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Mini-review

Molecular and genetic changes in asbestos-related lung cancer

Penny Nymark [a,*], Harriet Wikman [b], Tuija Hienonen-Kempas [a], Sisko Anttila [a,c]

[a] Finnish Institute of Occupational Health, 00250 Helsinki, Finland
[b] Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[c] Department of Pathology, Haartman Institute and HUSLAB, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

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Abstract

Asbestos-exposure is associated with an increased risk of lung cancer, one of the leading causes of cancer deaths worldwide. Asbestos is known to induce DNA and chromosomal damage as well as aberrations in signalling pathways, such as the MAPK and NF-κB cascades, crucial for cellular homeostasis. The alterations result from both indirect effects through e.g. reactive oxygen/nitrogen species and direct mechanical disturbances of cellular constituents. This review describes the current knowledge on genomic and pathway aberrations characterizing asbestos-related lung cancer. Specific asbestos-associated molecular signatures can assist the development of early biomarkers, molecular diagnosis, and molecular targeted treatments for asbestos-exposed lung cancer patients.

Keywords: Asbestos; Copy number; Gene expression; Lung cancer; Molecular changes

1. Introduction

Asbestos-exposure increases the risk of several malignancies, most of all the risk of malignant mesothelioma, followed by lung and laryngeal cancer. Lung cancer causes the most cancer-related deaths in the world and even if mesothelioma is commonly known as the primary asbestos-associated cancer type, it has been estimated that asbestos gives rise to an equal number or more lung cancers as compared to mesothelioma. The fraction of all lung cancers attributable to asbestos-exposure to date is estimated at approximately 5–7% [1,2].

In developed countries the use of asbestos in manufacture or building has been banned or under strict control for longer than a decade, and asbestos demolition work tightly regulated by law. Nevertheless, asbestos will continue to burden public health also in developed countries due to the long latency period, generally 30–40 years, between the initial exposure to asbestos and malignant disease. The manufacture and use of asbestos products peaked in the 1970s in Western Europe, North America, Japan and Australia. Today, a majority of the asbestos, produced worldwide is used in Eastern Europe, Latin America and Asia [1,3,4].

Asbestos-exposure and tobacco smoking have a synergistic, more than additive, effect on lung cancer

* Corresponding author. Tel.: +358 30 474 2210; fax: +358 30 474 2021.
E-mail addresses: penny.nymark@ttl.fi, penny.nymark@helsinki.fi (P. Nymark).

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risk, whereas asbestos-exposure is a sole risk factor for malignant mesothelioma [5,6]. It is not known, whether this difference reflects different carcinogenic mechanisms or different sensitivity of lung epithelial and mesothelial cell types to asbestos-induced carcinogenesis. The fact that most asbestos-exposed lung cancer patients are also tobacco smokers has made it very difficult to distinguish asbestos-related molecular changes from those related to tobacco carcinogens by molecular epidemiology [7].

Several genetic and molecular factors have been described to be involved in asbestos-induced carcinogenesis *in vitro* or *in vivo*, or identified in asbestos-related human cancers. The emerging methodology of genomics, transcriptomics and proteomics has provided new screening tools for specific carcinogen-associated molecular profiles, especially when applied to well-designed experimental settings or carefully characterized patient materials. The increasing knowledge of the molecular changes in asbestos-related lung cancer is crucial for the discovery and development of early biomarkers, molecular diagnosis, and molecular targeted treatments. In the following we will review experimental and human data on the molecular mechanisms of asbestos-induced lung carcinogenesis.

2. Toxicity and carcinogenicity of asbestos fibres

Asbestos is a generic term for industrially refined and produced fibrous silicate minerals. Asbestos is classified into six distinct mineralogical types, i.e., chrysotile, crocidolite, amosite, tremolite, anthophyllite, and actinolite. Chrysotile belongs to the serpentine group. It is a curly and thin fibre type. The other five types belong to the amphibole group of minerals, which are longer and needle-like. Chrysotile is the most commonly used and economically important asbestos type [1].

A number of animal experimental studies have shown that the longer the fibre, the more carcinogenic it is *per se* [8]. The genotoxicity depends also on the fibre's chemical composition and structure as well as the cell environment [9]. Amphibole fibres are chemically complex and contain variable amounts of associated mono-, di- and trivalent metals such as iron. It has been proposed that asbestos is not toxic by simply acting as a carrier of e.g. iron into the cell, but also by the particular way iron is bound to the fibre's surface enabling free radical generation [10,11]. Due to the metals, the fibre structure and their bio-persistence, the amphiboles are known to be more pathogenic in the human body compared to chrysotile. In contrast to chrysotile asbestos, which is fragmented and cleared from the lungs, amphiboles are considered insoluble in human lung [8,12].

In vitro studies have demonstrated that asbestos fibres are cytotoxic and clastogenic but surprisingly not mutagenic in Ames assay [13,14]. The main mechanisms behind these destructive effects are thought to be multiple, including generation of reactive oxygen (ROS) and nitrogen species (RNS), alteration in the mitochondrial function, physical disturbance of cell cycle progression, and activation of several signal transduction pathways [15,16].

Several mechanisms are likely to contribute to the synergistic carcinogenic effect of tobacco smoke and asbestos-exposure. It has been demonstrated that cigarette smoke augments the penetration of asbestos fibres in rat tracheal explants by an oxygen radical-mediated mechanism [17]. In addition, reactive oxygen species have been observed to alter the metabolism of a tobacco carcinogen, benzo[*a*]pyrene, by inhibiting the detoxification pathways [18]. On the other hand, tobacco carcinogens are known to be adsorbed on the surface of asbestos fibres increasing their uptake into the cells [19]. Furthermore, asbestos fibres induce cell proliferation, which may lead to clonal expansion of a cell with a heritable tobacco carcinogen-induced alteration in a critical gene [20].

3. Molecular changes in asbestos-induced carcinogenesis

In vitro studies have shown that all types of asbestos fibres are able to cause structural and/or numerical chromosomal aberrations, produce binucleated cells, and disturb cell division and homeostasis [21,22]. These alterations have been suggested to result from both an indirect effect of ROS and RNS on signalling cascades and a direct physiological interaction between the cell and the fibres [23] (Fig. 1). High-resolution time-lapse light microscopy studies on living cells have shown that asbestos fibres are actively transported along cytoplasmic microtubules to perinuclear regions where they can sterically block cytokinesis [21,24,25]. Recent results also show that asbestos fibres might directly bind to proteins that regulate the cell cycle, cytoskeleton, and mitotic processes contributing to significant spindle damage and chromosomal instability [26]. Furthermore, asbestos increases the frequency of

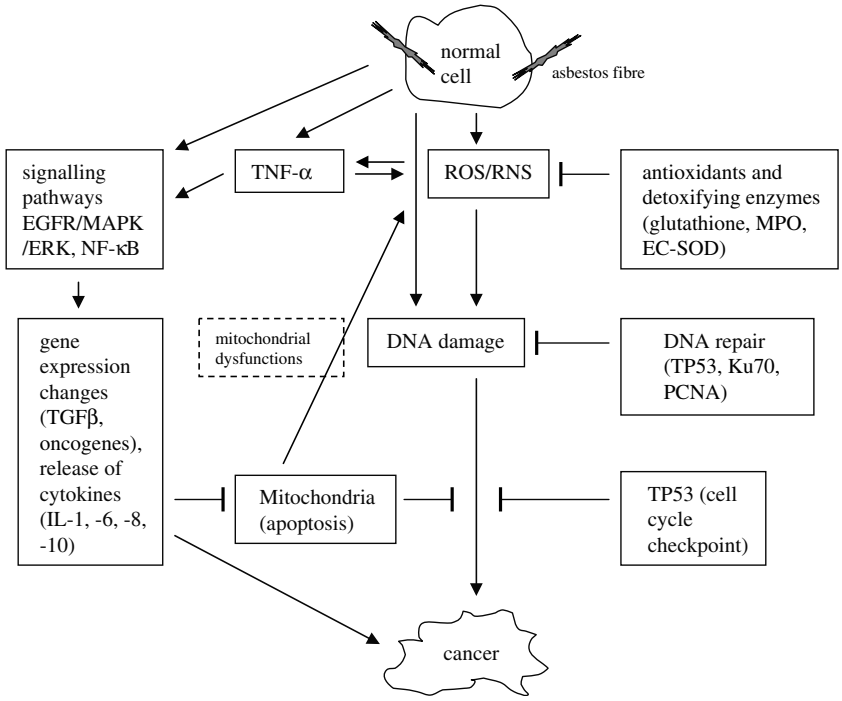

Fig. 1. Asbestos-related carcinogenic pathways in the lung.

homologous recombination (HR) suggested to be related with malignant transformation of cells carrying genetic alterations caused by other environmental mutagens and carcinogens [27].

To develop an *in vitro* model that would represent the actual *in vivo* progress of asbestos carcinogenesis has been challenging, since asbestos eventually causes apoptosis for many cell types *in vitro*. Nevertheless, a few malignant transformations by asbestos have been conducted on human bronchial epithelial cells, murine lung and hamster embryo cells [28–30]. Immortalized cell lines often show cytogenetic and molecular alterations and malignant transformation of such cells may be facilitated by these already existing changes. However, for example the HPV-18 immortalized human bronchial cell line, BEP2D, has been used in a variety of studies examining the transforming potential of various genotoxins, including asbestos [28,31,32]. The cell line has been shown to be karyotypically stable, although it does show some well documented abnormalities. The cells are also anchorage-dependent and do not form tumours in nude mice, not even in late passages [33]. Similar features apply to most cell lines used for this type of studies.

An animal model for *in vivo* experiments has also been difficult to establish, since in humans asbestos fibres usually act in combination with other environmental carcinogens, such as tobacco smoke and silica, and asbestos-related changes accumulate during many decades. *In vivo* studies have, however, validated some *in vitro* experiments by identifying asbestos-related gene expression changes such as activation of the NF-κB pathway, *p53* promoter activation, and cell proliferation induced by TNFα and -β as well as PDGF A and B [29,34–38]. Experimental and human data combined can give further insight into the central genes and pathways participating in the asbestos-induced malignant transformation of lung cells.

In the following sections the current knowledge on asbestos-induced chromosomal aberrations, disturbances in key genes and pathways, as well as point mutations in the lungs will be described. The most important known chromosomal loci, genes and pathways associated with asbestos-related lung cancer are listed in Table 1.

3.1. Chromosomal aberrations

The direct or indirect action of asbestos on DNA and proteins can cause many different types of DNA and chromosomal damage. Asbestos fibre-treated cells have shown increased amounts of

Table 1
Changes in chromosomes, genes and pathways, related to asbestos-associated lung carcinogenesis

Chromosome/gene/pathway	Aberration	Asbestos-related carcinogenic association	References[a]
Chrom. 1	Break at the centromere Copy number changes 1p36 and 1q21		[50,56,63]
Chrom. 3	LOH at 3p21 and 3p14	*FHIT* exon loss Possible down-regulation of tumour suppressors	[62–65]
Chrom. 5	Deletion or monosomy Deletion at 5q35.2–q35.3	Possible down-regulation of tumour suppressors	[33,63,64]
Chrom. 19	Monosomy AI at 19p13	Possible down-regulation of tumour suppressors	[33,63,64,68]
PPP (pentose phosphate pathway, e.g. *G6PD*)	Inhibition	Depletion of glutathione followed by a decreased resistance against oxidative stress	[73–75]
SOD	Redistribution of extracellular SOD	Decreased resistance against ROS/RNS	[72,77]
MPO	Increased activity G to A polymorphism in the 5′ untranslated region of *MPO*	Increased production of RNS Reduced lung cancer risk associated with MPO A-allele (G/A + A/A) genotype	[97,141,143]
NF-κB pathway	Up-regulation	Tumour promotion through activation of proto-oncogenes (e.g. *c-myc*)	[29,81,87,88,95]
TNFα	Up-regulation through NF-κB	Enhances the interactions between cells and fibres by increasing the binding of asbestos to tracheal epithelial cells Accumulative ROS generation through IL8	[80,81,149]
Interleukins (e.g. *IL1*, *IL6*, *IL8* and *IL10*)	Up-regulation	Elevated in asbestos-exposed workers who develop lung cancer (*IL6*) Accumulative ROS generation (*IL8*) Apoptosis resistance (*IL10*)	[81,82,84–86]
EGFR	Phosphorylation/activation	Cell proliferation through activation of the MAPK/ERK pathway	[82,90,91]
MAPK/ERK pathway (e.g. *ERK* genes)	Activation through EGFR	Tumour promotion through activation of AP-1 dependent target genes Cell proliferation through activation by PKCδ and adducin	[64,90–92,95,100,101]
AP-1 dependent target genes (e.g. *c-fos*)	Up-regulation by the NF-κB and MAPK/ERK pathways	Transcription factor for oncogenes	[29,92–95]
*MMP*s	Activation	Activation of EGFR	[37,99]
TGFβ	Up-regulation	Promotes fibrosis Immune tolerance towards mesothelioma	[104–107]
TP53	Up-regulation Mutations	Cell proliferation through regulation of *PCNA* Decreased tumour-suppressor activity	[38,114,123–127,129]
BCL2 and *BCL2*-like genes	Activation/inhibition	Apoptotic resistance	[86,117–119]

[a] Only asbestos-related aberrations mentioned in at least two studies have been included in this table.

abnormalities, such as, lagging chromosomes, bridges, and sticky chromosomes in anaphase or telophase, formation of micronuclei, DNA single-strand breaks and increased sister chromatid exchanges (SCE) [39–51]. The most typical asbestos-induced chromosomal aberrations in cell line experiments are deletions, breaks and fragments [14,52–57]. Most of these changes are found in a dose-dependent manner, but not surprisingly is the toxic dose highly dependent on the cell line and fibre type used [21,58,59]. In asbestos-exposed workers' white blood cells, increased levels of SCE and DNA double-strand breaks as well as anti-double-strand DNA antibodies have been reported [60,61].

Most studies describing chromosomal aberrations of human lung tumours have not reported the patients' asbestos-exposure, and thus it is impossible to decipher the association between the aberrations and asbestos based on these reports. However, a few studies have described asbestos-specific

aberrations in e.g. chromosomes 1, 3p, 5, 8, 9, and 19p. Recently, two studies have shown that a common early aberration in lung cancer, namely loss of 3p, occurs more frequently in the tumours of asbestos-exposed than in non-exposed patients. Marsit et al. showed that allelic imbalance (AI) in 3p21.3 was associated with occupational asbestos-exposure, *TP53* mutations as well as better patient survival [62,63]. We also detected 3p21.3 as one of the main regions differentiating lung tumours of asbestos-exposed and non-exposed patients in a whole genome aberration and expression screening [63,64].

In lung tumours, loss of another region in the short arm of chromosome 3, namely 3p14 containing the *FHIT* gene, has also been associated with asbestos-exposure and tobacco smoking [65]. However, Pylkkänen et al. detected reduced *FHIT* expression in both asbestos-exposed and non-exposed patients' lung tumours [66]. The region contains a fragile site, FRA3. In our array comparative genomic hybridization study we found that many of the regions with asbestos-related copy number changes were associated with fragile sites, demonstrating that asbestos may preferentially cause DNA damage at such sites [63].

Dopp et al. used fluorescence in situ hybridization (FISH) to investigate chromosomes 1 and 9 in human amniotic fibroblasts (AF) after asbestos treatment [50]. They reported the centromeric regions of these chromosomes to be affected by DNA breakage following asbestos-exposure. The same group treated the lymphocytes of smokers and non-smokers with asbestos fibres and again, damage in chromosome 1 was the main finding in non-smokers, whereas in smokers' lymphocytes other chromosomes were damaged as well [56]. These findings support the hypothesis that, at least in part, the synergistic carcinogenic effect of tobacco smoke and asbestos fibres results in similar aberration patterns as compared to either exposure alone, but with more frequent aberrations found when both exposures are present.

In a study by Suzuki et al., loss of one or two copies of chromosome 5, monosomy of chromosome 19, and trisomy of chromosome 8 were found to be common changes in five tumorigenic human bronchial epithelial cell lines transformed by chrysotile asbestos. The transformed cell lines showed especially high frequencies of the newly developed changes in chromosomes 5 and 19 which were not seen in the parental non-tumorigenic immortalized cell line BEP2D. Trisomy of chromosome 8 was not detected as a new change, but was seen more frequently in the tumourigenic cells. Furthermore, the non-tumorigenic parental BEP2D cell line generally showed hyperaneuploidy, with 46–50 chromosomes, while all five tumorigenic cell lines showed hypoaneuploidy with 42–44 chromosomes, demonstrating that asbestos causes loss of genomic material, consistent with other reports [33]. Interestingly, malignant transformation of the same cell line with radon-exposure did not cause the same type of aberrations, indicating that the changes could be asbestos-specific and not only cell line- or transformation-related [67]. Furthermore, in agreement with the results by Suzuki et al., we described loss of 5q35 and 19p combined with down-regulation of gene expression in asbestos-associated lung cancer [63,64,68].

3.2. Genes and pathways associated with asbestos-exposure

The main cellular functions that are affected by asbestos fibres include oxidative stress response, inflammation, DNA damage response, mitochondrial activity and apoptosis (Fig. 1). Many genes and pathways involved in these functions have been identified and investigated, usually gene by gene. However, asbestos triggers a large amount of cellular responses and the difficulty has lied in recognizing the primary asbestos-related responses and especially the changes related to oncogenesis.

3.2.1. Oxidative stress and inflammation

Asbestos-exposure causes oxidative stress through free radicals (ROS/RNS), which are released by macrophages upon unsuccessful phagocytosis of the fibres or generated directly through iron present on the surface of the fibres [69]. ROS cause DNA damage products, such as the mutagenic 8-hydroxy-2′-deoxyguanosine (8-OHdG) adducts, which have been found to be elevated in the white blood cells of asbestos-exposed workers [70]. Furthermore, asbestos induces gene expression changes through conversion of important signalling molecules, such as nitric oxide (NO), to free radicals (NO_2^-). NO is an important regulator of e.g. p53 and therefore affects a multitude of key cellular events [71].

3.2.1.1. Antioxidant pathways. An imbalance in oxidant–antioxidant levels has been proposed to underlie the pathogenesis of asbestos-related lung disease

[72]. Disturbances in antioxidant pathways reduce the cells capacity to protect itself against ROS/RNS. Studies have demonstrated that antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD) as well as antioxidant peptides including glutathione, can effectively protect cells *in vitro* against the mutagenic effects of asbestos-induced ROS/RNS production [55,73]. Indeed, lung carcinoma A549 cells, which contain a 3.5-fold glutathione content as compared to mesothelial Met-5A cells, have been shown to be more resistant against oxidants and fibres than the Met-5A cells [73]. Endocytosis of asbestos fibres by both macrophages and lung epithelial cells has been shown to be followed by a decrease in intracellular and an increase in extracellular glutathione [74]. The depletion of glutathione is caused by asbestos-induced inhibition of the enzyme G6PD involved in an antioxidant pathway (pentose phosphate pathway). Synthetic fibres, containing almost no iron, do not exert the same effect, indicating that the surface features of asbestos fibres trigger the changes in the cells' antioxidant activity [75,76]. In addition, another group showed that asbestos causes redistribution of extracellular SOD (EC-SOD) from the lung parenchyma to the air spaces in the lung, resulting in high levels of EC-SOD in bronchoalveolar lavage fluid in mice [72]. Increased sensitivity to asbestos-induced injury has been reported in mice lacking EC-SOD [77].

In contrast, increased manganese SOD (MnSOD) has been associated with higher sensitivity to oxidative stress in epithelial cells [78]. Janssen et al. found MnSOD to be increased both on mRNA and protein level in the lungs of asbestos-exposed rats [79]. Thus, the concept of oxidant–antioxidant imbalance in relation to asbestos-induced injury seems to be more complex than initially thought.

3.2.1.2. TNFα/NF-κB pathway. The inflammatory cytokine, TNFα, has been shown to be activated in macrophages after asbestos-exposure *in vitro* [80]. TNFα induces *IL8* expression in macrophages, which attracts neutrophils that in turn, release ROS and RNS. This leads to a feedback loop between ROS generation and increased TNFα expression, resulting in increased DNA damage [81]. Also, other interleukins are released by inflammatory cells [82] upon phagocytosis of fibres and e.g. *IL6* has been shown to be up-regulated in airway epithelial cells by NF-κB in response to asbestos-exposure [83]. Increased IL6 correlates with increased serum levels of C-reactive protein (CRP). CRP has been found to be significantly more elevated in the serum of asbestos-exposed workers who developed cancer (lung cancer and mesothelioma) than in those that did not develop cancer in a follow up study [84]. In addition, *IL1* and *IL10* have been found to be up-regulated by asbestos-induced oxidative stress *in vitro* [85,86].

NF-κB coordinates the inflammatory and cell proliferative responses to asbestos [29,87]. By exposing rat tracheal explants to asbestos, TNFα was found to increase the binding of fibres to the surface of tracheal epithelial cells via an NF-κB-dependent pathway, enhancing the interactions of the fibres with the tissue [81]. In mesothelial cells, treatment with TNFα activates NF-κB, which increases the percentage of cells that survive asbestos-exposure and thereby the amount of asbestos-damaged cells capable of undergoing malignant transformation [88]. Furthermore, NF-κB is a transcription factor for several oncogenes, and for example increased c-myc transcription has been detected upon asbestos-exposure in human embryonic lung cells [89].

3.2.1.3. EGFR/MAPK/ERK pathway. Asbestos-induced oxidative stress causes activation of the epidermal growth factor receptor (EGFR) by phosphorylation [82,90,91]. EGFR activates the MAPK/ERK pathway through phosphorylation of ERK1/2 and ERK5 [92]. Increased levels of phospho-ERK1/2 and -ERK5 induce proliferation and activation of the proto-oncogenes *c-fos*, *fra-1* and *c-Jun* (AP-1 family members) [29,93–95]. It has been proposed that the synergistic properties of asbestos and tobacco smoke may be caused by separate activation of the *ERK* genes and *JNK1/2*, respectively, which both transactivate AP-1 [92].

Reactivation of the cell cycle in a critical DNA repair stage may lead to DNA damage bypass allowing cells with oncogenic changes to continue proliferating. Indeed, low levels of asbestos have been shown to cause cytoplasmic localization of phospho-ERK1/2, which is followed by AP-1 dependent nuclear localization of cyclin D1 [96]. Cyclin D causes cell cycle re-entry through progression from G_1- to S-phase [97]. In addition, other growth factors such as the insulin-like growth factor (IGF) and platelet-derived growth factor (PDGF) are also known to promote S-phase after asbestos-exposure [98].

Activation of EGFR has also been shown to be caused by protein kinase C (PKC) activated matrix

metalloproteinases (MMP) [37], and e.g. *MMP2* has been found to be up-regulated after combined exposure to chrysotile and cigarette smoke *in vivo* [99]. In agreement, *PKCδ* and its substrate, adducin, was shown to cause cell proliferation through activation of ERK1/2 in response to asbestos-exposure [100,101]. Noticeably, adducin (*ADD1*) has been found to be up-regulated in lung tumours of asbestos-exposed patients compared to those of non-exposed patients [64].

Finally, ERK1/2 has been shown to be activated by Src family kinase [102]. Growth promoting tyrosine kinases, such as Src are activated by the urokinase plasminogen activator (PLAU) pathway, involved in tissue reorganization events such as wound healing. The PLAU pathway has been shown to be activated by asbestos [103].

3.2.1.4. TGFβ pathway. The transforming growth factor β (*TGFβ*) pathway has dual tumour suppressive and oncogenic effects depending on the stage of carcinogenesis [104]. High levels of the cytokine *TGFβ1* has been suggested to have an immunosuppressive role in mesothelioma, i.e., immune tolerance towards the tumour, allowing it to progress [105]. Asbestos causes over-expression of *TGFβ1* and it has been related to fibrogenesis in mice [106,107].

The TGFβ-induced gene, *βIGH3*, transcribes for an adhesion protein possibly involved in cell–collagen interactions. The gene has been shown to be up-regulated in human lung carcinoma [108]. In contrast, *βIGH3* was found to be down-regulated in five asbestos-transformed human bronchial epithelial cell lines, derived from the BEP2D cells [109]. The transformed cell lines consistently formed tumours in nude mice. Reintroduction of the gene in a highly malignant asbestos-transformed cell line inhibited cell growth and tumour formation in mice [110]. Thus, it is intriguing to hypothesize that the *βIGH3* gene may specifically be involved in asbestos-induced carcinogenicity. It is not known what causes the down-regulation of *βIGH3*, since *TGFβ1* is activated by asbestos [69,111,112]. However, it has been proposed that the *βIGH3* might be a target of asbestos-induced chromosome 5 losses [33,63]. The tumour suppressive role of *βIGH3* is still to be identified.

3.2.2. DNA damage repair

Initially cells attempt to adapt and repair damage caused by asbestos-exposure and consequently, many DNA repair genes, such as *TP53* and *GADD153*, are up-regulated in asbestos treated cells [113]. However, the accumulation of fibres in the lung and the continuous production of ROS/RNS causes repeated DNA damage, which may lead to increased genetic instability, a hallmark of neoplastic development. The DNA damage and instability may cause abnormal expression of DNA repair genes. In fact, *Ku70* involved in nonhomologous DNA repair, has been found to be down-regulated in asbestos-transformed tumorigenic lung cell lines [109].

Over-expression of *TP53* should assist the DNA repair processes. However, p53 has been shown to bind and regulate the proliferating cell nuclear antigen gene (*PCNA*), which is involved in DNA repair, but also in DNA damage bypass. Activated *PCNA* could together with growth factors, many of which are induced by asbestos, allow cells with DNA damage to continue proliferating. *PCNA* has been shown to be co-expressed with p53 in the lungs of asbestos-exposed rats [114], and it is also induced in lung epithelial cells after mechanical wounding of the cultured cell layer and after asbestos-exposure [100].

3.2.3. Mitochondrial function and apoptosis

Apoptosis protects against abnormal proliferation of cells with non-repairable DNA damage. Alterations in most of the previously mentioned asbestos-induced genes and pathways should eventually lead to apoptosis. However, the apoptotic pathways seem to be inhibited in asbestos-associated lung cancer as in many other cancers. Indeed, low doses of asbestos ($<0{,}5\ \mu g/cm^2$) promote S-phase entry and thereby cell proliferation through an *EGFR* dependent pathway instead of apoptosis [96]. If apoptosis is bypassed, the asbestos-associated dysfunctions in the mitochondrial respiratory chain maintain the increased release of ROS.

Genes in the apoptotic pathway could be potential target genes for therapy either through silencing or activation. For example, the expression and phosphorylation of cAMP responsive element binding protein (*CREB*) is thought to be an important regulator of apoptosis in asbestos-induced responses, and silencing of the gene dramatically increases asbestos-induced apoptosis in lung epithelial cells [115]. Similarly, over-expression of the 8-OHdG repair enzyme, OGG1 and its translocation to the mitochondria has been shown to reduce asbestos-induced apoptosis in HeLa cells [116].

Furthermore, gene expression profiling of asbestos-transformed tumorigenic lung cell lines has revealed down-regulation of an apoptosis-related putative tumour suppressor *DCC* (deleted in colorectal cancer) [109].

Miura et al. have produced an apoptosis resistant T-cell cell line through repeatedly exposing the cells to asbestos. By studying this cell line they proposed a model mechanism for acquiring of resistance to asbestos-induced apoptosis, involving activation of the genes *Src family kinase*, *IL-10*, *STAT3* and *BCL2*. Interestingly, *BCL2* was also found to be significantly up-regulated in the T cells of mesothelioma patients as compared to healthy volunteers and asbestosis patients, indicating a role in carcinogenesis [86]. Many other *BCL2* related genes have been implicated in asbestos-induced apoptotic resistance or carcinogenesis, such as *BNIP3L*, *Bax* and *Bcl-xl* [117–119].

3.3. Point mutations

Both crocidolite and chrysotile asbestos have been shown to increase the general mutation frequency in various hamster and rat cells [120,121]. However, the fibres are at most marginally positive for their ability to produce point mutations at specific loci *in vitro*. Nevertheless, some studies on human lung tumours have linked specific mutations to asbestos-exposure [48]. Some of these mutations could primarily be caused by tobacco specific carcinogens such as benzo[*a*]pyrene, which have been shown to have an enhanced mutagenic effect following co-exposure with amosite asbestos in rat lung [122].

TP53 is probably the most extensively studied gene in relation to asbestos-exposure due to its crucial role in DNA damage response. *TP53* is not only up-regulated after asbestos-exposure but abnormal accumulation of the protein has been detected more frequently in tumours and serum from exposed patients compared with patients without asbestos-exposure [114,123–125]. Increased levels of the protein could partially be caused by mutated p53. Indeed, mutations in p53 have been positively associated with asbestos-exposure in lung cancer patients in many [38,126,127] but not in all studies [62,128]. *TP53* mutations have also been identified in mouse fibroblasts after crocidolite exposure [129].

Also p16/INK4α, a regulator of p53, has been associated with asbestos-exposure. Inactivation of *p16/INK4α* by either methylation [130] or by homozygous deletion has been found to correlate with asbestos-exposure in non-small cell lung cancer patients [131].

The biomarker for oxidative stress, 8-OHdG adduct, is mutagenic and may cause G:C to T:A transversions. These nucleotide transversions have been found to be more frequent in tumours of asbestos-exposed individuals [132]. High levels of G to T transversions in codons 12, 13 and 61 of the *KRAS* gene were found in a study population of lung cancer patients exposed to asbestos, but the association could not be confirmed in a study with added cases [128,133]. However, in a later study with 355 cases, mutations in codon 12 of the *KRAS* gene were significantly associated with asbestos-related lung adenocarcinoma [134]. In contrast, no mutations could be found in the *KRAS* gene in five asbestos-transformed malignant cell lines, which suggests that these mutations may be a result of the synergistic effects of asbestos and tobacco carcinogens [28].

4. Genetic susceptibility to asbestos-related lung cancer

Tobacco is clearly the most important single environmental factor for lung cancer. As not all of even the heaviest smokers contract lung cancer, individual differences in several host factors including differences in metabolism, DNA repair capacity, altered expression of proto-oncogenes and/or inactivation of tumour-suppressor genes, are anticipated to modify individual susceptibility to the disease. Variations in an individual's capacity to detoxify tobacco carcinogens as well as in DNA repair capacity, due to genetic polymorphisms, are well documented. These individual variations have indeed been linked to different risks in contracting tobacco-related lung cancer. Most results have, however, shown that lung cancer risk is only moderately increased by single polymorphic genes, but can be considerably enhanced by specific combinations of susceptibility genes [135].

Individual susceptibility to asbestos-related lung cancer is much less studied. However, a few research groups have investigated the relation between asbestos-exposure and polymorphisms in xenobiotic metabolizing enzymes (GSTM1, GSTT1, MPO, CYP1A1, and CYP2E1) and ROS defence (SOD2) [52,136–140].

Glutathione *S* transferases (GST) catalyse conjugation of reduced glutathione (GSH) to a large

amount of different compounds, but may also play a role in the detoxification of ROS [136]. In tobacco-related lung cancer a deletion polymorphism in *GSTM1* seems to increase the risk of contracting the disease, whereas the role of *GSTT1* deletion is still controversial [135]. However, in a pooled study based on 651 lung cancer cases and 983 controls no relation between asbestos-exposure and either *GSTM1* or *GSTT1* null genotypes could be found [137]. Neither could an association be found between *CYP1A1* or *CYP2E1* polymorphisms and asbestos-related lung cancer [138,139].

Myeloperoxidase (MPO) is central to the microbicidal activity of neutrophils. The enzyme can activate a wide range of tobacco smoke pro-carcinogens, including benzo[*a*]pyrene, but is perhaps more importantly also involved in the generation of RNS [97]. In a case–control study based on 375 Caucasian lung cancer cases and 378 matched controls, Schabath et al. found that a genetic polymorphism (G to A in the 5′ untranslated region of the *MPO* gene), which reduces the MPO activity, modified the effect of asbestos-exposure on lung cancer risk. They found that for a similar level of exposure, individuals with the *MPO* A-allele genotypes (G/A and A/A) had a reduced risk of lung cancer, a similar effect which is found for lung cancer in general [141,142]. Enhanced MPO activity has been detected in rat and hamster lungs following asbestos-exposure and the enzyme has been found to be an important mediator of asbestos-induced oxidative stress [97,143].

Manganese superoxide dismutase (*SOD2*, MnSOD) is an important mitochondrial antioxidant enzyme and its activity is generally diminished in cancer cells, although it has been found to be induced by asbestos in various lung cells [79,144]. No association could, however, be found in a large case–control study between homozygous variant *SOD2* and increased lung cancer risk among asbestos-exposed lung cancer patients [145].

5. Conclusions

Since 1935 when Lynch, Smith and Gloyne first reported an association between lung cancer and asbestos-exposure [146,147], many studies have attempted to explain the extremely high risk of lung cancer among smoking asbestos-exposed workers. In 1999 it was estimated that past asbestos exposures in Western Europe alone will cause a quarter of a million deaths from lung cancers and an equal amount of deaths from mesotheliomas over the next 35 years [1]. Furthermore, while the World Health Organization has estimated that 125 million people worldwide are currently exposed to asbestos in their work environment [148], lung cancer screening by high-resolution computed tomography has not been confirmed to reduce lung cancer mortality, nor are any useful molecular markers available for early cancer detection. The current clinical methods for identification of asbestos-related lung cancer rely on occupational history and pulmonary asbestos fibre counts. Asbestos-specific molecular alterations in cancer could enable molecular diagnosis and identification of therapeutic targets.

The molecular changes related to asbestos-associated lung cancer may be both primary, caused by the asbestos-exposure itself, and secondary, caused by the early exposure-specific changes affecting the instability of the genome. This has made it difficult to elucidate the changes related to the asbestos-induced malignant transformation of lung epithelial cells. Many of the changes may be largely the same as with tobacco smoke-exposure alone, but accumulating at a higher rate following exposure to a co-carcinogen such as asbestos. In fact, some changes seem to be the same as in non-exposed patients, but more common in the asbestos-exposed patients, e.g. loss of 3p21 and *EGFR* activation. However, there is much evidence that asbestos does cause specific molecular changes that could accelerate the progression to lung cancer. Specific copy number aberrations, including loss of the whole chromosome 5 or regions on it and loss of 19p13, have been identified in asbestos-associated lung cancer [33,63,64,68]. Many changes in gene expression and pathways have also been recognized, however, asbestos-specificity for those changes is more difficult to elucidate than for chromosomal changes. For example, oxidative stress is caused by a wide variety of other genotoxins besides asbestos. Thus, changes in gene expression related to oxidative stress and the generation of ROS/RNS are theoretically less specific to asbestos itself. Nevertheless, asbestos has been shown to cause depletion of some antioxidants, such as EC-SOD and glutathione, which are normally elevated following oxidative stress [29,72].

In combination, both the more and the less specific changes may contribute to the carcinogenic effects of asbestos. Therefore, it is probable that a method combining several different asbestos-associated molecular changes is the way of creating a

reliable diagnostic test for asbestos-related lung cancer. Such molecular changes could naturally also contribute to the development of molecular treatment strategies.

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