Lung Cancer: Mechanisms of Carcinogenesis by Asbestos

12

Brooke T. Mossman and Alessandro F. Gualtieri

Introduction

It has been known for decades that occupational exposures to asbestos lead to an increased risk of lung cancers, especially in smokers. The complex nature of cigarette smoke, which contains hundreds of carcinogens and other toxins, has been the subject of many experimental studies over the past several decades (reviewed in [1]). Despite advances in understanding the etiology, biology, and evolution of lung cancers, tumors of the respiratory system continue to be the leading cause of cancer deaths worldwide [2]. Historically, asbestos fibers have been studied most frequently in the genesis of mesothelioma, a more infrequent tumor unrelated to cigarette smoke, and an understanding of the molecular mechanisms of mesothelioma, despite some progress, remains enigmatic [3].

Unraveling the roles of asbestos fibers in the induction and/or development of lung tumors and how these complex minerals interact with components of cigarette smoke have been daunting due to the lack of experimental inhalation models that allow one to map the development of lung cancers in rodents over time [4]. A confounding factor preventing the study of lung cancers in rodents is the more rapid development of asbestosis or pulmonary fibrosis which causes early death after co-exposures [4]. However, our present knowledge of the mechanisms of lung cancer development by asbestos has been spear-headed by short-term rodent studies as well as differentiated lung epithelial cells and tracheobronchial explants (organ cultures). These models permit identification of critical cell: cell interactions and the development of hyperplastic and metaplastic lesions, early events in the carcinogenic process. Most recently, human lung tissues and bronchial epithelial cells have been used to demonstrate epigenetic signatures of lung tumor development and the importance of a favorable tumor environment consisting of chronic inflammation and cell proliferation.

The objective of this chapter is to describe studies providing insight into the interactions between components of cigarette smoke and asbestos that are important in their accumulation in lung. We then focus on the roles of these agents in lung carcinogenesis with an emphasis on recent studies exploring genetic and epigenetic changes by asbestos in human lung cancers and epithelial cells of the respiratory tract. There are many properties of mineral fibers that have been linked to carcinogenic events by asbestos, and a quantitative model to predict lung cancer risk is presented.

Basic Concepts of Asbestos Mineralogy

Definitions of Asbestos

Asbestos is a broad term used to identify a few silicate minerals that can be found in nature as thin and flexible fibers when crushed. Some of these minerals were of industrial and economic importance and have been used widely in the past [5]. To date, a plethora of different and sometimes contradictory definitions of the term "asbestos" exists, depending upon its usage in commercial, mineralogical, regulatory, and other settings. Unfortunately, the inadequate and incomplete definition of "asbestos" results in the lack of standardized operating definitions for these mineral fibers. Ambiguity in the definition of asbestos minerals also leads to widespread confusion in social, health, and legal contexts [6].

In this chapter, we will refer to the mineralogical term coined in 1982 which applies to six minerals exploited commercially for their desirable physical properties, mostly due to their fibrous-asbestiform habit. The six minerals are the serpentine phase chrysotile and the amphibole minerals amosite, crocidolite, anthophyllite asbestos, tremolite asbestos, and actinolite asbestos [7, 8]. This definition is in line with regula-

B. T. Mossman (✉)
Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT, USA
e-mail: brooke.mossman@med.uvm.edu

A. F. Gualtieri
Chemical and Earth Sciences Department, University of Modena and Reggio Emilia, Modena, Italy

S. Anttila, P. Boffetta (eds.), *Occupational Cancers*, https://doi.org/10.1007/978-3-030-30766-0_12

tory and health agencies indicating the six minerals described above as carcinogenic to humans (Group 1) [9, 10].

Serpentine Asbestos

Chrysotile is a layer silicate which belongs to the serpentine group together with the other polymorphs lizardite and antigorite. Serpentine minerals are composed of Si-centered T sheets in a pseudo-hexagonal network joined to Mg-centered O sheets in units with a 1:1 (TO) ratio. The ideal chemical formula of serpentine minerals is $Mg_3(OH)_4Si_2O_5$. In chrysotile, substitutions may occur in both T and O sheets but are limited. Fe^{2+} and Fe^{3+} may substitute for Mg^{2+} in the O sheet while replacement for Si^{+4} in the T sheet is less frequent, with a preference for Al^{3+} [11, 12]. As a result of the misfit between the T and O sheets [13] and because of the polarity of the TO unit, a differential strain occurs between the two sides of the layer. In chrysotile, the strain is released by rolling the TO layer around the fibril axis to end up with a cylindrical lattice responsible for the fibrous crystal habit.

Amphibole Asbestos

The family of amphibole asbestos includes actinolite asbestos $Ca_2(Mg,Fe)_5Si_8O_{22}(OH)_2$, amosite (fibrous variety of grunerite) $(Fe^{2+},Mg)_7Si_8O_{22}(OH)_2$, anthophyllite asbestos $(Mg,Fe^{2+})_7Si_8O_{22}(OH)_2$, crocidolite (fibrous variety of riebeckite) $Na_2(Fe^{2+},Mg)_3Fe_2^{3+}Si_8O_{22}(OH)_2$, and tremolite asbestos $Ca_2Mg_5Si_8O_{22}(OH)_2$. Amphiboles are chain silicates with an ideal Si:O ratio of 4:11 whose structures consist of alternating tetrahedral (T) chains and octahedral band sheets that are parallel to the (100) plane. Tetrahedra form infinite double chains running parallel to the **c** axis. In amphiboles, the oxygen atoms of the chains coordinate not only with Si(Al) but a variety of other cations, leading to the general formula [14]: $A_{0-1}B_2C_5T_8O_{22}W_2$. In the most common *C*2/*m* monoclinic amphiboles, A (in the (12)-fold cavity with a complex nomenclature used to describe the positional disorder of the cations) may host vacancies, Na^+, K^+, Ca^{2+}, Li^+; B is the (8)-fold coordinated *M*(4) site with Na^+, Ca^{2+}, Mn^{2+}, Fe^{2+}, Mg^{2+}; C are the octahedrally coordinated sites *M*(1), *M*(2), *M*(3) with Mg^{2+}, Fe^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, Mn^{3+}, Ti^{4+}, Li; T are the tetrahedrally coordinated sites within the silicate chain with Si^{4+}, Al^{3+}; and W = OH^-, F^-,Cl^-, O^{2-} [14]. Due to the presence of strong bonds, amphibole crystals normally grow along the **c** axis and may display a fibrous habit due to the mono-dimensional character of their structural units (chains).

According to the IARC [10], there is *sufficient evidence* in humans for the carcinogenicity of all forms of asbestos; hence they all have been classified as *carcinogenic to humans* (*Group 1*).

Physical-Chemical and Crystallographic Characteristics of Minerals Important in Lung Cancers

This section discusses the multiple parameters of mineral fibers (morphometric, chemical, biodurability-related, and surface activity) considered to prompt the cellular processes related to lung carcinogenesis. More than 20 years ago, George D. Guthrie stated that "*Extensive research has focused on the biological mechanisms responsible for asbestos-induced diseases, but much less attention has been paid to the mineralogical properties that might influence a mineral's biological activity. Several important mineralogical characteristics are likely to determine its biological reactivity and play important roles in determining the toxicity and carcinogenicity of a particle*." [15]. In addition to the traditionally considered variables of particle size and shape that exert a major control on deposition, translocation, and clearance, other mineralogical properties with roles in determining the toxicity and carcinogenicity of a particle are:

- Surface reactivity and sample history. For example, differences between generation of oxidants from freshly fractured materials and aged materials exist [16].
- Sorption and ion exchange. Ion exchange occurs when a sorbed species on the mineral exchanges with a similarly charged species in fluids. Some minerals like zeolites have great capacities for cation exchange because the ions can diffuse rapidly from the surface of the mineral to its interior, thereby enabling the entire particle to provide a buffering capacity [15]. Cation exchange could play an important role in cellular responses through a number of mechanisms, including the buffering of Ca^{2+} activity at the surface of a cell. It was observed that cation-exchanged erionites (Na, K, Ca, and Fe^{3+}) can have an effect on cytotoxicity, gene response, and apoptosis in pleural mesothelial cells.
- Catalytic properties of mineral particles that can function in a manner similar to that of traditional enzymes.
- Surface oxidation/reduction with electron transfer that has the potential to produce a sustained or chronic redox condition to drive formation of $HO^{\bullet}$ in the fluid. For example, iron release to the fluid may drive Fenton-type reactions to maintain charge balance.
- Dissolution/leaching, a major component of particle clearance mechanisms that causes the release of ions (e.g., iron and other metals, see below) to the lung fluid.
- Surface reactivity.

Surface Properties

At first glance, the surface properties of native mineral fibers may be overlooked as they may be modified in lung fluids or

by cells. In cells, a complex protein corona surrounds some engulfed particles. As observed for nanoparticles (NPs), the formation of the protein corona is an unstable and reversible mechanism for which a hetero-aggregation model is applied [17]. In fact, the protein corona that surrounds a fiber is porous, and the properties and processes active at the surface such as dissolution, exchange, and surface activity may only be partly inhibited. The protein corona is porous because it is formed, for example, by globular proteins such as albumin with a diameter of about 8 nm that stick to the surface of the fiber (it is possible to align 1250 albumin proteins along a 10 μm long asbestos fiber). The globular proteins adapt their structure to the surface but will never be able to entirely cover it, forming a porous layer around the fiber that does not inhibit, for example, ion exchange.

Fiber Dimensions

Among the morphometric parameters of a fiber, length and diameter play a major role in the kinetics of inhalation and lung response. According to the "Stanton hypothesis" [18], the ideal morphology of fibers for inducing intrapleural tumors in rats consists of a diameter $D \leq 0.25$ μm and a length $L > 8$ μm. Elongated particles with $L > 8$ μm ("Stanton fibers") are not eliminated by phagocytic cells like alveolar macrophages [19] leading to "frustrated phagocytosis" which in turn prompts chronic inflammation and adverse effects. The curvature of the fibers plays a role as well because it affects protein binding and biological responses, as observed for NPs [20]. As a matter of fact, protein adsorption on curved surfaces like that of chrysotile asbestos can be suppressed up to the point when it no longer occurs.

Crystal Structure and Adsorptive Properties

The fiber crystal habit also influences its toxic and pathogenic potential as curled vs. needle-like fibers have different deposition patterns. Compared to needle-like fibers, curled chrysotile fibers tend to deposit in the upper airways where they are more efficiently cleared [21]. The density of a fiber is used for the calculation of its aerodynamic diameter [22] and influences the deposition depth of inhaled particles in the airways [23].

The hydrophilicity/hydrophobicity of fibers affects their adsorption of biopolymers and interaction with human phagocytic cells. Hydrophobic surfaces adsorb biopolymers more strongly than hydrophilic surfaces and are more prone to cell uptake [24]. The surface area of a fiber is a factor that affects not only its biodurability and dissolution rate but also its availability for interaction with cells.

Iron and Trace Metals

Concerning the different chemical parameters of fibers, iron and especially active Fe^{2+} sites available at the surface of asbestos minerals promote the formation of hydroxyl radicals ($HO^{\bullet}$) and have been associated with cytotoxic and genotoxic effects [25]. The surface availability of iron favors the production of $HO^{\bullet}$ through the Haber–Weiss cycle, whenever H_2O_2, the radical species superoxide (O_2^-) and free oxygen are released in vivo by macrophages during the inflammatory burst, following frustrated phagocytosis [26]. The activity of surface iron is also dependent upon its nuclearity at the catalytic site, the number of iron atoms joined in a single coordination entity by bridging ligands. Cluster nuclearity is indicated by monomeric (single iron atom, no other iron atoms in the second shell coordination), dinuclear or dimeric (a cluster of two iron atoms, connected by a bridging oxygen atom), trinuclear or trimeric (a cluster of three iron atoms, connected by bridging oxygen atoms), and so on [22]. The sites with isolated $(FeO)^{2+}$ structures are the preferred candidate active sites $((H_2O)_5FeO)^{2+}$ as they have a low iron nuclearity [27].

The rate of fiber dissolution controls the amount of bulk iron that becomes available for the production of HO• at the surface of the fibers [22]. Despite the huge difference in iron content between iron-poor chrysotile and the iron-rich amphiboles, crocidolite and amosite, the much faster dissolution rate of chrysotile compared to amphiboles prompts comparable amounts of available active surface iron within a short time frame [22].

The content and association of asbestos fibers with trace metals are important as these elements are capable of inducing lung cancer [28]. Asbestos fibers can act as carriers of trace elements [29] as well as PAH as described later in this chapter. Because chrysotile undergoes faster dissolution in comparison to amphibole asbestos, it may release its metal cargo in the lung environment, mimicking the phenomenon that explains the toxicity of nanoparticles. Hence, a non-biodurable fiber (e.g., chrysotile) should be undeniably considered less hazardous than a biodurable fiber (e.g., crocidolite: [30, 31]) but its rapid dissolution may prompt acute release of toxic metals in the intracellular/extracellular medium.

Biodurability

As introduced in the previous paragraph, a basic property of mineral fibers is their biodurability (see above), one of the two components of biopersistence [32, 33] which play a key role in the fibers' toxicity paradigm [34]: a fiber rapidly dissolving in lung fluids has a low biopersistence and is considered less harmful. It is long known that the

biodurability of chrysotile is much lower than that of amphibole asbestos [35, 36]. For long fibers that cannot be fully phagocytosed, biopersistence is a key determinant of potential toxicity over time. If long fibers are biosoluble in lung fluids, they can either dissolve or break apart into shorter fibers and be cleared. Long fibers which are not biosoluble will persist in the lung and initiate inflammatory and carcinogenic responses.

The amount of silica-rich reactive relicts produced during the dissolution of mineral fibers is another critical parameter that should be taken into account in assessing the toxicity/pathogenicity potential of a mineral fiber. In chrysotile, the first step of dissolution produces a "pseudomorphic" Si-rich amorphous phase [37] characterized by silanol groups (Si–OH) and ionized silanol groups ($Si–O^-$) that may prompt the production of HO• [38]. If this proviso is correct, when rating the toxicity/pathogenicity of a mineral fiber, one should consider the rate of production of reactive silica-rich relicts during the dissolution process [22]. The rate of release of metals must also be carefully evaluated as they display a catalytic activity with production of $HO^{\bullet}$ and other reactive species when they are available at the surface of the particles.

A re-evaluation of the content of metals in mineral fibers and their possible adverse effects in vivo considers the so-called "Trojan horse-type effect" observed for NPs [39]. In NPs, intracellular ion release elicited by the acidic conditions of the lysosomal cellular compartment is responsible for the sequence of events associated with their intracellular toxicity [40]. For a wide class of NPs, the acidic environment of the lysosomes triggers the release of relatively toxic ions in the cell and these ions can be the true mediators responsible for the observed intracellular toxicity profiles [40].

Surface Charge

Concerning the surface activity of mineral fibers, the ξ potential, a measure of the surface charge of particles, may correlate with a number of phenomena responsible for adverse effects [22]. A negative ξ potential may prompt the formation of $HO^{\bullet}$ in contact with peroxide and may favor the binding of collagen and redox-activated Fe-rich proteins. It may also affect crosstalk phenomena and apoptosis [41]. The ξ potential of mineral fibers also affects their agglomeration. This is a critical point as conditions having the highest degree of agglomeration induce highest biological responses [42]. Hence, fibers with low absolute values of ξ potential (i.e., tendency to agglomerate) are virtually more prone to cause adverse effects such as frustrated phagocytosis compared to fibers with high absolute values of ξ potential (i.e., stable) [22].

Interactions Between Cigarette Smoke and Asbestos Fibers Affecting Deposition in the Lung

Inhalation is the primary route of entry of asbestos fibers and components of cigarette smoke into the lung. The normal human lung is equipped with a battery of effective clearance mechanisms including a mucociliary escalator comprised of ciliated and mucin-secreting cells, alveolar and interstitial macrophages, and a lymphatic system allowing transfer of particles to distal sites and elimination from the body. Inhaled fibers first encounter a variety of inflammatory cell types and are also taken up by epithelial cells lining the airways and alveoli (Fig. 12.1). These are the cell types developing into bronchogenic and peripheral lung carcinomas.

Recent reviews point to the importance of asbestos fiber type, geometry, length, and high aspect (length to diameter) ratio as important determinants of cancer risk [43–46]. One reason is because long (>15–20 μm), thin amphibole fibers exceeding the cell diameters of human and rodent alveolar macrophages are cleared less effectively and remain in the lung. In contrast, both macrophages and lung epithelial cells engulf shorter fibers and transport them intra- or intercellularly to distal sites including the lung interstitium [47, 48].

Effects on Clearance Mechanisms

Several studies show that toxic components of cigarette smoke impair clearance of asbestos and other particles from the upper airways [49–52]. For example, smoking hinders the removal of amosite asbestos fibers after their intratracheal injection into rats [50, 51]. This is accompanied by toxicity to lung epithelial cells and increased penetration of fibers into airway walls. A comparison between asbestos fiber burdens in cigarette smokers versus never smokers, both groups with heavy occupational asbestos exposures, showed that cigarette smoking caused enhanced accumulation of both amosite and chrysotile asbestos in the airway mucosa [52]. Cigarette smoke or amosite exposures produced increased bromodeoxyuridine (BrdU) labeling, a marker of unscheduled DNA synthesis, in small airway walls, epithelial cells, and pulmonary artery cells, and a brief synergistic increase in cell labeling in the small airways was noted with both agents [53]. Tracheal organ cultures showed that amosite fiber binding to epithelial cells was a rapid process that was enhanced in the presence of cigarette smoke [54]. These authors concluded that iron on the surface of fibers was important in cellular adhesion of fibers to epithelial cells.

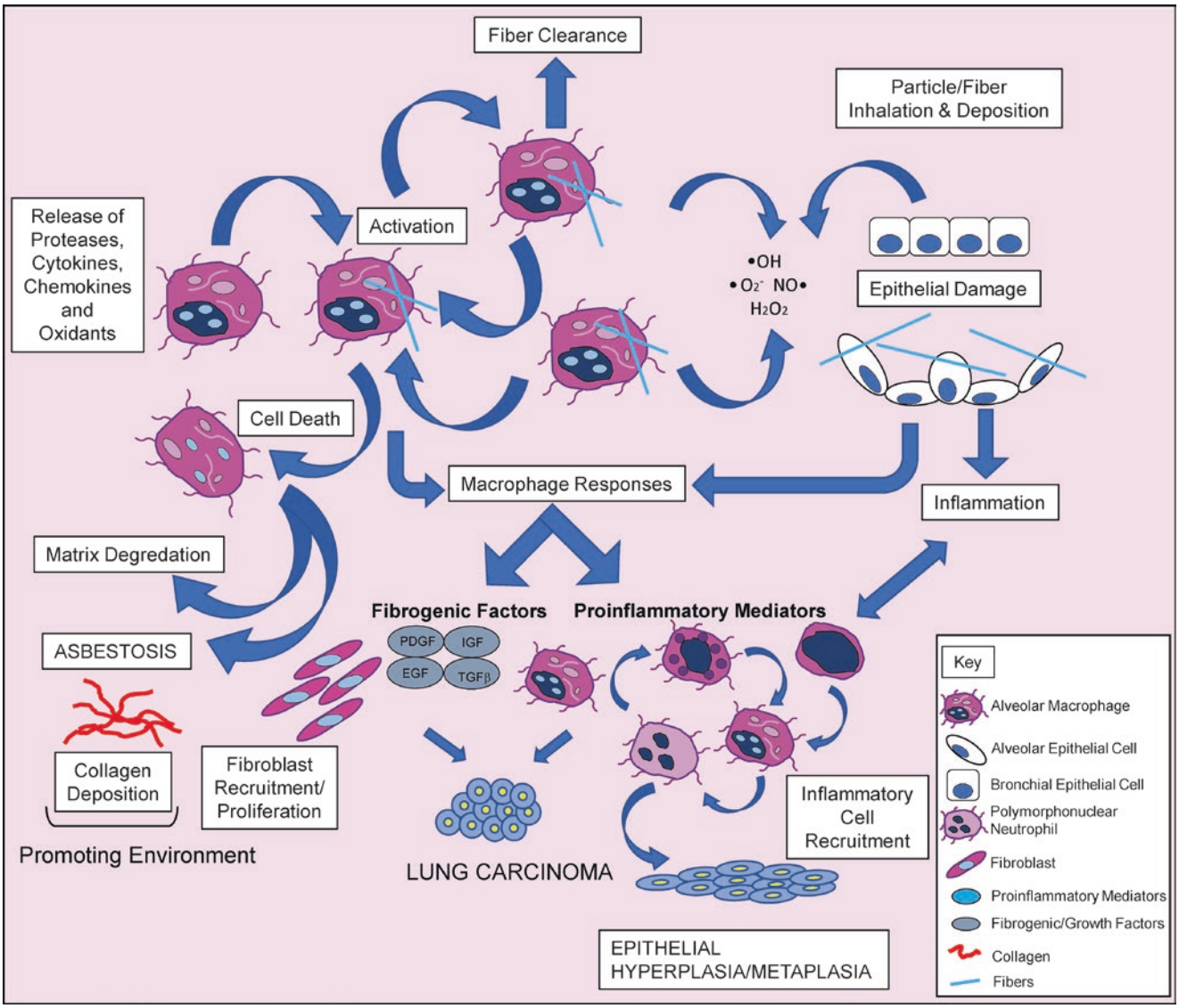

Fig. 12.1 A diagram illustrating the complex cellular responses in the lung after inhalation of asbestos fibers. The development of chronic inflammation and fibrosis (asbestosis) creates a lung microenvironment favoring lung carcinomas

Metabolism of Chemical Carcinogens

Asbestos fibers cause increases in uptake and metabolism of polycyclic aromatic hydrocarbons (PAH) by lung epithelial cells. PAH are perhaps the most widely studied chemical carcinogens of the many mutagenic and carcinogenic substances found in the particulate or vapor phases of cigarette smoke. They are known to form adducts with DNA that are linked to their carcinogenicity. PAH adhere to asbestos fibers and other particles in the atmosphere and are eluted from the particle surfaces in the upper airways [55]. In vitro studies have shown that dispersions of PAH alone are not readily taken up by tracheal epithelial cells. However, epithelial cell uptake and retention over time, as evidenced by adduct formation of PAH with DNA, are substantially increased when PAH are pre-adsorbed to chrysotile or crocidolite asbestos before their addition to cell cultures [56, 57].

Crocidolite asbestos and PAH interact synergistically to cause cell proliferation and squamous metaplasia, a preneoplastic lesion, in tracheal organ cultures [58]. Both agents are required for the development of tumors after implantation of explants into syngeneic animals [59, 60]. In assessing a number of particles (crocidolite asbestos, kaolin, carbon, hematite) as carriers of PAH in these studies, no tumors were observed with either particles or PAH alone. However, a direct relationship was observed between numbers of tumors and the amount of PAH adsorbed to particles when explants were exposed to PAH-coated particles.

The studies above suggest that asbestos and other particles act as vehicles for adsorption and delivery of chemical

carcinogens to lung tissues. Thus, doses to epithelial cells, the progenitor cell types of lung cancer, are increased. In addition, PAH and asbestos may cooperatively activate cellular pathways that are important in initiating hyperplasia or cell proliferation as well as squamous metaplasia, critical early lesions in the development of lung carcinomas. (See also Chap. 13: Co-carcinogenesis of PAH and inhaled particulates)

The induction of squamous metaplasia by cigarette smoke was noted in humans by Auerbach [61] and has been widely studied in hamster and human tracheal explants using a variety of particles of different geometries and dimensions [58, 62–66]. In these models, the severity and extent of squamous metaplasia are dose-related when long (>10 μm length) rod-like fibers are added to explants. These fibers serve as matrices for epithelial cell proliferation, whereas short fiber analogs and cleavage fragments do not.

Modern Concepts of Carcinogenesis

The majority of chemical carcinogens tested in bacterial and mammalian cell assays are mutagens that directly interact with DNA or require cell metabolism to do so. Mutations are also caused by replication errors and heredity, giving rise to a hypothesis in which the overwhelming drivers of cancer risk are accumulated mutations [67]. This model has been criticized by some as it does not reflect the importance of tissue microenvironments, evolutionary processes, and epigenetic events in tumor development [68].

In a two-step model of carcinogenesis developed in the 1960s [69], "initiation" of cancers was viewed as an irreversible effect caused by a heritable mutation in DNA, whereas the second stage, "promotion" encompassed a series of events during the period from initiation to the demonstration of frank cancers. This model has evolved into a contemporary multi-step model of tumor progression defined broadly as a stepwise series of events favoring increased genomic instability of cells during which they acquire invasive and metastatic properties. During tumor promotion and progression, premalignant cells are rapidly dividing, and additional errors in DNA replication and repair accrue. Emphasis in cancer research has shifted from studying mutations and genetic changes in DNA to revealing proteins and transcription factors that stimulate cell signaling and mitochondrial pathways necessary for malignant tumor development [70].

The term "epigenetics" has evolved over time to explain traits not involving alterations in the primary structure or sequence of DNA. According to definition, "an epigenetic trait can be a stable inheritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" [71]. Epigenetic events also can be reversible as a result of many repair pathways.

Multiple modes of epigenetic signaling have been recognized including DNA methylation, histone modifications, chromatin remodeling, and effects induced by noncoding RNAs, a class of regulatory molecules that control gene expression by binding to complementary sites on target messenger RNA (mRNA) transcripts. Noncoding RNAs can be long (lncRNAs) or short (miRNAs) and can alter expression of multiple mRNAs. Downregulation of certain miRNAs is observed in a number of human cancers, suggesting their functional similarities to tumor suppressor genes. Other miRNAs can regulate cell differentiation and programmed cell death, i.e., apoptosis. For these reasons, they are under investigation as biomarkers, prognostic factors, and therapeutic targets in lung cancers (see below).

It is important to recognize that there are many different manifestations of toxic injury at the cell and tissue level that are dependent on concentration, type, and other properties of mineral fibers. For example, at high concentrations of agents, cell death frequently occurs, precluding transfer of mutagenic and other heritable alterations to cell progeny. However, at low concentrations, cells may remain intact or exhibit uncontrolled cell proliferation and other heritable, functional and phenotypic changes that may be critical to tumor development. Often the term "genotoxicity," i.e., alterations in the genome of cells resulting in cell death, altered function or division of cells, is used incorrectly and synonymously with "carcinogenicity" in the scientific literature.

Genetic Alterations in Human Lung Cancers by Cigarette Smoke and Asbestos

Certain oncogenes and tumor suppressor genes are the likely targets of somatic alterations resulting from tobacco smoke carcinogens including PAH, nitrosamines, and aromatic amines [reviewed in [1]]. Karyotypic analyses and molecular screening show that lung cancer cells typically demonstrate dozens of genetic lesions including aneuploidy gene copy number alterations, and alterations in proto-oncogenes or their encoded proteins. These changes include mutations in growth factor receptors, tyrosine and serine-threonine protein kinases (both receptor and non-receptor-related), membrane-associated G proteins, and nuclear transcription factors.

Oncogenes

The most common aberrations in lung cancers are overexpression (due to mutations and/or chromosomal rearrangements) of members of the epidermal growth factor receptor (ErbB family) of tyrosine kinases. These include ERBB1 (also known as EGFR) and ERBB2. Chromosomal

rearrangements involving the tyrosine kinase anaplastic lymphoma kinase (ALK), ROS1, and an orphan receptor tyrosine kinase also are noted. Mutations in KRAS correlate with smoking history and occur more frequently in tumors from former or current smokers [72], and mutations in BRAF (a downstream effector of the RAS pathway) are also observed. Amplification and/or mutations in the nuclear transcription factors MYC, MYB, JUN, and FOS also occur in lung tumors although their precise roles in lung carcinogenesis are unknown [1].

Tumor Suppressor Genes

A number of tumor suppressor genes (TSG) also undergo structural abnormalities and loss of function in lung cancers. These include TP53, genes in the RB1/Cyclin D1/CDK4/CDKN2A pathway, candidate chromosomal 3p tumor suppressor genes, and the LKB1/STK11 gene. Involvement of other tumor suppressor genes has been suggested based upon the loss of many corresponding chromosomal regions in lung cancers [1].

Modern technology has enabled genome-wide investigation of somatic mutations as well as gene expression profiling of lung cancers. Most recently, knowledge of gene deregulation caused by cigarette smoking and persisting after smoking cessation has been obtained from a gene expression (mRNA profiling) study that examined human airway epithelial cells isolated from bronchoscopy in smokers and never smokers [73–75]. A gene biomarker panel could distinguish between smokers with and without lung cancers [75].

A recent examination of mutations by exome sequencing in lung adenocarcinomas in Finnish patients with occupational exposures to asbestos suggests that smoking is an overriding confounder in interpretation of results [76]. Only 1 tumor from 26 patient samples was from a never smoker. KRAS mutations occurred in 42% of patients with and without exposures to asbestos, and less frequent BRAF mutations were also observed. Both mutations were associated with smoking, but not asbestos exposures. Moreover, no activating EGFR mutations could be attributed to asbestos exposures.

Another study explored the occurrence of somatic mutations (EGFR, ERBB2, HER2, KRAS, BRAF, PIK3 kinase, and ALK) in lung cancers from never smokers with occupational exposures to asbestos, silica, diesel exhaust fumes, chrome, and paints [77]. Asbestos-exposed patients exhibited a significantly lower rate of EGFR mutations but a higher rate of less frequent HER2 mutations. These investigators concluded that occupational exposures "slightly affect the molecular pattern of lung cancers in never smokers" [77]. The studies summarized above indicate that driving mutations by asbestos in lung cancers are absent or obscured by changes rendered by cigarette smoking.

Epigenetic Effects of Asbestos in Lung Cancers and Human Bronchial Epithelial Cells

Epigenetic markers include: (1) Noncoding RNAs, including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs); (2) Histone modifications, DNA methylation changes, and chromatin remodeling.

Noncoding RNAs

Small single-stranded RNA molecules have been widely studied in lung cancers [reviewed in [78–80]]. Over 30% of exons (protein-coding human genes) are regulated by miRNAs, and an estimated 1000 or more human miRNAs exist [81]. Nucleotide precursors, i.e., pre-miRNAs, are transported from the nucleus into the cytoplasm where they are further processed to generate a mature, double-stranded duplex (miRNA/miRNA) as part of an RNA-induced silencing complex (RISC). RISC and its miRNA complex then bind to a number of target mRNAs to cause cleavage or translational repression. miRNA loss and downregulation have been observed in a number of tumor types including lung cancers [78–80] and mesotheliomas [82]. In contrast to miRNAs, long noncoding RNAs (lncRNAs) have not been studied as intensely but are important in epithelial mesenchymal transition (EMT), tumor progression, and metastases [83]. LncRNAs function as chromatin modulators in that they target histone-modifying enzymes to repress homeobox transcription factor (HOX) genes aberrantly expressed in some tumors [84] as well as genes suppressing metastases [85].

Histone/DNA Modifications

Histone acetylation (addition of $–COCH_3$) and removal, i.e., via deacetylation and methylation, affect nucleosome–DNA interactions and result in altered gene expression. In general, acetylation has been linked to increased accessibility of DNA as euchromatin, whereas methylation causes condensation of chromatin, making it inaccessible for transcription. The most frequently studied epigenetic marker is DNA methylation, a process catalyzed by DNA methyl transferases (DNMT) and resulting in covalent attachment of a methyl group to cytosine. This also occurs at sites of CpG dinucleotides located within the promoter regions of genes.

Aberrant DNA methylation, characterized by hypermethylation of CpG islands, as well as hypomethylation of other regions occurs commonly in several tumor types. These alterations lead to silencing of tumor suppressor gene and/or genomic instability [86]. Overall, human tumors show global hypomethylation or hypermethylation of CpG islands. Methyl-DNA binding domain (MBD) proteins interact with different chromatin-modifying proteins to form compact chromatin with repression of transcription. Different CpG island methylation patterns recruit different sets of MBD proteins that may assume unique functions. These changes may also be important in epithelial cell gene silencing, the development of EMT, and the evolution of tumors [87].

DNA Methylation Changes in Lung Cancers and Mesotheliomas

Although epigenetic signatures have been widely studied in lung cancers in general [reviewed in [1, 78–80, 88, 89]], and less frequently in mesotheliomas [reviewed in [3, 82, 90]], little information is available on epigenetic changes by cigarette smoke or asbestos in lung tumors or human bronchial epithelial cells. Recently, asbestos and smoking associated genome-wide DNA methylation were examined in lung cancer tissues from asbestos-exposed or non-asbestos-exposed patients [91]. Both groups consisted of mostly smokers. Hypomethylation was an overall characteristic of differentially methylated regions (DMR) in lung cancers from asbestos-exposed patients. Moreover, when patterns of methylation in asbestos-related vs. "mostly smoking related" tumors were compared, novel methylation changes appeared to be specific for each of the two risk factors.

Aberrant methylation of the CDKN2A/p16INK4A gene promoter region and other TSGs that have been associated with cell cycle control has been reported in human mesotheliomas [92]. The *CDKN2A* locus encodes the tumor suppressor proteins, p16INK4 and p14ARF known to regulate the Rb and p53 cell cycle regulatory pathways. In these patients, lung content of asbestos (ferruginous) bodies was measured as an indication of exposures to asbestos. These studies are important as they show a direct relationship between numbers of asbestos bodies and increases in methylation changes related to gene silencing, thus providing a causal link between asbestos, methylation of TSGs, and the development of tumors. Loss of CDK2NKB function has also been noted in both lung cancers [reviewed in [1]] and experimental models of mesothelioma, where increased sensitivity to crocidolite asbestos reflected increased numbers of tumors with decreased latency periods in knockdown animals [93].

Methylation status of the CDKN2A gene has also been evaluated in precancerous bronchial lesions from a series of 37 patients at high risk for lung cancer [94]. Aberrant methylation of the CDKN2A promoter was found in 19% of preinvasive lesions. Increases in frequency occurred with the severity of lesions, suggesting its causal relationship to the development of lung cancers.

Asbestosis is a lung disease where increased interstitial accumulation of asbestos fibers occurs in the lung with the development of chronic inflammation and thickening of lung matrix. A recent study evaluated the functional ramifications of loss of CDKN2B function in lung tissues and isolated lung fibroblasts from patients with idiopathic pulmonary fibrosis (IPF), an interstitial disease with many similarities to asbestosis in that proliferation of lung fibroblasts and their differentiation to myofibroblasts occur [95]. In comparison to normal controls, fibroblasts from patients with IPF showed hypermethylation at the CDKN2B gene locus, and decreased protein expression in lungs was localized to regions of myofibroblast and fibroblast accumulation. Targeted overexpression or silencing of CDKN2B caused inhibition of or increased myofibroblast differentiation, respectively, but did not affect cell proliferation *per se*. Cdkn2b knockout mice also developed more fibrosis after exposures to bleomycin when compared to wild-type rodents. Although CDKN2B is traditionally regarded as a cell cycle inhibitor, its roles in proliferation and altered differentiation may be multi-faceted.

DNA Methylation Changes by Asbestos

A very recent study has documented global and gene-specific DNA methylation effects of different types of asbestos fibers on immortalized human bronchial epithelial cells [96]. DNA methylation on CpG sites was evaluated as these are the most common sites of altered DNA methylation in cancers. Global DNA methylation on total cytosine residues was quantified over a range of asbestos concentrations, and subsets of differentially methylated genes at a single concentration of each fiber type (amosite, crocidolite, and chrysotile) were examined. Since asbestos exposures in vitro are typically at high concentrations of fibers that induce chromosomal aberrations, micronuclei formation, and DNA strand breaks in rodent cells, a COMET assay for DNA strand breaks was used to show the correlations between DNA damage and cell viability in human cells. Comparisons here showed that chrysotile asbestos was most damaging to cells at equal weight concentrations when compared to both amphibole types of asbestos. Also noteworthy was the detection of dose-related DNA damage at amounts of dusts not affecting cell viability, suggesting the effectiveness of DNA repair processes at lowest concentrations of asbestos fibers.

Others have shown that chrysotile is more cytotoxic than crocidolite or amosite asbestos on an equal mass or fiber concentration basis in rodent and human lung epithelial and

mesothelial cells [97–99]. Moreover, large-scale deletions incompatible with cell viability have been noted by chrysotile in a hamster-human hybrid cell mutation assay [100]. However, despite the increased cytotoxicity of chrysotile, global DNA methylation was only observed after exposures to crocidolite or amosite asbestos—no changes were observed at the lowest concentrations of amphibole fibers indicating a threshold effect [96]. Exposure to either amphibole type induced global hypo- and hypermethylation at CpG sites, whereas exposure to chrysotile induced differential methylation only in gene promoter regions with a different frequency distribution. Hierarchical clustering of gene-specific DNA methylation patterns also showed differential clustering in chrysotile-exposed cells. Gene functional classification of shared genes methylated after exposure to all types of asbestos revealed five common clusters related to: (1) nuclear (homeobox or HOX) transcription factors that control embryogenesis; (2) ATP binding functions; (3) Rho proteins and serine-threonine and tyrosine protein kinases; (4) Wnt signaling family members; and (5) Ankyrin repeat domains and NF-KB inhibition.

Epigenetic signatures and RNA profiling (described below) are promising as they detect changes specific to smoking or asbestos exposures. In addition, they appear to reveal differences between types of asbestos and inhaled minerals that may reflect their respective pathogenic potentials in lung and pleural diseases. Dose–response experiments suggest a threshold for responses as has been demonstrated for chrysotile exposures in human lung cancers [101]. Lastly, once gene promoter methylation targets, i.e., specific genes, are identified, overexpression and inhibition studies can be performed to determine the functional significance of these events in carcinogenesis.

RNA Profiling Studies and Asbestos-Induced Pulmonary Responses in Animals

Inhalation is the physiological route of exposures to mineral fibers, but long-term inhalation experiments are expensive and time-consuming [reviewed in [99]]. Whereas experiments using intratracheal instillation and injection of particles have many limitations [99, 102], oropharyngeal aspiration provides dissemination of materials throughout the lung without impairment of clearance mechanisms.

Gene expression profiles (mRNA profiling) have been recently examined in mice after a single oropharyngeal aspiration of asbestos fibers (crocidolite, tremolite), erionite (a non-asbestos fiber associated with increased mesotheliomas and lung cancers in humans), and wollastonite (a fiber not associated with adverse health effects) [103]. Inflammatory cell and cytokine changes and tissue responses were evaluated at days 1, 7, and 56 days post exposures, and a high-throughput mRNA microarray analysis was performed at 7 days. To identify pathways and networks perturbed by various fiber preparations, ingenuity pathway analysis was performed on differentially expressed gene expression. The targeted dose of each fiber preparation was calculated as 8.8×10^7 fibers/mouse although it was noted that the total numbers of fibers for erionite or wollastonite were less than asbestos fibers. The crocidolite preparation had the greatest range of fiber lengths and high aspect ratios followed by tremolite, erionite, and wollastonite that consisted almost exclusively of shorter, low aspect ratio fibers. Overall, the severity of both inflammation and fibrosis was greatest with crocidolite, but cytokine responses were different with erionite and wollastonite exposures as compared to the two asbestos types. Analyses of the top 10 significantly upregulated vs. downregulated genes in each of the four treatment groups showed only one common gene (chloride channel accessory 1 or CLCA1) that was upregulated in all mineral groups. The variability in data may reflect the fact that whole lung homogenates consisting of multiple cell types and the small numbers of animals (N = 3/group) were analyzed in gene profiling experiments.

Cell Signaling Pathways

Cell signaling pathways are other routes linked to altered cell proliferation and differentiation that are perturbed by asbestos fibers as they come in contact with lung epithelial cells. Many of these pathways are also stimulated after interaction of fibers with receptors on cells or by active oxygen and nitrogen species (ROS/RNS) that are generated by fibers (see below). Alternatively, crosstalk between macrophages and/or lung fibroblast and epithelial cells stimulates many cell signaling pathways as well as cytokine circuits that may cause epithelial cell proliferation or injury [reviewed in [99]]. As indicated in Fig. 12.1, circuits of activated macrophages and other immune cell types may amplify these responses.

After exposures in vitro to long (>10 μm), thin fibers, epithelial cells exhibit many alterations in mitogenic signaling pathways that are linked to increased survival, proliferation, and disruption of cell cycle control. Activation of receptor tyrosine kinases (RTK), mitogen activated protein kinases (MEK1 or Ras/Extracellular Signal Regulated Kinases (ERK1/2)), and phosphatidyl 3-kinase (PI3)-kinase/AKT pathways are events observed after exposures to asbestos fibers [reviewed in [99, 104–113]]. These signaling pathways and their protein targets can be stimulated by: (1) increased activity of RTKs or their receptors and ligands; (2) phosphorylation or dephosphorylation of specific kinases; (3) increased activation and binding of transcription factor proteins to target genes; and (4) inactivation of negative regulators in these cascades.

Lung epithelial cells express a number of cytokine and chemokine receptors that trigger inflammation and cell proliferation. Cells also respond to a broad array of growth factors that are stimulated by autocrine or paracrine mechanisms, including epidermal growth factor (EGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), tumor necrosis factor α (TNF-α), interleukin 8 (IL-8), fibroblast growth factors (FGFs), transforming growth factor β (TGF-β), and insulin-like growth factor 1 (IGF-1) [reviewed in [99]].

Activation of the MEK1/ERK1/2 cascade results in induction of AP-1, a heterodimeric transcription factor comprised of members of the c-Fos and c-Jun proto-oncogene families. These kinases in turn phosphorylate a number of intracellular substrates and increase gene expression of respective proto-oncogenes as well as other proliferation-related genes such as *cyclin D1*. After inhalation of crocidolite asbestos, cell-specific increases in unphosphorylated and phosphorylated ERK1 and ERK2 are noted in bronchiolar and alveolar type II epithelial cells in areas of epithelial cell hyperplasia [104]. Asbestos-exposed transgenic mice expressing a dominant-negative MEK1 targeted to lung epithelial cells to inhibit this signaling pathway show less cell proliferation in response to asbestos, suggesting a causative role of ERK1/2 signaling in lung epithelial cell proliferation [105]. Related studies have indicated that crocidolite asbestos causes increased c-Jun expression in tracheal epithelial cells [106] and in lung homogenates after inhalation in a dose-related fashion [107]. These changes are not observed after exposures to riebeckite or polystyrene beads in vitro [106, 109].

Crocidolite asbestos fibers also cause dose-dependent proliferation of lung epithelial and pleural mesothelial cells after inhalation that are sustained after cessation of inhalation [reviewed in [99, 107, 108, 110]]. Epithelial cell proliferation at high airborne concentrations of crocidolite is accompanied by inflammatory and fibrotic changes that are known to perpetuate lung cancers.

EGFR Receptors

Mutation or activation of EGFR receptors is linked to stimulation of a number of cell signaling cascades including MEK1/ERK1/2 and the AKT pathway. Long crocidolite asbestos fibers activate the EGFR via direct membrane interactions or by affecting the kinetics of EGFR binding to its ligands [111, 112]. A direct link between the EGFR and expression of Fos and Jun family members has been shown in mutant EGFR mice exhibiting loss of function in pulmonary epithelial cells [113]. After inhalation of crocidolite asbestos, mice with downregulation of EGFR exhibit loss of epithelial cell proliferation and no increases in Fos/Jun expression [113]. As emphasized above, gain of function mutations of the EGFR receptor family and consequent upregulated signaling cascades are a feature of many lung cancers, and blockade of EGFR signaling is an approach used in patient populations demonstrating mutations and other anomalies in this pathway [114].

Uptake of Asbestos Fibers by Lung Epithelial Cells

In tracheal or lung epithelial cells, short asbestos fibers and fragments are incorporated into membrane-bound phagolysosomes without morphologic or quantitative decreases in cell viability [47, 97, 99]. Fibers less than 5 μm in length accumulate in the perinuclear region of lung epithelial cells and are presumably transported away from a forming mitotic spindle [115]. However, long, thin crocidolite fibers may orient parallel to the mitotic spindle and attach to the nuclear envelope, sterically blocking cytokinesis when cells divide. Interactions between lung epithelial cells and crocidolite asbestos fibers were studied using high resolution time lapse video-enhanced microscopy during mitosis [116]. These studies showed that physical interactions between long crocidolite fibers and chromosomes occurred randomly and infrequently with most crocidolite-containing cells completing mitosis normally. Although physical interactions of crocidolite asbestos fibers with DNA have been suggested as a mechanism of aneuploidy, studies with lung fibroblasts show that intracellular asbestos fibers induce aneuploidy by binding to a subset of intracellular proteins that regulate the cell cycle and cytoskeleton [117].

The kinetics of uptake of asbestos fibers by different cell types in the lung and pleura may be different. For example, human mesothelial cells are more sensitive than human bronchial epithelial cells and fibroblasts to the cytotoxic and genetic changes triggered by amosite asbestos, phenomena linked to increased uptake of fibers by human mesothelial cells [98]. Species-specific differences in DNA repair may also be relevant to cell response. For example, in contrast to crocidolite, chrysotile is 100 to 300 times more toxic to human bronchial epithelial cells but does not induce significant numbers of chromosome changes [118]. Micronuclei formation, i.e., small fragments of chromosomes, is observed as a consequence of chrysotile but not of crocidolite exposures. Overall, changes in chromosomal stability are more infrequent than reports in the literature using rodent cells.

The Role of the Lung Microenvironment in Lung Cancer Development

Lung epithelial cells are critical in repair of the lung after exposures to cigarette smoke or asbestos. Epithelial cells interact with macrophages and other cells of the immune

system as well as other cell types to maintain the normal architecture of the lung. However, epithelial cell perturbations and lung cancers occur when the normal defense mechanisms of the lung are overwhelmed.

Chronic Inflammation

The interplay between alveolar macrophages, polymorphonuclear leukocytes (PMNs), and epithelial cells in early injury by asbestos fibers has been studied historically in acute inhalation studies [reviewed in [99, 119, 120]]. Tumor-associated macrophages are also critical to establishing and maintaining lung cancers as well as promotion of metastases [reviewed in [121, 122]].

Early inflammation after exposure to asbestos is characterized by activation of multiple signaling pathways in activated macrophages and epithelial cells that produce a number of cytokines and chemokines affecting cell function and repair [reviewed in [99]]. At concentrations of fibers causing overload of defense mechanisms, more cells may be recruited to sites of fiber injury, leading to chronic inflammation and disease. For example, NADPH oxidases are upregulated and activated in cells after frustrated phagocytosis of long fibers [123] and in epithelial cells forming carcinomas [124]. Oxidants are generated via an NADPH-dependent process, inducing genetic and epigenetic changes [reviewed in [125]].

Inflammatory processes including ROS induce a number of epigenetic events linked to tumorigenesis, and fibrosis [121, 126, 127]. Moreover, chronic inflammation is associated with the development of fibrosis and lung cancers both in animal models [reviewed in [99]] and humans [119, 124]. Rodent inhalation studies by Davis and colleagues emphasize the importance of long fibers in inflammation, asbestosis, and lung cancers [128–130].

Inflammasomes

Inflammasomes play critical roles in the development of chronic inflammation, pulmonary fibrosis, and lung cancers. "Inflammasomes" are cytoplasmic protein complexes activated upon recognition of a number of diverse "danger signals." Their assembly and activation are associated with exposures to pathogenic particles and fibers in a number of cell types [131]. Macrophages have been studied most frequently with regard to the mechanisms of uptake of exogenous crystals such as silica, asbestos, and nanomaterials and consequent activation of the NLRP3 inflammasome [reviewed in [132]]. Priming and activation of the NLRP3 inflammasome are linked causally to early inflammation and cytokine release after inhalation of chrysotile asbestos in rodents [123], and a number of particle characteristics have been linked to inflammasome activation by different pathogenic fibers and particles [reviewed in [131]].

A recent review summarizes the multi-faceted roles of different inflammasomes in lung cancers and other tumors, emphasizing their distinct roles in release of inflammatory cytokines, cell death, and tissue repair [133]. Cigarette smoke causes activation and release of IL-1B and CXCL-8, critical inflammatory cytokines produced after inflammasome activation, from human bronchial epithelial cells [134]. A number of other alterations in immune response and cell proliferation have been linked to inflammasome activation in lung cancers including establishment of a lung microenvironment that permits growth, progression, and metastases of lung tumors [reviewed in [133]].

ROS, including mitochondrial-derived oxidants, are known effectors of inflammasome activation and function [reviewed in [131]]. Mitochondrial DNA damage and apoptosis are noted in alveolar epithelial cells after exposure to amosite asbestos [135] and mitochondrial-derived oxidants contribute to crocidolite asbestos-induced gene expression of NF-κB and MIP-2 [136]. Accumulation of the NLRP3 inflammasome in cytosol is dependent upon its production by NF-κB signaling and removal by autophagy. The priming, assembly, and activation of inflammasomes are threshold-like responses in cells exposed to asbestos fibers as are the stability of cytokine and inflammation networks, and the spread of inflammation [137]. These damage thresholds reflect cooperativity of a number of antioxidant pathways and repair responses at low exposures to asbestos fibers. However, at high occupational exposures, interstitial disease or asbestosis can occur and this may create a lung environment conducive to the development of lung tumors (see Fig. 12.1).

Interstitial Accumulation of Asbestos and Formation of Asbestos Bodies

If both mucociliary clearance of inhaled fibers and phagocytosis of fibers by macrophages fail, the ultimate response to interstitial fibers is isolation of the invading fiber via encapsulation inside the so-called *asbestos body* (AB). The first AB was observed in 1906 in a human lung as pigmented crystal [138], but was at that time called *asbestosis body* [139]. Only later the term was substituted by *asbestos body* when these aggregates were discovered in patients with lung diseases other than asbestosis [140].

When ABs were found to grow also around fibers other than asbestos (e.g., Al-silicates and glass fibers) or around particles of uncertain nature, the term *ferruginous body* was applied [141]. The term *asbestos body* is now generally used to indicate bodies containing asbestos fibers while the term *ferruginous body* or *pseudoasbestos body* is applied to all non-asbestos-containing aggregates [142].

The coating process of the particles is mediated by iron with the ferritin core of ABs composed of ferric oxyhydroxide (FeOOH or $FeOOPO_3H_3$ if phosphate is present [143]). Besides iron and phosphorus, calcium and magnesium may also participate in the coating process (see, for example, [144]). AB formation is extracellular and the various configurations found around the fibers might reflect repeated contact of the same AB with different macrophages [145]. ABs can be formed within 2–3 months of exposure in rats, with a time span of formation in animals similar to humans [146]. A shared mechanism of formation of ABs points to a biological origin via intracellular coating that begins with deposition of a ferritin layer around the fibers. The formation of ABs is certainly a complex and not yet fully understood mechanism that involves many distinct parameters such as the nature of the inhaled fiber, its morphometry, the coating efficiency of the animal host, and the fiber entry process. Differences in accumulation of amphibole asbestos vs. chrysotile fibers within the lungs of different animals following long-term inhalation exposures indicated that the relative retention of amphibole asbestos fibers in the lungs was higher than that of chrysotile [147].

In a recent study aimed at understanding the process of formation of ABs, FEG–SEM (field emission gun-scanning electron microscopy) and μ-Raman were used to investigate the characteristics of both fibers and ABs formed in rats after a single intraperitoneal or intrapleural injection of selected mineral fibers [148]. Regarding the residual fibers found in the tissues of the rats, chrysotile showed a mean fiber length ranging from 14.3 to 15.8 μm with diameters in the range 0.45–0.54 μm. The average size of chrysotile fibers encapsulated in ABs was 29.6 μm in length and 0.5 μm in diameter. Leaching of Mg from chrysotile was also observed in agreement with reports in the literature data [149]. Remarkable variations in size and morphology of ABs formed around chrysotile fibers were noted. Their size ranged from 1.5 to 20 μm in length and from 0.6 to 15 μm in diameter. Uncoated fibers were detected in all samples. The percentage of coated fibers was 3.3%. This relative amount did not change over time, indicating that the number of ABs does not increase with time.

Crocidolite fibers displayed a mean length ranging from 13.7 to 18.6 μm with diameters in the range 0.54–0.71 μm. The mean size of crocidolite fibers producing ABs was 41.0 μm in length and 0.86 μm in diameter. For crocidolite, the size of ABs varied in length from 4 μm to 25 μm, and from 4 μm to 8 μm in diameter. ABs were predominantly formed on long crocidolite fibers and could occur around a single fiber as well as around clusters of particles. Most of the observed fibers were uncoated. The percentage of coated fibers was 6.0% and the relative amount did not change with time.

There were no differences in the characteristics of ABs formed in the pleural and peritoneal cavities. ABs appeared around chrysotile and crocidolite fibers in less than 40 weeks. Such short times of formation are in line with human observations [150]. The large morphological variability of ABs suggests that a high concentration of fibers prompts changes in the shape of ABs and favors the appearance of new forms [151].

Diminished generation of oxidant species and reduced toxicity of coated fibers with respect to uncoated fibers have been reported by many authors [150]. The limited number of coated fibers observed in the tissue of the rats after intrapleural or intraperitoneal injection may be due to both fiber overload and the lack of nutrients, specifically Fe, P, and Ca, to form the asbestos coating. Fibers found in human lungs also display variable degrees of coating and morphologies of ABs. Figure 12.2 portrays examples of naked (a) and coated (b) crocidolite fibers found in the lungs of a patient with occupational exposure to asbestos.

More extensive reviews on ferruginous bodies are provided [150, 152–154].

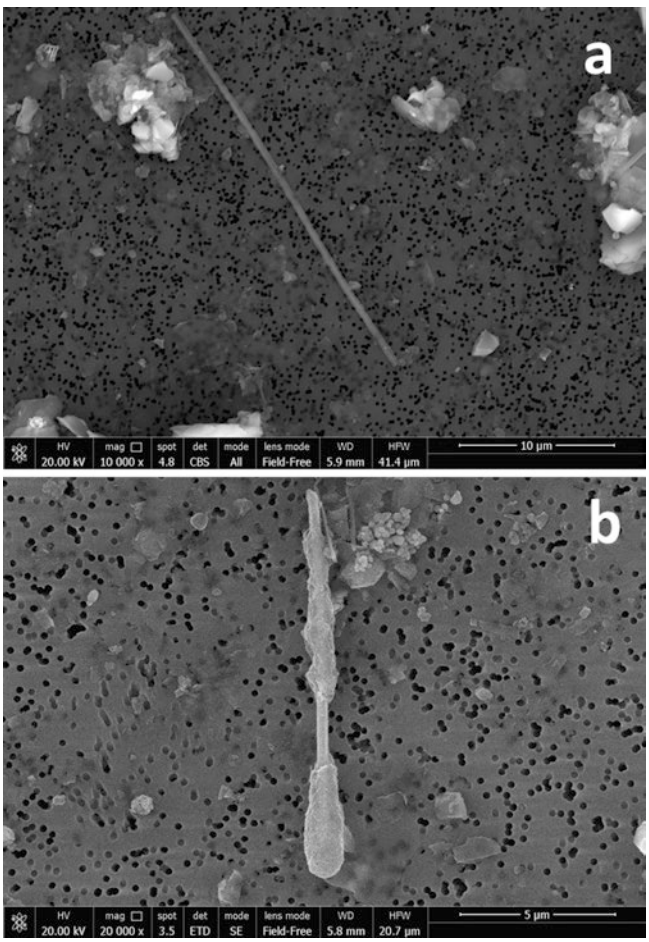

Fig. 12.2 Naked (**a**) and coated (**b**) crocidolite fibers found in the lung lobe of the human lung of a patient developing lung cancer after occupational exposure to asbestos

Towards a Predictive Model of the Potential of Mineral Fibers to Induce Lung Cancer

Recently, a quantitative predictive model for toxicity/pathogenicity of mineral fibers has been developed based upon the physical/chemical and morphological parameters described above [155]. The model derives a Fiber Potential Toxicity Index (FPTI) to predict and rank the toxic and pathogenic potential of asbestos fibers, unregulated/unclassified fibers, and other elongated mineral particles (EMP). The parameters of the model that have been considered are: 1. *Morphometric parameters*: (1,1) mean fiber length, (1,2) mean fiber diameter, (1,3) crystal curvature, (1,4) crystal habit, (1,5) density, (1,6) hydrophobic character, (1,7) specific surface area; 2. *Chemical parameters*: (2,1) iron content, (2,2) content of ferrous iron, (2,3) surface iron and its nuclearity, (2,4) content of metals other than iron; 3. *Biodurability-related parameters*: (3,1) dissolution rate, (3,2) rate of iron dissolution/release, (3,3) rate of silica dissolution/release, (3,4) rate of release of metals from the fiber; 4. *Surface activity-related parameters*: (4,1) ζ potential, (4,2) aggregation state of the fibers in suspension, (4,3) cation exchange capacity (from fibrous zeolite species).

A score is assigned to each parameter depending on its measured value. For example, the mean fiber length L of a fiber species (1,1) takes a score $T_i = 0.1$ if 5 μm < L < 10 μm, $T_i = 0.2$ if 10 μm < L < 20 μm, and $T_i = 0.4$ if L > 20 μm. Because the parameters of the model can be correlated with each other, a hierarchical scheme taking into account cross-correlations was developed. Figure 12.3 [modified after [155]] depicts the scheme of the hierarchical clustering of the FPTI model. A weighing scheme is associated with each parameter of the model according to its step/hierarchy H where $w_1 = 1/H$ with H = 1, 2, or 3. A weight defined as $w_2 = 1/U$ is also applied to each parameter of the model. It accounts for the uncertainty in the determination of a specific parameter (n,m) and is defined by the penalty parameter U (1 = low to null uncertainty, 2 = some degree of uncertainty, 3 = high uncertainty). Having defined the weighing scheme of the parameters, the FPTI_i of each fiber is calculated according to the equation:

$$\mathrm{FPTI}_i = \sum_{i=1}^{n} w_1 \cdot w_2 \cdot T_i$$

with T_i = class value of the parameter i of the model; $w_1 = 1/H$ weight of the parameter according to its hierarchy H; $w_2 = 1/U$ weight of the parameter according to the uncertainty U of its determination. In the example above of the mean fiber length L, both $w_1 = 1/H$ and $w_2 = 1/U$ are = 1 because $H = 1$ and $U = 1$.

The FPTI has been calculated for some mineral fibers of social and economic importance [155], and it was found that all the amphibole asbestos species (amosite UICC standard, South African, NB #4173–111-4; anthophyllite UICC standard asbestos, Finnish NB #4173–111-5; crocidolite UICC standard, South African, NB #4173–111-3; fibrous fluoroedenite from Biancavilla, Sicilia (Italy); and tremolite asbestos from Val d'Ala, Turin, Italy) display FPTI values >2.50, whereas chrysotile asbestos samples (chrysotile from Balangero, Torino, Italy; chrysotile "B" asbestos UICC standard; chrysotile from Valmalenco, Sondrio, Italy) have values in the range 2.00–2.30. FPTI values <2.00 have been derived from nonpathogenic mineral fibers (fibrous sepiolite from Vallecas (Spain) and wollastonite NYAD G [156]).

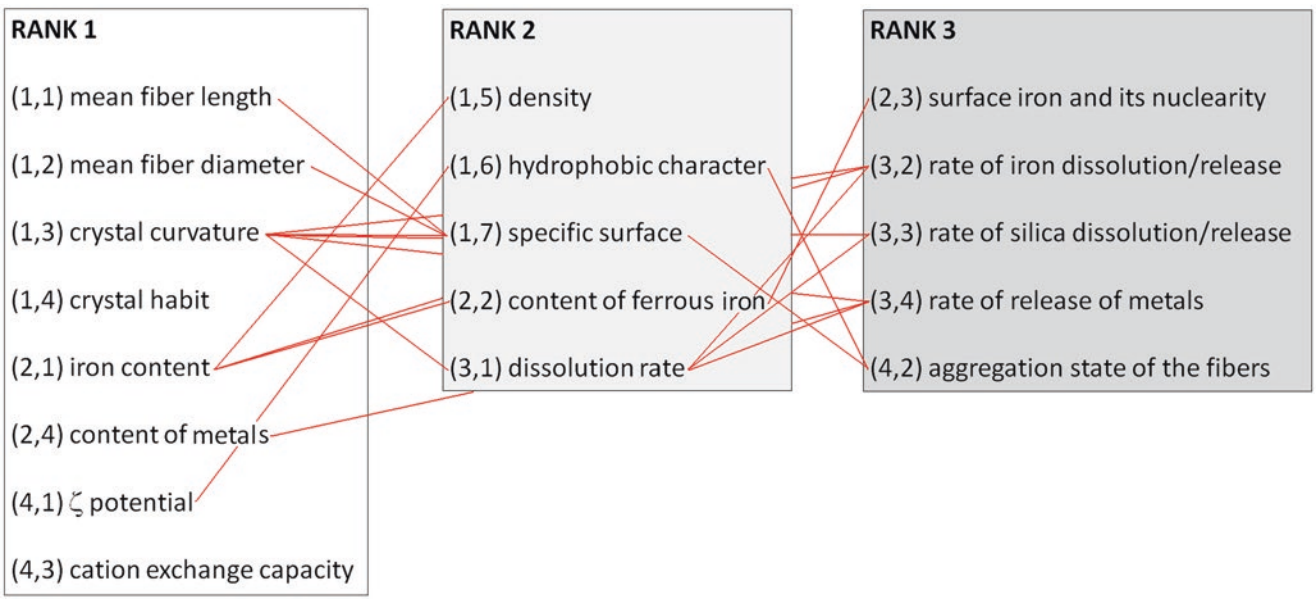

Fig. 12.3 The hierarchy (rank 1, 2, and 3) of various parameters of fibers considered in the Fiber Potential Toxicity Index (FPTI) model

This model quantitatively supports the concept of a different pathogenic potential range for amphibole asbestos as compared to chrysotile asbestos. The difference in biodurability between amphibole asbestos and chrysotile asbestos [157] is the key to explaining why chrysotile is less pathogenic than amphiboles. In fact, the low biopersistence of chrysotile determines its disintegration in the lungs with fibers becoming shorter [157]. Nevertheless, the FPTI indices for both amphibole and chrysotile asbestos are higher than nonpathogenic mineral fibers. Work is in progress to validate the model in collaboration with international organizations, and to deliver a FPTI model-based user-friendly code.

Conclusions

Occupational exposures to asbestos are associated with an increased risk of lung cancers, especially in smokers. The complexity of asbestos fibers, distinct minerals with different chemical, physical, and structural features, coupled with the thousands of chemicals and particles in cigarette smoke, have made study of the interactions between these agents difficult. However, several commonalities between smoking and asbestos exist that can be related to their additive or multiplicative potencies. For example, both impede normal clearance mechanisms of the lung. Both agents also cause chronic inflammation and lung fibrosis that favor the development of lung tumors. Most importantly, both agents can cause proliferation and metaplasia of lung epithelial cells through epigenetic mechanisms including stimulation of cell signaling pathways. Understanding the properties of asbestos minerals and their contributions to the development of lung cancers will facilitate predictive models for prediction of the potential of other mineral types in lung diseases.

References

1. Fong KM, Larsen JE, Wright C, et al. Molecular basis of lung carcinogenesis. In: Coleman WB, Tsongalis GJ, editors. The molecular basis of human cancer. New York: Springer; 2017. p. 447–96.
2. Coleman WB, Tsongalis GJ. Cancer epidemiology: incidence and etiology of human neoplasms. In: Coleman WB, Tsongalis GJ, editors. The molecular basis of human cancer. New York: Springer; 2017. p. 1–24.
3. Testa JR. Asbestos and mesothelioma. Cham: Springer International; 2017. 407 p.
4. Wehner AP, Felton DL, Company RJRT, Institute EPR. Biological interaction of inhaled mineral fibers and cigarette smoke: proceedings of an International Symposium/workshop, held at the Battelle Seattle Conference Center, April 10–14, 1988. Seattle, Washington: Battelle Press; 1989.
5. Case BW, Abraham JL, Meeker G, Pooley FD, Pinkerton KE. Applying definitions of "asbestos" to environmental and "low-dose" exposure levels and health effects, particularly malignant mesothelioma. J Toxicol Environ Health B Crit Rev. 2011;14(1–4):3–39.
6. Lee RJ, Strohmeier BR, Bunker KL, Van Orden DR. Naturally occurring asbestos: a recurring public policy challenge. J Hazard Mater. 2008;153(1–2):1–21.
7. Mossman BT, Alleman JE. Asbestos revisited. Sci Am. 1997;277(1):70–5.
8. Gualtieri AF. Introduction (mineral fibres). In: Mineral fibres: crystal chemistry, chemical-physical properties, biological interaction and toxicity. London: European Mineralogical Union; 2017. p. 1–16.
9. IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man: asbestos. IARC Monogr Eval Carcinog Risk Chem Man. 1977;14:1–106.
10. IARC. Arsenic, metals, fibres, and dusts. IARC Working Group. Lyon; 17–24 March 2009. IARC Monogr Eval Carcinog Risk Chem Hum C. 2012; 100C:219–316.
11. O'Hanley DS, Dyar MD. The composition of chrysotile and its relationship with lizardite. Can Mineral. 1998;36(3):727–39.
12. Gualtieri AF, Andreozzi GB, Tomatis M, Turci F. Iron from a geochemical viewpoint. Understanding toxicity/pathogenicity mechanisms in iron-bearing minerals with a special attention to mineral fibers. Free Radic Biol Med. 2019;13:321–37.
13. Bailey SW, America MSo. Hydrous phyllosilicates: (exclusive of micas). Chantilly, VA: Mineralogical Society of America; 1988.
14. Hawthorne FC, Oberti R, Harlow GE, et al. Nomenclature of the amphibole supergroup. Am Mineral. 2012;97(11–12):2031–48.
15. Guthrie GD Jr. Mineral properties and their contributions to particle toxicity. Environ Health Perspect. 1997;105(Suppl):51003–11.
16. Vallyathan V, Shi XL, Dalal NS, Irr W, Castranova V. Generation of free radicals from freshly fractured silica dust. Potential role in acute silica-induced lung injury. Am Rev Respir Dis. 1988;138(5):1213–9.
17. Liu W, Rose J, Plantevin S, Auffan M, Bottero JY, Vidaud C. Protein corona formation for nanomaterials and proteins of a similar size: hard or soft corona? Nanoscale. 2013;5(4):1658–68.
18. Stanton MF, Layard M, Tegeris A, et al. Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. J Natl Cancer Inst. 1981;67(5):965–75.
19. Churg A. Asbestos-related disease in the workplace and the environment: controversial issues. Monogr Pathol. 1993;36:54–77.
20. Deng ZJ, Liang M, Toth I, Monteiro MJ, Minchin RF. Molecular interaction of poly(acrylic acid) gold nanoparticles with human fibrinogen. ACS Nano. 2012;6(10):8962–9.
21. Harris RL Jr, Timbrell V. Relation of alveolar deposition to the diameter and length of glass fibres. Inhaled particles IV. Oxford: Pergamon Press; 1977.
22. Gualtieri AF, Mossman BT, Roggli VL. Towards a general model to predict the toxicity and pathogenicity of mineral fibres. In: Mineral fibres: crystal chemistry, chemical-physical properties, biological interaction and toxicity: European Mineralogical Union–EMU notes in mineralogy; 2017. p. 501–32.
23. Yeh HC, Phalen RF, Raabe OG. Factors influencing the deposition of inhaled particles. Environ Health Perspect. 1976;15:147–56.
24. van Oss CJ, Naim JO, Costanzo PM, Giese RF, Wu W, Sorling AF. Impact of different asbestos species and other mineral particles on pulmonary pathogenesis. Clays Clay Miner. 1999;47(6):697–707.
25. Hardy JA, Aust AE. Iron in asbestos chemistry and carcinogenicity. Chem Rev. 1995;95(1):97–118.
26. Kamp DW. Asbestos-induced lung diseases: an update. Transl Res. 2009;153(4):143–52.
27. Zecchina A, Rivallan M, Berlier G, Lamberti C, Ricchiardi G. Structure and nuclearity of active sites in Fe-zeolites: comparison with iron sites in enzymes and homogeneous catalysts. Phys Chem Chem Phys. 2007;9(27):3483–99.

28. Wei B, Yang L, Zhu O, et al. Multivariate analysis of trace elements distribution in hair of pleural plaques patients and health group in a rural area from China. Hair Ther Transpl. 2014;4:125.
29. Dixon JR, Lowe DB, Richards DE, Cralley LJ, Stokinger HE. The role of trace metals in chemical carcinogenesis: asbestos cancers. Cancer Res. 1970;30(4):1068–74.
30. Bernstein DM, Hoskins JA. The health effects of chrysotile: current perspective based upon recent data. Regul Toxicol Pharmacol. 2006;45(3):252–64.
31. Bernstein DM, Donaldson K, Decker U, et al. A biopersistence study following exposure to chrysotile asbestos alone or in combination with fine particles. Inhal Toxicol. 2008;20(11):1009–28.
32. Bernstein D, Rogers R, Smith P. The biopersistence of Canadian chrysotile asbestos following inhalation: final results through 1 year after cessation of exposure. Inhal Toxicol. 2005;17(1):1–14.
33. Utembe W, Potgieter K, Stefaniak AB, Gulumian M. Dissolution and biodurability: important parameters needed for risk assessment of nanomaterials. Part Fibre Toxicol. 2015;12:11.
34. Pott F. Asbestos use and carcinogenicity in Germany and a comparison with animal studies. Ann Occup Hyg. 1994;38(4):589–600, 420.
35. Hesterberg TW, Chase G, Axten C, et al. Biopersistence of synthetic vitreous fibers and amosite asbestos in the rat lung following inhalation. Toxicol Appl Pharmacol. 1998;151(2):262–75.
36. Bernstein D, Pavlisko EN. Differential pathological response and pleural transport of mineral fibres. In: Gualtieri AF, editor. Mineral fibres: crystal chemistry, chemical-physical properties, biological interaction and toxicity: European Mineralogical Union and the Mineralogical Society of Great Britain & Ireland; 2017. p. 417–34.
37. Wypych F, Adad LB, Mattoso N, Marangon AA, Schreiner WH. Synthesis and characterization of disordered layered silica obtained by selective leaching of octahedral sheets from chrysotile and phlogopite structures. J Colloid Interface Sci. 2005;283(1):107–12.
38. Pollastri S, Gualtieri AF, Vigliaturo R, et al. Stability of mineral fibres in contact with human cell cultures. An in situ muXANES, muXRD and XRF iron mapping study. Chemosphere. 2016;164:547–57.
39. Studer AM, Limbach LK, Van Duc L, et al. Nanoparticle cytotoxicity depends on intracellular solubility: comparison of stabilized copper metal and degradable copper oxide nanoparticles. Toxicol Lett. 2010;197(3):169–74.
40. Sabella S, Carney RP, Brunetti V, et al. A general mechanism for intracellular toxicity of metal-containing nanoparticles. Nanoscale. 2014;6(12):7052–61.
41. Pollastri S, Gualtieri AF, Lassinantti Gualtieri M, Hanuskova M, Cavallo A, Gaudino G. The zeta potential of mineral fibres. J Hazard Mater. 2014;276:469–79.
42. Roggli VL, Sharma A. Analysis of tissue mineral fiber content. Pathology of asbestos-associated diseases. Berlin: Springer; 2014. p. 253–92.
43. Lippmann M. Toxicological and epidemiological studies on effects of airborne fibers: coherence and public [corrected] health implications. Crit Rev Toxicol. 2014;44(8):643–95.
44. Barlow CA, Grespin M, Best EA. Asbestos fiber length and its relation to disease risk. Inhal Toxicol. 2017;29(12–14):541–54.
45. Roggli VL. The so-called short-fiber controversy: literature review and critical analysis. Arch Pathol Lab Med. 2015;139(8):1052–7.
46. Oberdorster G, Graham U. Predicting EMP hazard: Lessons from studies with inhaled fibrous and non-fibrous nano- and microparticles. Toxicol Appl Pharmacol. 2018;361:50–61.
47. Mossman BT, Kessler JB, Ley BW, Craighead JE. Interaction of crocidolite asbestos with hamster respiratory mucosa in organ culture. Lab Invest. 1977;36(2):131–9.
48. Hansen K, Mossman BT. Generation of superoxide (O2-.) from alveolar macrophages exposed to asbestiform and nonfibrous particles. Cancer Res. 1987;47(6):1681–6.
49. Keeling B, Hobson J, Churg A. Effects of cigarette smoke on epithelial uptake of non-asbestos mineral particles in tracheal organ culture. Am J Respir Cell Mol Biol. 1993;9(3):335–40.
50. McFadden D, Wright JL, Wiggs B, Churg A. Smoking inhibits asbestos clearance. Am Rev Respir Dis. 1986a;133(3):372–4.
51. McFadden D, Wright J, Wiggs B, Churg A. Cigarette smoke increases the penetration of asbestos fibers into airway walls. Am J Pathol. 1986b;123(1):95–9.
52. Churg A. The uptake of mineral particles by pulmonary epithelial cells. Am J Respir Crit Care Med. 1996;154(4 Pt 1):1124–40.
53. Sekhon H, Wright J, Churg A. Effects of cigarette smoke and asbestos on airway, vascular and mesothelial cell proliferation. Int J Exp Pathol. 1995;76(6):411–8.
54. Churg A, Sun J, Zay K. Cigarette smoke increases amosite asbestos fiber binding to the surface of tracheal epithelial cells. Am J Physiol. 1998;275(3 Pt 1):L502–8.
55. Kennedy AR, Little JB. The transport and localization of benzo(a) pyrene-hematite and hematite-210Po in the hamster lung following intratracheal instillation. Cancer Res. 1974;34(6):1344–52.
56. Eastman A, Mossman BT, Bresnick E. Formation and removal of benzo(a)pyrene adducts of DNA in hamster tracheal epithelial cells. Cancer Res. 1981;41(7):2605–10.
57. Eastman A, Mossman BT, Bresnick E. Influence of asbestos on the uptake of benzo(a)pyrene and DNA alkylation in hamster tracheal epithelial cells. Cancer Res. 1983;43(3):1251–5.
58. Mossman BT, Eastman A, Bresnick E. Asbestos and benzo[a] pyrene act synergistically to induce squamous metaplasia and incorporation of [3H]thymidine in hamster tracheal epithelium. Carcinogenesis. 1984;5(11):1401–4.
59. Mossman BT, Craighead JE. Use of hamster tracheal organ cultures for assessing the cocarcinogenic effects of inorganic particulates on the respiratory epithelium. Prog Exp Tumor Res. 1979;24:37–47.
60. Mossman BT, Craighead JE. Comparative cocarcinogenic effects of crocidolite asbestos, hematite, kaolin and carbon in implanted tracheal organ cultures. Ann Occup Hyg. 1982;26(1–4):553–67.
61. Auerbach O, Stout AP, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. N Engl J Med. 1961;265:253–67.
62. Mossman BT, Adler KB, Craighead JE. Interaction of carbon particles with tracheal epithelium in organ culture. Environ Res. 1978;16(1–3):110–22.
63. Mossman BT, Craighead JE, MacPherson BV. Asbestos-induced epithelial changes in organ cultures of hamster trachea: inhibition by retinyl methyl ether. Science. 1980;207(4428):311–3.
64. Woodworth CD, Mossman BT, Craighead JE. Interaction of asbestos with metaplastic squamous epithelium developing in organ cultures of hamster trachea. Environ Health Perspect. 1983a;51:27–33.
65. Woodworth CD, Mossman BT, Craighead JE. Squamous metaplasia of the respiratory tract. Possible pathogenic role in asbestos-associated bronchogenic carcinoma. Lab Invest. 1983b;48(5):578–84.
66. Woodworth CD, Mossman BT, Craighead JE. Induction of squamous metaplasia in organ cultures of hamster trachea by naturally occurring and synthetic fibers. Cancer Res. 1983c;43(10):4906–12.
67. Tomasetti C, Li L, Vogelstein B. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. Science. 2017;355(6331):1330–4.
68. DeGregori J. Connecting cancer to its causes requires incorporation of effects on tissue microenvironments. Cancer Res. 2017;77(22):6065–8.

69. Berenblum I. A re-evaluation of the concept of cocarciongenesis. Prog Exp Tumor Res. 1969;11:21–30.
70. Martín-Subero JI, Esteller M. Epigenetic mechanisms in cancer development. In: The molecular basis of human cancer. Berlin: Springer; 2017. p. 263–75.
71. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes Dev. 2009;23(7):781–3.
72. Riely GJ, Kris MG, Rosenbaum D, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res. 2008;14(18):5731–4.
73. Spira A, Beane J, Shah V, et al. Effects of cigarette smoke on the human airway epithelial cell transcriptome. Proc Natl Acad Sci U S A. 2004;101(27):10143–8.
74. Shah V, Sridhar S, Beane J, Brody JS, Spira ASIEGE. Smoking induced epithelial gene expression database. Nucleic Acids Res. 2005;33(Database issue):D573–9.
75. Spira A, Beane JE, Shah V, et al. Airway epithelial gene expression in the diagnostic evaluation of smokers with suspect lung cancer. Nat Med. 2007;13(3):361–6.
76. Mäki-Nevala S, Sarhadi VK, Knuuttila A, et al. Driver gene and novel mutations in asbestos-exposed lung adenocarcinoma and malignant mesothelioma detected by exome sequencing. Lung. 2016;194(1):125–35.
77. Paris C, Do P, Mastroianni B, et al. Association between lung cancer somatic mutations and occupational exposure in never-smokers. Eur Respir J. 2017;50(4):1700716.
78. Gyoba J, Shan S, Roa W, Bedard EL. Diagnosing lung cancers through examination of micro-RNA biomarkers in blood, plasma, serum and sputum: a review and summary of current literature. Int J Mol Sci. 2016;17(4):494.
79. Qi J, Mu D. MicroRNAs and lung cancers: from pathogenesis to clinical implications. Front Med. 2012;6(2):134–55.
80. Mizuno K, Mataki H, Seki N, Kumamoto T, Kamikawaji K, Inoue H. MicroRNAs in non-small cell lung cancer and idiopathic pulmonary fibrosis. J Hum Genet. 2017;62(1):57–65.
81. Zhang W, Dahlberg JE, Tam W. MicroRNAs in tumorigenesis: a primer. Am J Pathol. 2007;171(3):728–38.
82. Reid G. MicroRNAs in mesothelioma: from tumour suppressors and biomarkers to therapeutic targets. J Thorac Dis. 2015;7(6):1031–40.
83. Schmitz SU, Grote P, Herrmann BG. Mechanisms of long noncoding RNA function in development and disease. Cell Mol Life Sci. 2016;73(13):2491–509.
84. Homminga I, Pieters R, Meijerink JP. NKL homeobox genes in leukemia. Leukemia. 2012;26(4):572–81.
85. Richards EJ, Zhang G, Li ZP, et al. Long non-coding RNAs (LncRNA) regulated by transforming growth factor (TGF) beta: LncRNA-hit-mediated TGFbeta-induced epithelial to mesenchymal transition in mammary epithelia. J Biol Chem. 2015;290(11):6857–67.
86. Sandoval J, Esteller M. Cancer epigenomics: beyond genomics. Curr Opin Genet Dev. 2012;22(1):50–5.
87. Sun L, Fang J. Epigenetic regulation of epithelial-mesenchymal transition. Cell Mol Life Sci. 2016;73(23):4493–515.
88. Mehta A, Dobersch S, Romero-Olmedo AJ, Barreto G. Epigenetics in lung cancer diagnosis and therapy. Cancer Metastasis Rev. 2015;34(2):229–41.
89. Di Paolo A, Del Re M, Petrini I, Altavilla G, Danesi R. Recent advances in epigenomics in NSCLC: real-time detection and therapeutic implications. Epigenomics. 2016;8(8):1151–67.
90. Sage AP, Martinez VD, Minatel BC, et al. Genomics and epigenetics of malignant mesothelioma. High Throughput. 2018;7:3.
91. Kettunen E, Hernandez-Vargas H, Cros MP, et al. Asbestos-associated genome-wide DNA methylation changes in lung cancer. Int J Cancer. 2017;141(10):2014–29.
92. Christensen BC, Marsit CJ, Houseman EA, et al. Differentiation of lung adenocarcinoma, pleural mesothelioma, and nonmalignant pulmonary tissues using DNA methylation profiles. Cancer Res. 2009;69(15):6315–21.
93. Altomare DA, Menges CW, Xu J, et al. Losses of both products of the Cdkn2a/Arf locus contribute to asbestos-induced mesothelioma development and cooperate to accelerate tumorigenesis. PLoS One. 2011;6(4):e18828.
94. Lamy A, Sesboue R, Bourguignon J, et al. Aberrant methylation of the CDKN2a/p16INK4a gene promoter region in preinvasive bronchial lesions: a prospective study in high-risk patients without invasive cancer. Int J Cancer. 2002;100(2):189–93.
95. Scruggs AM, Koh HB, Tripathi P, Leeper NJ, White ES, Huang SK. Loss of CDKN2B promotes fibrosis via increased fibroblast differentiation rather than proliferation. Am J Respir Cell Mol Biol. 2018;59(2):200–14.
96. Oner D, Ghosh M, Moisse M, et al. Global and gene-specific DNA methylation effects of different asbestos fibres on human bronchial epithelial cells. Environ Int. 2018;115:301–11.
97. Craighead JE, Mossman BT, Bradley BJ. Comparative studies on the cytotoxicity of amphibole and serpentine asbestos. Environ Health Perspect. 1980;34:37–46.
98. Lechner JF, Tokiwa T, LaVeck M, et al. Asbestos-associated chromosomal changes in human mesothelial cells. Proc Natl Acad Sci U S A. 1985;82(11):3884–8.
99. Mossman BT, Lippmann M, Hesterberg TW, Kelsey KT, Barchowsky A, Bonner JC. Pulmonary endpoints (lung carcinomas and asbestosis) following inhalation exposure to asbestos. J Toxicol Environ Health B Crit Rev. 2011;14(1–4):76–121.
100. Hei TK, Piao CQ, He ZY, Vannais D, Waldren CA. Chrysotile fiber is a strong mutagen in mammalian cells. Cancer Res. 1992;52(22):6305–9.
101. Pierce JS, Ruestow PS, Finley BL. An updated evaluation of reported no-observed adverse effect levels for chrysotile asbestos for lung cancer and mesothelioma. Crit Rev Toxicol. 2016;46(7):561–86.
102. Drummond G, Bevan R, Harrison P. A comparison of the results from intra-pleural and intra-peritoneal studies with those from inhalation and intratracheal tests for the assessment of pulmonary responses to inhalable dusts and fibres. Regul Toxicol Pharmacol. 2016;81:89–105.
103. Yanamala N, Kisin ER, Gutkin DW, Shurin MR, Harper M, Shvedova AA. Characterization of pulmonary responses in mice to asbestos/asbestiform fibers using gene expression profiles. J Toxicol Environ Health A. 2018;81(4):60–79.
104. Cummins AB, Palmer C, Mossman BT, Taatjes DJ. Persistent localization of activated extracellular signal-regulated kinases (ERK1/2) is epithelial cell-specific in an inhalation model of asbestosis. Am J Pathol. 2003;162(3):713–20.
105. Manning CB, Sabo-Attwood T, Robledo RF, et al. Targeting the MEK1 cascade in lung epithelium inhibits proliferation and fibrogenesis by asbestos. Am J Respir Cell Mol Biol. 2008;38(5):618–26.
106. Heintz NH, Janssen YM, Mossman BT. Persistent induction of c-fos and c-Jun expression by asbestos. Proc Natl Acad Sci U S A. 1993;90(8):3299–303.
107. Quinlan TR, Marsh JP, Janssen YM, et al. Dose-responsive increases in pulmonary fibrosis after inhalation of asbestos. Am J Respir Crit Care Med. 1994;150(1):200–6.
108. Shukla A, Vacek P, Mossman BT. Dose-response relationships in expression of biomarkers of cell proliferation in in vitro assays and inhalation experiments. Nonlinearity Biol Toxicol Med. 2004;2(2):117–28.
109. Janssen YM, Heintz NH, Marsh JP, Borm PJ, Mossman BT. Induction of c-fos and c-Jun proto-oncogenes in target cells of

the lung and pleura by carcinogenic fibers. Am J Respir Cell Mol Biol. 1994;11(5):522–30.
110. Quinlan TR, BeruBe KA, Marsh JP, et al. Patterns of inflammation, cell proliferation, and related gene expression in lung after inhalation of chrysotile asbestos. Am J Pathol. 1995;147(3):728–39.
111. Pache JC, Janssen YM, Walsh ES, et al. Increased epidermal growth factor-receptor protein in a human mesothelial cell line in response to long asbestos fibers. Am J Pathol. 1998;152(2):333–40.
112. Taylor ES, Wylie AG, Mossman BT, Lower SK. Repetitive dissociation from crocidolite asbestos acts as persistent signal for epidermal growth factor receptor. Langmuir. 2013;29(21):6323–30.
113. Manning CB, Cummins AB, Jung MW, et al. A mutant epidermal growth factor receptor targeted to lung epithelium inhibits asbestos-induced proliferation and proto-oncogene expression. Cancer Res. 2002;62(15):4169–75.
114. Sequist LV, Martins RG, Spigel D, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. J Clin Oncol. 2008;26(15):2442–9.
115. Cole RW, Ault JG, Hayden JH, Rieder CL. Crocidolite asbestos fibers undergo size-dependent microtubule-mediated transport after endocytosis in vertebrate lung epithelial cells. Cancer Res. 1991;51(18):4942–7.
116. Ault JG, Cole RW, Jensen CG, Jensen LC, Bachert LA, Rieder CL. Behavior of crocidolite asbestos during mitosis in living vertebrate lung epithelial cells. Cancer Res. 1995;55(4):792–8.
117. MacCorkle RA, Slattery SD, Nash DR, Brinkley BR. Intracellular protein binding to asbestos induces aneuploidy in human lung fibroblasts. Cell Motil Cytoskeleton. 2006;63(10):646–57.
118. Kodama Y, Boreiko CJ, Maness SC, Hesterberg TW. Cytotoxic and cytogenetic effects of asbestos on human bronchial epithelial cells in culture. Carcinogenesis. 1993;14(4):691–7.
119. Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. Am J Respir Crit Care Med. 1998;157(5 Pt 1):1666–80.
120. Robledo R, Mossman B. Cellular and molecular mechanisms of asbestos-induced fibrosis. J Cell Physiol. 1999;180(2):158–66.
121. Peden DB. The role of oxidative stress and innate immunity in O(3) and endotoxin-induced human allergic airway disease. Immunol Rev. 2011;242(1):91–105.
122. Conway EM, Pikor LA, Kung SH, et al. Macrophages, inflammation, and lung cancer. Am J Respir Crit Care Med. 2016;193(2):116–30.
123. Dostert C, Petrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science. 2008;320(5876):674–7.
124. Wu Y, Antony S, Meitzler JL, Doroshow JH. Molecular mechanisms underlying chronic inflammation-associated cancers. Cancer Lett. 2014;345(2):164–73.
125. Shukla A, Gulumian M, Hei TK, Kamp D, Rahman Q, Mossman BT. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. Free Radic Biol Med. 2003;34(9):1117–29.
126. Horsburgh S, Robson-Ansley P, Adams R, Smith C. Exercise and inflammation-related epigenetic modifications: focus on DNA methylation. Exerc Immunol Rev. 2015;21:26–41.
127. Vento-Tormo R, Alvarez-Errico D, Garcia-Gomez A, et al. DNA demethylation of inflammasome-associated genes is enhanced in patients with cryopyrin-associated periodic syndromes. J Allergy Clin Immunol. 2017;139(1):202–11 e6.
128. Donaldson K, Brown GM, Brown DM, Bolton RE, Davis JM. Inflammation generating potential of long and short fibre amosite asbestos samples. Br J Ind Med. 1989;46(4):271–6.
129. Davis JM, Cowie HA. The relationship between fibrosis and cancer in experimental animals exposed to asbestos and other fibers. Environ Health Perspect. 1990;88:305–9.
130. Davis JM, Jones AD. Comparisons of the pathogenicity of long and short fibres of chrysotile asbestos in rats. Br J Exp Pathol. 1988;69(5):717–37.
131. Sayan M, Mossman BT. The NLRP3 inflammasome in pathogenic particle and fibre-associated lung inflammation and diseases. Part Fibre Toxicol. 2016;13(1):51.
132. Nakayama M. Macrophage recognition of crystals and nanoparticles. Front Immunol. 2018;9:103.
133. He Q, Fu Y, Tian D, Yan W. The contrasting roles of inflammasomes in cancer. Am J Cancer Res. 2018;8(4):566–83.
134. Mortaz E, Henricks PA, Kraneveld AD, Givi ME, Garssen J, Folkerts G. Cigarette smoke induces the release of CXCL-8 from human bronchial epithelial cells via TLRs and induction of the inflammasome. Biochim Biophys Acta. 2011;1812(9):1104–10.
135. Kim SJ, Cheresh P, Williams D, et al. Mitochondria-targeted Ogg1 and aconitase-2 prevent oxidant-induced mitochondrial DNA damage in alveolar epithelial cells. J Biol Chem. 2014;289(9):6165–76.
136. Driscoll KE, Carter JM, Howard BW, Hassenbein D, Janssen YM, Mossman BT. Crocidolite activates NF-kappa B and MIP-2 gene expression in rat alveolar epithelial cells. Role of mitochondrial-derived oxidants. Environ Health Perspect. 1998;106(Suppl 5):1171–4.
137. Cox LAT Jr. Biological mechanisms of non-linear dose-response for respirable mineral fibers. Toxicol Appl Pharmacol. 2018;361:137–44.
138. Marchand F. Uber eigentumliche Pigmentkristalle in den Lungen. Verh Dtsch Ges Pathol. 1906;17:223–8.
139. Stewart MJ, Haddow AC. Demonstration of the peculiar bodies of pulmonary asbestosis ("asbestosis bodies") in material obtained by lung puncture and in the sputum. J Pathol Bacteriol. 1929;32:172.
140. Roggli VL. Pathology of human asbestosis: a critical review. Adv Pathol. 1989;2:31–60.
141. Gross P, de Treville RTP, Cralley LJ, Davis JMG. Pulmonary ferruginous bodies. Development in response to filamentous dusts and a method of isolation and concentration. J Occup Environ Med. 1969;11(4):208–9.
142. Churg AM, Warnock ML. Asbestos and other ferruginous bodies: their formation and clinical significance. Am J Pathol. 1981;102(3):447–56.
143. Harrison PM, Fischbach FA, Hoy TG, Haggis GH. Ferric oxyhydroxide core of ferritin. Nature. 1967;216(5121):1188–90.
144. Pascolo L, Gianoncelli A, Kaulich B, et al. Synchrotron soft X-ray imaging and fluorescence microscopy reveal novel features of asbestos body morphology and composition in human lung tissues. Part Fibre Toxicol. 2011;8(1):7.
145. Koerten HK, Hazekamp J, Kroon M, Daems WT. Asbestos body formation and iron accumulation in mouse peritoneal granulomas after the introduction of crocidolite asbestos fibers. Am J Pathol. 1990;136(1):141–57.
146. Morgan A, Holmes A. Concentrations and dimensions of coated and uncoated asbestos fibres in the human lung. Br J Ind Med. 1980;37(1):25–32.
147. Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of the inhalation of asbestos in rats. Br J Cancer. 1974;29(3):252–69.
148. Gandolfi NB, Gualtieri AF, Pollastri S, Tibaldi E, Belpoggi F. Assessment of asbestos body formation by high resolution FEG-SEM after exposure of Sprague-Dawley rats to chrysotile, crocidolite, or erionite. J Hazard Mater. 2016;306:95–104.
149. Jaurand MC, Bignon J, Sebastien P, Goni J. Leaching of chrysotile asbestos in human lungs. Correlation with in vitro studies using rabbit alveolar macrophages. Environ Res. 1977;14(2):245–54.
150. Roggli VL. Asbestos bodies and non-asbestos ferruginous bodies. Pathology of asbestos-associated diseases. Berlin: Springer; 2014. p. 25–51.

151. Morgan A, Holmes A. The enigmatic asbestos body: its formation and significance in asbestos-related disease. Environ Res. 1985;38(2):283–92.
152. Ghio AJ, Churg A, Roggli VL. Ferruginous bodies: implications in the mechanism of fiber and particle toxicity. Toxicol Pathol. 2004;32(6):643–9.
153. Pavlisko EN, Carney JM, Sporn TA, Roggli VL. Mesothelioma pathology. Asbestos and mesothelioma. Berlin: Springer; 2017. p. 131–60.
154. Attanoos RL, Churg A, Galateau-Salle F, Gibbs AR, Roggli VL. Malignant mesothelioma and its non-asbestos causes. Arch Pathol Lab Med. 2018;142(6):753–60.
155. Gualtieri AF. Towards a quantitative model to predict the toxicity/pathogenicity potential of mineral fibers. Toxicol Appl Pharmacol. 2018;361:89–98.
156. Bellmann B, Muhle H. Investigation of the biodurability of wollastonite and xonotlite. Environ Health Perspect. 1994;102(Suppl 5):191–5.
157. Gualtieri AF, Pollastri S, Bursi Gandolfi N, Gualtieri ML. In vitro acellular dissolution of mineral fibres: a comparative study. Sci Rep. 2018;8(1):7071.