pulmonary fibre count to determine the probability of their cancer being caused by asbestos exposure. A patient may be eligible to receive compensation due to lung cancer with a causal association with occupational exposure (Jamrozik et al., 2011). A biomarker that would objectively indicate the association of a given lung tumour with asbestos exposure would greatly benefit the identification of such tumours.

Genomic changes are known to accumulate during lung tumour progression, resulting in complex genomic alterations typical of LC. Very few studies describe asbestos-associated molecular genetic alterations in LC, probably due to the fact that pulmonary asbestos fibre counts in LC patients are defined infrequently, albeit that asbestos-related LC is among the most significant of occupational cancers (Karjalainen et al., 1994). Furthermore, tobacco smoke exposure often interferes with molecular correlates. Nevertheless, some genomic alterations, such as extensive allelic loss in 3p21.3 in LC have associated with patients' earlier asbestos exposure (Marsit et al., 2004). In addition, homozygous deletions and loss of heterozygosity (LOH) in *CDKN2A/P16* have proved to be more frequent among LC patients with asbestos exposure, while hypermethylation of the gene's promoter region is more frequent among patients without exposure (Andujar et al., 2010; Kraunz et al., 2006). Recently, up-regulation of *ADAM28* in 8p21.2, encoding a disintegrin and metalloproteinase domain protein, was shown to associate with asbestos exposure in patients suffering from adenocarcinoma (AC) of the lung (Wright et al., 2010).

In contrast to the above mentioned studies, we used a systems biology approach to study asbestos-associated molecular alterations in LC. This approach included studies of genome-wide DNA copy number alterations (CNA), gene expression profiling, miRNA expression profiling as well as signalling pathways of well-characterized material from asbestos-exposed and non-exposed LC patients (Nymark et al., 2011, 2006; Ruosaari et al., 2008a; Ruosaari et al., 2008b; Wikman et al., 2007). Several chromosomal regions such as 2p21–p16.3, 3p21.31, 5q35.2–q35.3, 9q33.3–34.1, 16p13.3, 19p13.3–p13.1, and 22q12.3–q13.1 were affected by asbestos-related genomic alterations in LC. We further studied the three that showed the most significant associations, i.e. 19p13, 2p21–p16, and 9q31.3–34.3, with allelic imbalance (AI) and CNA. The asbestos-associated CNA in 2p and 9q were narrowed down to 2p16 and 9q33.1 (Kettunen et al., 2009; Nymark et al., 2009). Moreover, asbestos exposure in human bronchial epithelial cells (BEAS-2B) induced micronuclei harbouring 19p fragments (Ruosaari et al., 2008b). Together these studies suggest that 19p13, 2p16, and 9q33.1 are significant in asbestos-related LC.

Molecular abnormalities such as LOH have been seen in the histologically normal bronchial epithelium of smokers or LC patients, but they have not been previously studied in specifically asbestos-related LC (Wistuba et al., 1997, 2002). Our study investigated whether AI in 19p13, 2p16, and 9q33.1 could be detected in histologically normal bronchial and bronchiolar epithelium microdissected from a set of asbestos-exposed and non-exposed LC patients. We also studied DNA copy numbers and AI in 19p13, 2p16, and 9q33.1 in a larger number of lung tumours and found that concurrent alterations in these genomic sites accumulate and characterize the lung tumours of patients with a history of asbestos exposure, showing a dose–response with increasing asbestos fibre count.

2. Material and methods

2.1. *Lung tumour material and normal tissue*

The tumour material consisted of primary lung tumours, classified according to the latest WHO classification, from 225 Finnish caucasians (9 females, 216 males; Table 1). Figure 1 shows an overview of the study cohorts.

Two different collections of lung tumours were used. The first set consisted of 64 tumour samples (fifty-six fresh frozen (FF) and eight formalin-fixed paraffin-embedded (FFPE) samples) from patients operated on in the Department of Thoracic and Cardiovascular Surgery of the Helsinki University Central Hospital in 1990–1996. These 64 patients were interviewed regarding their smoking and work history as described in detail earlier (Karjalainen et al., 1993). Twenty-four patients had a probable or definite history of occupational asbestos exposure, whereas 40 patients acknowledged no asbestos history. The difference for distribution of smoking status was insignificant between the asbestos-exposed and non-exposed patients ($P = 0.36$, Fisher's exact test). These two groups showed also insignificant difference for pack-years ($P = 0.73$, *t*-test). The patients gave their informed consent to use their tissue and data. The Ethical Review Board for Research in Occupational Health and Safety, and the Coordinating Ethical Review Board, Helsinki and Uusimaa Hospital District (75/E2/2001) approved the study protocol, the collection of samples, and the personally interviewed data.

The second set was formed of FFPE specimens from 161 patients whose lung tissue samples were originally sent to the