

# Analyzing biological and molecular characteristics and genomic damage induced by exposure to asbestos

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Diana Ospina<sup>1</sup>  
Victoria Eugenia Villegas<sup>1</sup>  
Giovanni Rodríguez-  
Leguizamón<sup>2</sup>  
Milena Rondón-Lagos<sup>3</sup>

<sup>1</sup>Biology Program, Faculty of Natural Sciences and Mathematics, Universidad del Rosario, Bogotá 111221, Colombia;

<sup>2</sup>Hospital Universitario Mayor Méderi – Universidad del Rosario, School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, 111221, Colombia; <sup>3</sup>School of Biological Sciences, Universidad Pedagógica y Tecnológica de Colombia, Tunja 150003, Colombia

**Abstract:** Asbestos is one of the most important occupational carcinogens. Currently, about 125 million people worldwide are exposed to asbestos in the workplace. According to global estimates, at least 107,000 people die each year from lung cancer, mesothelioma, and asbestosis as a result of occupational exposure to asbestos. The high pathogenicity of this material is currently known, being associated with the development of pulmonary diseases, of which lung cancer is the main cause of death due to exposure to this mineral. Pulmonary diseases related to asbestos are a common clinical problem and a major health concern worldwide. Extensive research has identified many important pathogenic mechanisms; however, the precise molecular mechanisms involved, and the generated genomic damage that lead to the development of these diseases, are not completely understood. The modes of action that underlie this type of disease seem to differ depending on the type of fiber, lung clearance, and genetics. This evidences the need to increase our knowledge about these effects on human health. This review focuses on the characteristics of asbestos and the cellular and genomic damage generated in humans via exposure.

**Keywords:** occupational exposure, cellular damage, genomic damage, cancer

## Introduction

The term asbestos, or earth flax, was assigned generically to a group of fibrous minerals characterized by their resistance to high temperatures and isolation of heat and noise.

Due to their physical characteristics, their properties of tension and resistance to heat, the chemical structure, and their lower cost, asbestos has been used since antiquity, especially in twentieth-century industry.<sup>1</sup> Since the beginning of the twentieth century, the relationship between exposure to asbestos and lung damage has been known. Since then, several studies have been conducted that have demonstrated the degree of danger represented by the constant use of this fiber for human health.<sup>2</sup> In 1987, the International Agency for Research in Cancer (IARC) classified asbestos as a Group 1 human carcinogen (defined) by direct, indirect, and domestic exposure.<sup>3,4</sup> Exposure to asbestos occurs through the inhalation of fibers, mainly from contaminated air in the work environment, as well as from ambient air in the neighborhood of point sources, or air inside homes and buildings containing friable asbestos materials.<sup>5</sup>

The high pathogenicity of this material is currently known, being associated with the development of pulmonary diseases of which lung cancer is the main cause of death due to exposure to this mineral. Pulmonary diseases related to asbestos are

Correspondence: Milena Rondón-Lagos  
School of Biological Sciences, Universidad  
Pedagógica y Tecnológica de Colombia,  
Tunja 150003, Colombia  
Tel +571 297 0200 (ext. 4029)  
Fax +571 297 0200  
Email [sandra.rondon01@uptc.edu.co](mailto:sandra.rondon01@uptc.edu.co)

a common clinical problem and a major concern for health worldwide. Epidemiological studies have established that exposure to asbestos fibers causes pleural abnormalities (effusions and plaques), pulmonary fibrosis (asbestosis), and malignancies (bronchogenic carcinoma and mesothelioma).<sup>6-9</sup> Extensive research has identified many important pathogenic mechanisms; however, the precise molecular mechanisms involved and the generated genomic damage that lead to the development of these diseases are not completely understood. The modes of action that underlie this type of disease seem to differ depending on the type of fiber, lung clearance, and genetics. This evidences the need to deepen and increase our knowledge about the effects of asbestos on human health.

### Asbestos: general considerations

The term asbestos, or earth flax, was generically assigned to a group of fibrous minerals characterized by their resistance to high temperatures and isolation of heat and noise. This mineral is found naturally in rocks and soils, is extracted from mines, and its processing is cheap. Asbestos is used to make clothing (gloves, anti-flame jogging suit, aprons, mittens, and ropes), in construction (fiber cement, tiles, slabs, etc.), in rubber, and in some household appliances (irons, toasters, hair dryers, and coffee makers).

Asbestos is one of the most important occupational carcinogens. Currently, about 125 million people worldwide are exposed to asbestos in the workplace.<sup>5</sup> According to global estimates, at least 107,000 people die each year from lung cancer, mesothelioma, and asbestosis as a result of occupational exposure to asbestos.<sup>5</sup> Almost 400 deaths have been attributed to non-professional exposure to asbestos. The number of asbestos-related diseases continues to rise, even in countries that banned its use in the early 1990s. Due to the long latency periods associated with the diseases in question, suspending from now the use of asbestos, will result in a decrease in the number of deaths related to asbestos alone after several decades.<sup>5</sup>

According to the IARC, asbestos and earth flax were classified as Group 1 human carcinogens by direct, indirect, and domestic exposure.<sup>3,4</sup> Many industrialized countries have introduced legislation that prevents factories from using compounds such as asbestos in production due to its high carcinogenic risk. Additionally, it has been proven that joint exposure to tobacco smoke and asbestos fibers increases the risk of lung cancer – the more one smokes, the greater this risk.

Exposure to asbestos occurs by inhalation of fibers dispersed in the air and can be of three types: occupational (people who manipulate asbestos or who work in places of exploitation or its use), domestic (people living with workers exposed to asbestos, also those living in houses or buildings built with materials based on it), or environmental (people who live or have lived in the proximity of sites that use asbestos).<sup>10</sup> Nevertheless, the occupational exposure has always been and remains the most likely source of human exposure. Asbestos is dispersed in air due to the extraction of the mineral, its production, the inadequate disposal of the material, and the repair of facilities containing asbestos. Taking into account that asbestos is harmful to health in the phases in which it is dispersed in the air, this mineral has been considered a carcinogen by the IARC.<sup>3,4</sup>

Two groups of asbestos are distinguished: serpentines, which include chrysotile; and amphiboles, among which are crocidolite, amosite, tremolite, anthophyllite, and actinolite.<sup>11</sup> The first type consists of curled, wavy, flexible, long, and easily breakable fibers, soluble in the tissues, with diameters of 0.02–0.03 microns.<sup>6</sup> The second type, amphibole, are rigid, short, sharp, and highly resistant fibers to chemical and biological solutions, and have a greater biological persistence compared to chrysotile.<sup>6,12</sup>

### Etiopathology related to asbestos

The determinants of the toxicity of asbestos fibers depend on multiple factors, including dose, dimension, biopersistence, surface reactivity, and genetic history of those exposed. The dose of asbestos is a crucial factor triggering inflammation: high doses during short periods promote a predominant acute inflammation characterized by neutrophil accumulation, whereas low doses during prolonged exposure periods promote a chronic inflammation linked to accumulation of alveolar macrophages (AMs).<sup>6</sup> The dimensions of the fibers and their chemical characteristics seem to determine the biological potency of fibrogenesis. It is thought that these characteristics, together with the surface properties, are also important for carcinogenesis. Thin and long fibers are more active than short fibers and amphiboles are more active than chrysotile – a property attributed to its greater biological persistence.

The ability of the inhaled fibers to penetrate into the lung spaces depends on their size, so fibers with aerodynamic diameters equal to or  $<5 \mu\text{m}$  show a penetration of more than 80%, but also a lower retention (10–20%).<sup>13</sup> The dimensions of the fiber are important because only the very thin

fibers (diameter  $<0.4 \mu\text{m}$  and length  $<10 \mu\text{m}$ ) are respirable in the distal alveolar space; the long fibers cannot be swallowed by the AM because they are biodurable. Phagocytosis of the fibers is limited by the size of the AMs (generally  $14\text{--}21 \mu\text{m}$ ). In general, although fibers longer than  $20 \mu\text{m}$  in length are associated with asbestosis, fibers longer than  $10 \mu\text{m}$  are the most carcinogenic. However, the carcinogenicity of amphiboles is two orders of magnitude greater than that of chrysotile.<sup>6</sup> Additionally, it has been reported that fibers  $<5 \mu\text{m}$  in length can also promote pulmonary fibrosis and malignancy, especially when administered as a pulmonary overload condition, as can occur in dust clouds.<sup>7</sup> When the fibers are too long to be completely phagocytosed, the AMs try to swallow them, which results in their death when their membrane is crossed, which is called “frustrated phagocytosis”. This process results in the release of digestive enzymes, reactive oxygen species (ROS), reactive nitrogen species (RNS), proteases and cytokines that affect the lungs and other tissues.<sup>7</sup> Considering that frustrated phagocytosis by phagocytic cells is associated with an increase in the release of ROS and RNS, long and thin fibers are considered more genotoxic and mutagenic, which has been related to the alteration of mitosis by interfering with cytokinesis<sup>8</sup> by breaking the mitotic spindle.<sup>14</sup> These fibers can penetrate deep into the lungs, unlike short fibers that are completely wrapped by AMs and are eliminated as any particle.<sup>7</sup> However, the smaller-diameter fibers are likely deposited in the alveoli.<sup>5</sup>

The biopersistence of the fibers depends on the site and speed of deposition, the rates of elimination by AMs or mucociliary transport, their solubility in pulmonary fluids, their rate of rupture, and transport through the biological membranes.<sup>7</sup> The biopersistence of chrysotile fibers is greater than that of amphibole fibers (months vs years, respectively), but chrysotile has a smaller surface area ( $27$  vs  $\sim 8 \text{ m}^2\text{g}^{-1}$ , respectively).<sup>12</sup> For fibers whose chemical composition makes them totally or partially soluble inside the lung, it is possible that they completely dissolve or weaken sufficiently to be broken into shorter fibers, which can be eliminated through macrophage-mediated phagocytosis and mucociliary transport<sup>7</sup> through the nasal and tracheo-bronchial region.<sup>5</sup> The relatively low biopersistence of chrysotile could be explained by the fact that the leached fibers break into shorter fibers that are eliminated more easily. The leaching of chrysotile occurs in acidic or strong chelating conditions, which produces the elimination of magnesium (Mg), as in phagocytosis by AMs, thereby decreasing its biological potential.<sup>5</sup>

## Cellular damage induced by exposure to asbestos

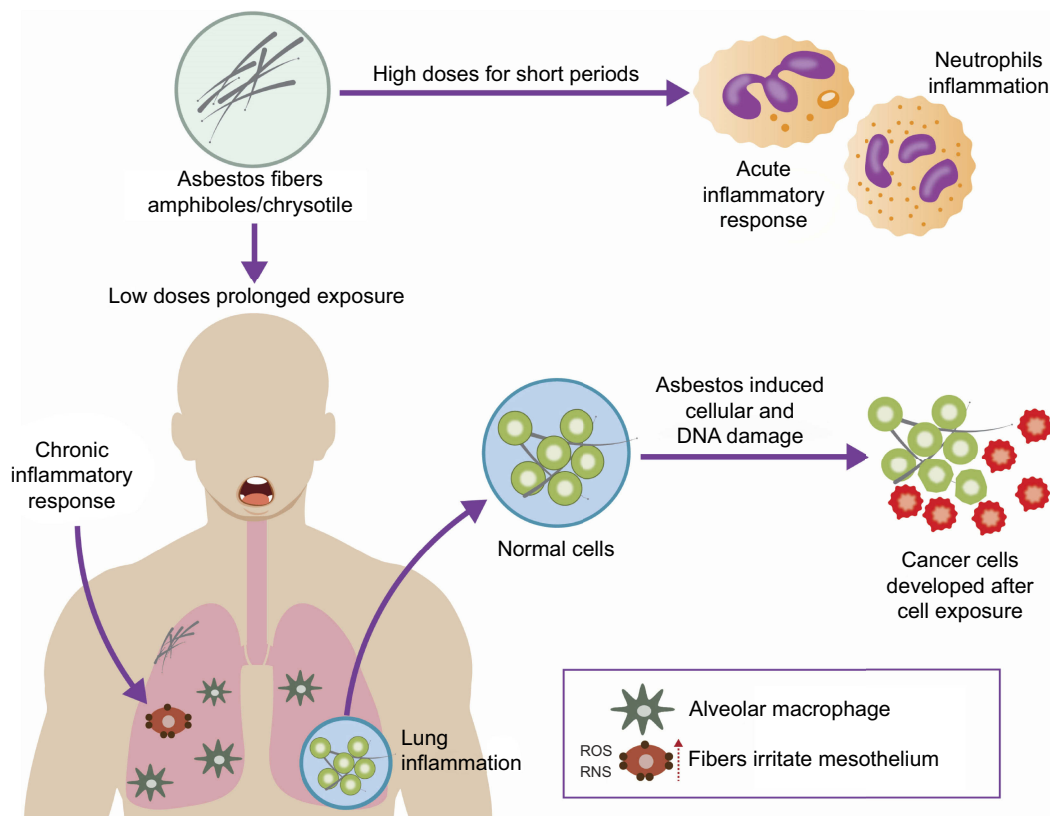
Exposure to asbestos has been shown to cause damage at both the cellular and genomic levels.<sup>8</sup> Notably, several studies have shown that the damage to the organism caused by asbestos differs depending on the concentration, the exposure, and the type of fiber – chrysotile is the most pathogenic fiber, followed by crocidolite.<sup>15</sup> At the cellular level, the accumulation of fibers causes, among other effects, oxidative stress, fibrosis, and chronic inflammation (Figure 1).<sup>8</sup>

### Oxidative stress

Asbestos fibers tend to accumulate on the pleural surface and interact with the mesothelial cell layer, leading to the generation of ROS and RNS, and the formation of free radicals.<sup>5</sup> The formation of ROS and RNS results from the chronic inflammation generated by the prolonged phagocytic activity by macrophages that function in the elimination of biopersistent fibers (Figure 1).<sup>7</sup> The catalytic iron (Fe) associated with asbestos fibers is one of the main sources of ROS production.<sup>14</sup> The amphiboles have a higher Fe content than serpentines, which are more mutagenic. This difference in harm potential may be due to the variation in the activity of the superficial Fe. Importantly, the valence and mobility status of Fe are determining factors in the mutagenic potential of the fiber.<sup>14</sup> It is estimated that free radicals derived from Fe produce other effects such as lipid peroxidation, the release of tumor necrosis factor, cellular apoptosis, adhesion, and an increase in the absorption of fibers by epithelial cells.<sup>5</sup> It has been suggested that the generation of free radicals, ROS, RNS, growth factors, and the induction of inflammatory cytokines secondary to fiber accumulation cause desoxyribonucleic acid (DNA) damage and induce the activation of proto-oncogenes, cell proliferation, and susceptibility to mutations.

### Fibrosis

The mode of action of the long fiber is mechanical and not chemical; its length is essential in the production of fibrosis since the short fibers are unable to produce such reaction. The retention time determines its development.<sup>7</sup> The target lung cells that work in the elimination of fibers, especially AMs and epithelial cells, produce cytokines, proteases, and growth factors that promote cell proliferation and tissue repair, their prolonged activation by chronic exposure leads to pulmonary fibrosis.<sup>8</sup>



**Figure 1** Cellular damage induced by exposure to asbestos. High doses of asbestos during short periods promote acute inflammation of neutrophils. Low doses during prolonged exposure periods promote neutrophil accumulation and thus acute inflammation. Free radicals, ROS and RNS result from the chronic inflammation generated by the prolonged phagocytic activity by macrophages. This condition causes DNA damage inducing the activation of proto-oncogenes, cell proliferation, and susceptibility to mutations. **Abbreviations:** ROS, reactive oxygen species; RNS, reactive nitrogen species.

An early event of fibrogenesis is the injury of Type I epithelial cells, followed by the hypertrophy of Type II epithelial cells. Increases in epithelial cells proliferation and that of fibroblasts are determining factors in the repair and regeneration of tissue that, when not controlled, can lead to fibrosis.<sup>16</sup> Therefore, this non-mutagenic process can be related to excessive apoptosis caused by the genotoxicity of ROS that results in lung tissue injury and uncontrolled and prolonged cell proliferation.<sup>8</sup>

## Chronic inflammation

Chronic inflammation is a recognized risk factor for human cancer<sup>17–19</sup> that can promote all stages of tumorigenesis, including DNA damage, continuous replication, evasion of apoptosis, prolonged angiogenesis, resistance to signaling of anti-growth, and invasion/metastases. Furthermore, crocidolite asbestos fibers are capable of inducing cell proliferation, cell cycle detention, and apoptosis in diverse populations of mesothelial cells<sup>20</sup> and epithelial cells of the lung.<sup>21</sup> The inflammatory response and the release of ROS and RNS are triggered by the

frustrated phagocytosis of the long asbestos fibers by the AMs.<sup>22,23</sup> ROS and RNS recruit more macrophages and other inflammatory cells to the lung (Figure 1). Therefore, the persistence of asbestos fibers in the lungs can trigger the prolonged production of free radicals and chronic inflammation at the sites of fiber deposition.

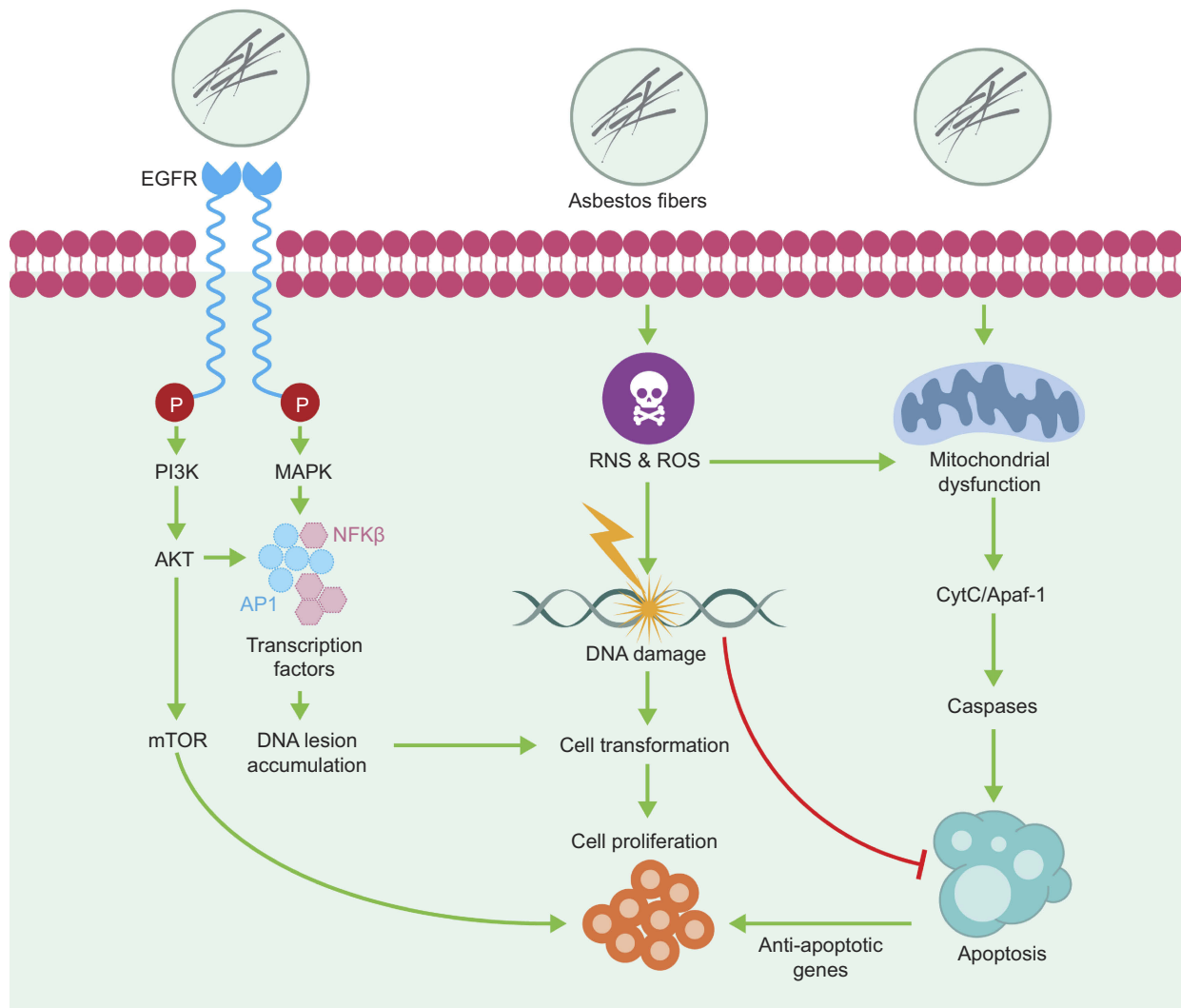
## Signaling pathways involved

In addition to inducing direct DNA damage and mutagenesis, chronic inflammation (induced by asbestos, ROS, and RNS) activates multiple signaling cascades,<sup>24–26</sup> including the signaling pathway of the mitogen-activated protein kinase (MAPK) (Figure 2). A rapid increase in signaling of the MAPK pathway subsequently activates transcription factors, such as the activator protein-1 (AP-1) and the nuclear transcription factor kappa-B (NFκB), in target cells exposed to asbestos (Figure 2). AP-1 is a family of transcription factors comprised of homo- and heterodimers of the *JUN* and *FOS* early response protooncogenes. It is a redox-sensitive transcription factor classically associated with the development of cell proliferation and tumor promotion.<sup>27</sup>

NF- $\kappa$ B is a critical transcription factor in inflammation and responses in target cells of asbestos-related diseases, since its activation is crucial in the upregulation of many genes related to proliferation and apoptosis.<sup>28</sup> Moreover, asbestos fibers caused transcriptional activation of a number of NF $\kappa$ B dependent genes, including *c-MYC*, through an oxidant-dependent pathway.<sup>29</sup>

The properties of asbestos fibers eliciting these cell signaling cascades and the consequences of asbestos-induced AP-1 and NF $\kappa$ B dependent gene expression may be related to the initiation of asbestos-associated cell responses and lung/pleural diseases. Cross-talk between these cell signaling pathways also exists, and may be relevant to asbestos-induced inflammation and proliferation.<sup>30,31</sup>

Extensive studies by Mossman et al<sup>7,28</sup> identified additional cellular signaling pathways involved in the response to asbestos inhalation, especially the Epidermal growth factor receptor (EGFR) regulated signaling pathway (Figure 2). In 2010, Heintz et al<sup>28</sup> showed that asbestos, chrysotile, and crocidolite fibers activate the EGFR in mesothelial cells - an event related to the activation of extracellular signal regulated protein kinases (ERK) 1 and 2 (ERK1/2).<sup>32</sup> ERK1/2 partially regulates the transcriptional activity of *FOS*, and the mRNA levels of both *FOS* and *JUN* induced by earth flax in the distal bronchial epithelium are reduced in mice without EGFR.<sup>33</sup> These studies provide a link between the activation of EGFR and ERK1/2. Additional studies have shown that phosphorylation of EGFR also occurs with other types of



**Figure 2** Chronic inflammation activates multiple signaling cascades. The signaling pathway of the mitogen-activated protein kinase (MAPK) and the signaling pathway regulated by the epidermal growth factor receptor (EGFR) are some of the cellular signaling pathways involved in the response to asbestos inhalation.

**Abbreviations:** PI3K, phosphatidylinositol 3-kinase; AKT, AKT serine/threonine kinase; mTOR, mechanistic target of rapamycin kinase; AP-1, activator protein-1; NF $\kappa$ B, nuclear transcription factor kappa-B; ROS, reactive oxygen species; RNS, reactive nitrogen species; CytC, cytochrome C; Apaf-1, apoptotic peptidase activating factor 1.

cancer fibers and may be related to the generation of oxidants after an incomplete phagocytosis of long fibers.<sup>34</sup> The activation of these signaling pathways promotes the proliferation of fibroblasts and epithelial cells of the lung as a result of lung inflammation after chronic inhalation of earth flax (chrysotile and crocidolite).

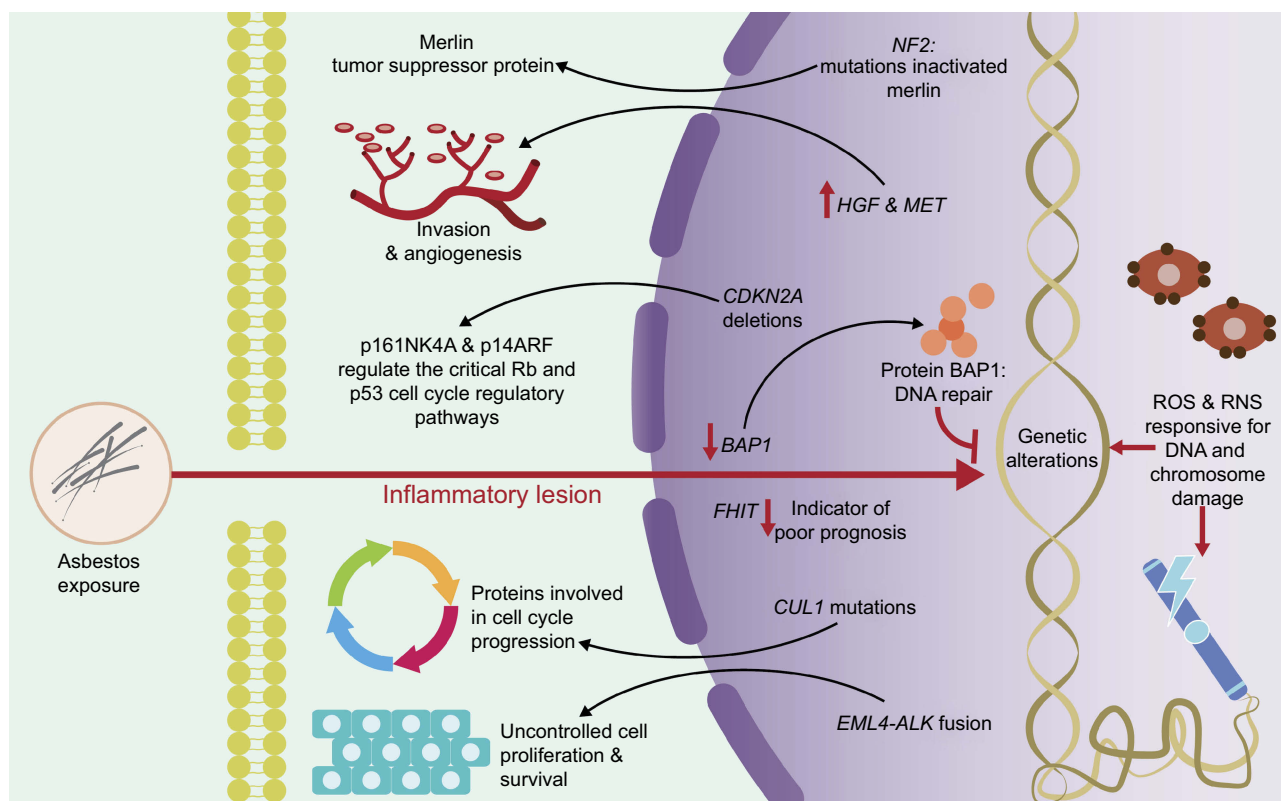
## Genomic damage induced by exposure to asbestos

At the genomic level, asbestos fibers can directly induce mutagenicity and genotoxicity through physical interaction with the mitotic machinery of dividing cells after they are phagocytosed by the target cells, or indirectly as a result of DNA damage (genetic damage) and to chromosomes (chromosomal damage) (Figure 3) by ROS and RNS.<sup>19,23,24,26</sup> ROS and RNS are responsible for producing a wide variety of DNA and chromosome damage and generate single chain breaks, chromosomal fragments, and 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a product of DNA oxidation.<sup>8,35</sup>

## Genetic damage

The activation or deactivation of certain genes by amplification or structural rearrangements (deletions or inversions) has been associated with the progressive development and treatment of pleuro-pulmonary diseases. Genes, such as BRCA1 Associated Protein 1 (*BAP1*), anaplastic lymphoma kinase (*ALK*), and mesenchymal-epithelial transition (*MET*) factor, are highly related to these diseases and play well-established roles within them (Figure 3).

*BAP1* gene has been proposed as a tumor suppressor gene, with important functions in cell proliferation and growth inhibition.<sup>36</sup> This gene is located on the short arm of chromosome 3 (3p21.1), a region that harbors germline mutations associated with an inherited multicancer syndrome with an autosomal dominant transmission<sup>37</sup> (Table 1). *BAP1* is the first and only gene that has been proposed to influence environmental carcinogenesis, such that a germinal mutation in the *BAP1* gene leads to a greater susceptibility to asbestos, which favors the



**Figure 3** Genomic damage induced by exposure to asbestos. ROS and RNS are responsible for producing a wide variety of DNA and chromosome damage. The activation or deactivation of certain genes has been associated with the progressive development and treatment of pleuro-pulmonary diseases.

**Abbreviations:** RNS, reactive nitrogen species; p16INK4A and p14ARF, cyclin-dependent kinase Inhibitor 2A; p53, protein p53; Rb, retinoblastoma protein; *NF2*, neurofibromin 2 gene; HGF, hepatocyte growth factor; MET, mesenchymal-epithelial transition factor; CDKN2A, cyclin-dependent kinase inhibitor 2A; *BAP1*, BRCA1 associated protein 1 gene; *FHIT*, fragile histidine triad gene; *CUL1*, cullin 1 gene; *EML4*, echinoderm gene associated with microtubules 4 gene; *ALK*, anaplastic lymphoma kinase gene.

**Table 1** Genes commonly altered in lung and pleural diseases associated with asbestos exposure

Chromosomal Region	Gene symbol	Gen name	Function	Alteration	Disease	References
2p23	<i>ALK</i>	Anaplastic Lymphoma Kinase gene	Oncogen	Paracentric Inversion	Lung Cancer	47,48
3p14	<i>FHIT</i>	Fragile Histidine	Regulation of apoptosis	Deletion	MPM	81,121,122
3p21.1	<i>BAP1</i>	BRCA1 Associated Protein 1	Tumor suppressor gene	Gene deletion	MPM	36,78
7q31.2	<i>MET</i>	Mesenchymal-epithelial transition factor	Cell growth/differentiation	Gene amplification	Lung Cancer	50
7q36.1	<i>CUL1</i>	Cullin 1	Ubiquitin ligase complex	Somatic mutation	MPM	78
9p21	<i>CDKN2A</i>	Cyclin Dependent Kinase Inhibitor 2A	Tumor suppressor gene	Deletion	MPM	73,74,121
	<i>CDKN2B</i>	Cyclin Dependent Kinase Inhibitor 2B	Tumor suppressor gene	Deletion	MPM	121
22q12	<i>NF2</i>	Neurobrimonin 2	Tumor suppressor gene	Deletion	MPM	121,123

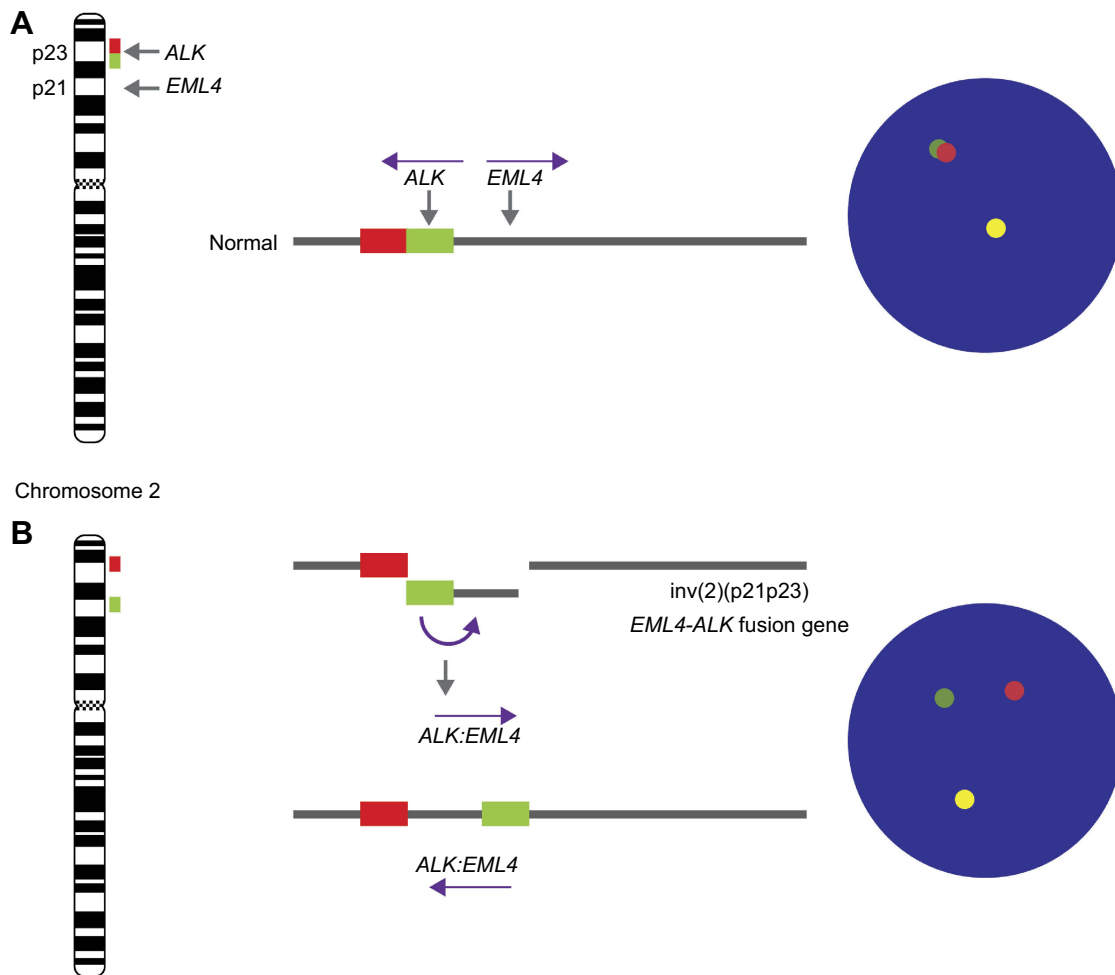
clinical onset of Malignant Mesothelioma (MM).<sup>36,38–40</sup> This gene encodes the protein BAP1, a nuclear deubiquitinate enzyme,<sup>41</sup> which plays important roles in the ubiquitin-proteasome pathway, in the deubiquitination of histones, in the regulation of cell cycle progression, and DNA repair (Figure 3). The loss of *BAP1*, independent of the mechanism that leads to such loss, including deletion or point mutation (detected with a high incidence in MM),<sup>42</sup> translates into nuclear negativity for the expression of *BAP1* assessed by immunohistochemistry.<sup>43–45</sup> The loss of the expression of the BAP1 nuclear protein is useful for differentiating both MM and malignant pleural imitators (lung and ovarian cancers) and malignant vs reactive mesothelial proliferation with a high specificity despite the variable sensitivity.<sup>45,46</sup>

*ALK* gene, located on the short arm of chromosome 2 (2p23), encodes for a receptor with tyrosine kinase activity. *ALK* regulates several signaling pathways including mitogen-activated protein kinase RAS (MAPK), phosphatidylinositol 3-kinase (PI3K)-AKT, and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways. Within the rearrangements that involve the *ALK* gene, the most frequently observed in lung cancer is the fusion of the *ALK* gene with the echinoderm gene associated with microtubules 4 (*EML4*) (Figure 3). The *EML4-ALK* fusion results in a paracentric inversion within the 2p chromosomal region that fuses different parts of the *EML4* gene with a portion of the *ALK* gene<sup>47</sup> (Table 1 and Figure 4). Such rearrangement

leads to fusion of the 5' end of *EML4* with the intracellular tyrosine kinase domain of *ALK*, leading to the constitutive activation of the *ALK* kinase and its downstream signaling pathways, and hence to uncontrolled cell proliferation and survival.

Considering that this gene rearrangement involves large chromosomal inversion and translocation, fluorescence in situ hybridization (FISH) has become the method of choice for detecting all forms of *ALK* gene rearrangement, so that a cell is considered normal (*ALK* negative) when the 5' and 3' signals are fused, whereas a cell is considered positive when 5' and 3' signals are separated (*ALK* positive) (Figures 4 and 5). *EML4-ALK* is the predominant *ALK* fusion in lung cancer, with several studies demonstrating that *ALK* fusion proteins are oncogenic and enough to induce pulmonary tumorigenesis in vivo.<sup>48</sup> Thus, the presence of *EML4-ALK* gene fusion (*ALK* positive) (Figure 5) has not only been associated with several distinctive clinicopathological features in lung diseases, including the absence of a history of smoking, but is considered an important therapeutic target, sensitive to treatment with small-molecule *ALK* kinase inhibitors, such as crizotinib.<sup>47,49</sup> The early performance of FISH tests at the time of diagnosis of diseases such as MM, adenocarcinomas and large cell lung carcinomas, can determine the appropriate treatment directed to *ALK*.

*MET* gene, located on the long arm of chromosome 7 (7q31.2), encodes for a high-affinity receptor for hepatocyte



**Figure 4** ALK Break-Apart FISH Probe for testing for the presence of *EML4* (2p21) - *ALK* (2p23) fusion gene (*ALK* rearrangement) in lung cancers. The *ALK* break-apart probe is typically designed by labeling the 3' (telomeric) part of the fusion breakpoint with one fluorochrome (orange signal) and the 5' (centromeric) part with another fluorochrome (green signal). **(A)** In normal cells, the genomic areas homologous to the 3' and 5' probes are molecularly very close and these signals are seen as fused or adjacent. In contrast, **(B)** in abnormal cells, as result of the paracentric inversion on short arm of chromosome 2 (*inv(2)(p21p23)*), a gene fusion occurs between the *AML* and *ALK* genes. When the *EML4-ALK* fusion gene is present, the 5' *ALK* green signal becomes far removed from the 3' *ALK* red signal (by approximately 12.5 Mb), and the signals are seen as being split. The *inv(2)(p21p23)* is present when a green/orange fusion signal, specific for *ALK*, splits into separate green and orange signals.

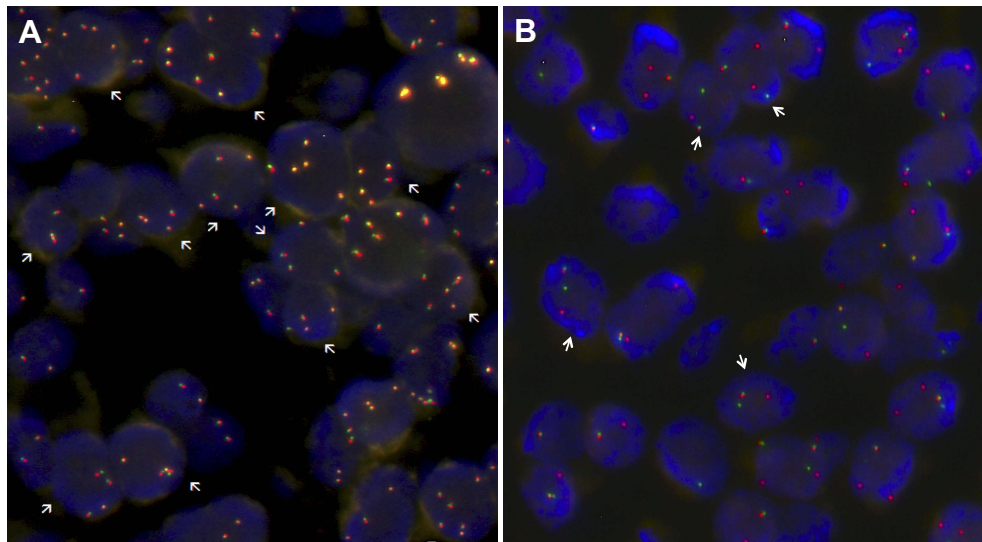
**Abbreviations:** *ALK*, anaplastic lymphoma kinase gene; *EML4*, echinoderm gene associated with microtubules 4 gene; *inv*, chromosomal inversion.

growth factor (HGF), also known as a dispersion factor, and is involved in cell growth and differentiation, neovascularization, and tissue repair in normal tissues (Table 1).<sup>50</sup> The deregulation of *MET* and HGF has been implicated in tumor development, invasion, and angiogenesis for a variety of malignancies<sup>51</sup> (Figure 3). Such deregulation can be caused by different mechanisms, including overexpression of the *MET* protein, amplification of the *MET* gene, mutations, or rearrangements. Amplification of the *MET* gene has been associated with poor prognosis, tumor development, invasion, and angiogenesis in a variety of malignancies including ovarian,<sup>52</sup> lung,<sup>53</sup> thyroid, stomach, and colon cancer.<sup>51</sup> Also, amplification of this gene has been associated with secondary resistance to the tyrosine kinase inhibitor and with aggressive anatomopathological features

in lung adenocarcinoma, such as increased tumor size, pleural invasion, and invasion of lymphatic vessels.<sup>54,55</sup> Additionally, studies in lung cancer cell lines with *MET* gene amplification have shown a significantly higher sensitivity to *MET* inhibitors, suggesting that patients with tumors harboring amplified *MET* may present clinical responses to *MET* inhibitors.<sup>56</sup> Considering the above findings, the *MET* gene has been postulated as a poor independent prognostic marker in lung adenocarcinoma,<sup>54</sup> and as a promising target for the treatment of lung cancer.<sup>57,58</sup>

Asbestos has been indicated to act as a tumor promotor and facilitates the mutagenic effects in synergy with other carcinogens,<sup>59</sup> such as cigarette smoke, generating significant DNA damage that increases in proportion to the dose of exposure to earth flax, size, and biodurability of the





**Figure 5** Signal patterns in lung tumor nuclei hybridized with ALK break-apart FISH. **(A)** A cell is interpreted as having a normal pattern (ALK negative) when the 5' and 3' signals are fused (indicated by arrows); **(B)** A cell is interpreted as having a split pattern (ALK positive) when the 5' and 3' signals are separated (indicated by arrows), regardless of the number of actual isolated signals.

**Abbreviation:** ALK, anaplastic lymphoma kinase gene.

fibers. There are two possible mechanisms by which cigarette smoke act: absorbing the carcinogens on the surface of the fibers, favoring their retention time; and increasing the penetration of the target cells due to the chemical carcinogens of the smoke.<sup>7</sup> In addition, it has been established that asbestos can inactivate the *p53* gene in epithelial, mesothelial,<sup>8</sup> and alveolar<sup>5</sup> cells in the lungs, stopping the cell cycle and apoptosis to allow time for DNA repair.

## Chromosomal damage

Some studies have shown that the direct interaction between asbestos fibers with the mitotic spindle and chromosomes during mitosis in vitro<sup>60</sup> can lead to the induction of chromosomal instability (CIN)<sup>23,61,62</sup> (Figure 3). The main chromosomal alterations reported as a result of exposure to asbestos, include numerical (aneuploidy, polyploidy, and hyperploidy) and structural alterations (deletions, translocations, inversions, duplications, chromosomal ruptures, and exchange of sister chromatids).<sup>15</sup> However, these alterations were observed in rat embryos,<sup>15</sup> with information in humans being scarce.

CIN, defined as the rate of gain or loss of complete chromosomes or fractions of chromosomes, has been recognized as a hallmark of cancer and a source of genetic variation that favors the adaptation of the tumor to stressful environments. CIN favors the simultaneous growth of various tumor subpopulations, leading to inter- and intra-tumor genomic heterogeneity (clonal heterogeneity).<sup>63–65</sup>

CIN and clonal heterogeneity lead to gene regulatory interactions and variable concentrations of proteins, which could affect the cellular response to drug treatment.<sup>66</sup>

Although several studies in humans have been aimed at determining the induction of CIN by exposure to asbestos, such studies have been limited to the identification of micronucleus (MN) and sister chromatid exchange (SCE), demonstrating an increase in the frequency of the same.<sup>35,67–71</sup> In general, the results of these studies were extremely heterogeneous in terms of type of exposure (occupational, domestic, and environmental), type of fibers, and duration of exposure. Only a few of the investigated populations showed significantly higher levels of MN. These results suggest the existence of differences in the level of DNA damage and repair between the different types of asbestos fibers. These findings suggest that the damage induced by chrysotile (white asbestos) could be repaired more easily than asbestos,<sup>71,72</sup> causing minor damage. However, few studies have described the type and frequency of specific chromosomal alterations induced by exposure to asbestos.

For instance, in MM, a rare aggressive neoplasm arising from the pleural, peritoneal, or pericardial lining, 40% to 70% of both pleural and peritoneal mesotheliomas harbor loss of 9p including loss of the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene, or 22q, including loss of the neurobrimonin 2 (*NF2*) gen.<sup>73,74</sup> Specifically, in

Malignant Pleural Mesothelioma (MPM), losses of chromosome arms 1p, 3p, 4q, 6q, 9p, 13q, 14q, and 22q and gains of chromosome arms 1q, 5p, 7p, 8q, and 17q,<sup>75</sup> deletions in *CDKN2A*, cyclin-dependent kinase inhibitor 2B (*CDKN2B*), and *NF2* genes<sup>76,77</sup> and mutations in *BAP1* and *Cullin 1 (CUL1)* genes<sup>78</sup> have been reported (Table 1 and Figure 3). Additional studies have demonstrated losses in chromosomal regions 3p14–p21, 8p12–pter, and 17p12–pter or gain in 7q.<sup>79</sup> Losses of the short arm of chromosome 3 (3p) have been reported as an early common aberration in lung cancer, observed more frequently in tumors of patients exposed to asbestos than in unexposed patients.<sup>80</sup> This chromosomal region (3p14), contains the fragile histidine triad (*FHIT*) gene, has also been associated with exposure to asbestos and smoking (Table 1).<sup>81</sup> In this disease (MPM), significant correlations have been described between high contents of asbestos fibers in lung tissue and partial or total losses of chromosomes 1, 4, and 9, and chromosomal rearrangements involving a breakpoint at 1p11–p22.<sup>82,83</sup> Comparison between recurrent altered regions in asbestos-exposed and unexposed patients showed a significant difference in the 14q11.2–q21 region, which is also lost in fiber-induced murine mesothelioma.<sup>84</sup> Chromosomal regions and genes altered in MPM are indicated in Table 1.

In Malignant Peritoneal Mesothelioma, analysis by comparative genomic hybridization showed the presence of CIN, which was characterized by losses of the chromosomal regions 3p21, 9p21, and 22q12. Interestingly, Chirac et al (2016)<sup>85</sup> reported that in patients with malignant peritoneal mesothelioma exposed to asbestos, the proportion of chromosomal losses and gains was higher than that observed in patients without asbestos exposure. Additional studies have been performed on cell lines, including V79 lung fibroblasts. Such studies demonstrated that the chrysotile and rock wool fibers cause chromosome aberrations, as indicated by a dose-dependent increase in MN frequency.<sup>86</sup> However, studies that describe the type and frequency of specific chromosomal alterations induced by exposure to asbestos are limited.

## Asbestos and disease

Prolonged exposure to asbestos fibers, the accumulation of these in the lungs, and the sum of other risk factors, such as smoking, lead to the development of various diseases, which are mainly pulmonary.<sup>3–5,87</sup> Usually, people who have diseases related to the mineral do not show signs of the disease until a long time after the first exposure. It can take 10 to 40 years or more for the symptoms of an

asbestos-related condition to appear.<sup>5,6</sup> Among the diseases generated by exposure to asbestos fibers are pulmonary fibrosis (asbestosis) and malignant tumors (lung cancer and mesothelioma),<sup>6–9</sup> among others.

## Asbestosis

Asbestosis is a chronic lung disease caused by the inhalation exposure to asbestos after a latent period of more than 20 years.<sup>88</sup> Prolonged exposure to these fibers and their deposition in the lungs triggers an inflammatory process that can lead to the formation of scars (fibrosis) inside the lung. In this process, the fibrosis is of an interstitial and diffuse type, tends to affect primarily the lower lobes and the peripheral areas, and, in advanced cases, is associated with the obliteration of the normal architecture of the lung. Fibrosis of the adjacent pleura is common. None of the histological features of asbestosis differentiates it from interstitial fibrosis due to other causes, except for the presence of earth flax in the lung in the form of asbestos bodies, visible under an optical microscope, or uncoated fibers, most of which are too thin to be visualized except by electron microscopy.<sup>13</sup>

Sometimes fibrosis can be limited to relatively few areas, mainly affecting the peribronchiolar regions, causing disease of the small airways related to earth flax. In this case, none of the histological changes of this process distinguishes it from disease of the small airways due to other causes (such as tobacco use or exposure to other mineral powders), except for the presence of earth flax in the lung. The disease of the small airways may be the only manifestation of asbestos-related pulmonary fibrosis, or it may coexist with varying degrees of interstitial fibrosis.<sup>13</sup>

The initial stage of asbestosis is characterized by discrete foci of fibrosis within the respiratory bronchiole walls and alveolar duct bifurcations associated with the accumulation of earth flax bodies.<sup>7,89,90</sup> Asbestos triggers the accumulation of AMs and an inflammatory reaction, followed by a more diffuse pulmonary involvement characterized by 1) loss of the alveolar epithelium type I and Alveolar Type II (AT2) cells, 2) proliferation of fibroblasts, and 3) collagen deposition. Pulmonary fibrosis of asbestosis is associated with fibrosis of the walls of the respiratory bronchioles and alveolar ducts. The site of asbestos-induced inflammation occurs in the area of fiber deposition along the airways and in the alveolar spaces.<sup>89,90</sup>

The ingestion of asbestos fibers by macrophages triggers a fibrogenic response of fibroblasts by the release of growth factors, such as TGF- $\beta$  and the platelet-derived growth factor, as well as cytokines, such as the TNF- $\alpha$  and IL-1 $\beta$ , which collectively promote collagen deposition.<sup>16,91</sup>

Indicative characteristics for the diagnosis of asbestosis include reliable exposure to asbestos; an appropriate latency period, typically >20 years; where exertional dyspnea and dry cough together with the late inspiratory crackles are the most frequent symptoms and signs; abnormal chest images showing subpleural reticular abnormalities with basal predominance, typically with pleural plaques (80–90%); and restrictive pulmonary physiology with reduced gas exchange.<sup>90</sup> In cases of occupational exposure, a chest X-ray with reading is used with applying the radiological classification of the International Labor Organization.<sup>13</sup>

## Lung cancer

Lung cancer remains the leading cause of incidence and mortality due to cancer worldwide (with 2.1 million new cases of lung cancer and 1.8 million deaths expected in 2018).<sup>92</sup> Even if mesothelioma is commonly known as the primary type of cancer related to asbestos, it has been estimated that asbestos results in an equal or greater number of lung cancer cases compared to mesothelioma.<sup>80</sup> The IARC concluded that there is sufficient evidence of carcinogenicity in humans for all types of asbestos, including chrysotile.<sup>5</sup>

Asbestos fibers have been indicated to interact with other genotoxic agents, such as tobacco smoke, increasing not only CIN<sup>93</sup> but also the risk of lung cancer.<sup>94</sup>

## Malignant Mesothelioma (MM)

MM is an aggressive and fatal tumor strongly associated with asbestos exposure. MM is responsible for ~3,000 deaths per year in the United States and 5,000 deaths in Western Europe.<sup>95</sup> According to Montanaro et al,<sup>91</sup> MM incidence or mortality predicts a steady growth in the number of cases among industrialized countries, following a plateau or decline as a consequence of the restriction on the use of asbestos. In addition, the demography of MM has changed; the age of MM patients has decreased and there is an increased incidence in women, likely reflecting exposure from non-occupational sources.<sup>96</sup> MM is a highly aggressive, fast-growing type of cancer, associated with a low rate of patient survival, poor prognosis,

and low overall survival, relatively resistant to chemotherapy and radiotherapy, with limited therapeutic options.<sup>97</sup> The median overall survival for MM following frontline chemotherapy with pemetrexed and cisplatin is only ~12 months.<sup>98</sup>

This type of neoplasm results from the uncontrolled proliferation of mesothelial cells lining pleural, pericardial, and peritoneal cavities. According to the IARC,<sup>5</sup> MM has been related to occupational, domestic, and environmental exposure to asbestos. Thus, in at least 376 cases of MM, the causative agent was non-occupational (domestic) exposure to asbestos.<sup>99</sup>

The populations most exposed to the development of this type of neoplasm are those who work in the automobile industry, fiber cement products factories, and construction, in combination causing 70–80% of cases of mesothelioma. However, the development of diseases due to exposure to asbestos is not only occupational, but also domestic or even environmental. It has been reported that the families of these aforementioned workers and the communities surrounding the factories can also develop harmful symptoms and diseases.<sup>100</sup>

Although it has been observed that 10% of those who died due to MM did not present asbestos or earth flax residues in their biopsy, 90% of patients who have MM attribute it to exposure to these compounds. The rare cases of MM without exposure to asbestos have been related to exposure to factors such as ionizing radiation, other fibrous minerals, and genetic predisposition.<sup>101</sup> The time from exposure to asbestos to the diagnosis is considerably long, but the time from the onset of the disease to the malignancy is short. In addition, the affected organism shows symptoms soon after the initial growth.

At the genetic level, the activation or deactivation of certain genes allows the progressive development of MM. Genes, such as *CDKN2A*, *NF2*, and *BAP1*, are highly related to this disease and play well-established roles within it. The next-generation sequencing data indicate that *NF2* and *BAP1* genes are the most frequently mutated genes in MM.<sup>73,78,102</sup>

## Biomarkers of asbestos exposure in the evaluation of cancer risk

The identification of biomarkers for the evaluation of the carcinogenic risk in populations exposed to asbestos and also for an early diagnosis of malignant diseases, has been the topic of research of several studies. Among the most

**Table 2** Biomarkers of asbestos exposure in the evaluation of cancer risk

Asbestos biomarker	Biomarker symbol	Description	References
Soluble mesothelin-related protein	SMRP	Mesothelin is the only blood-based biomarker approved by Food and Drug Administration in MM diagnosis	<sup>103,104</sup>
Osteopontin	None	Osteopontin is an integrin-binding protein involved in tumorigenesis, progression and metastasis	<sup>105</sup>
Fibulin-3	Fb-3	Fb-3 could play a role in the development of neoplastic and non-neoplastic diseases of the respiratory tract in subjects exposed to asbestos and/or asbestos-like fibers	<sup>106,107</sup>
High Mobility Group Box 1 (HMGB1) protein	HMGB1 protein	Total level of HMGB1 in the blood was significantly higher in patients with MM and in patients exposed to asbestos compared to healthy controls	<sup>108,124</sup>
Aquaporin I	AQPI	The expression of aquaporins has been shown to play a role in the growth and metastatic potential of different tumors, including pulmonary adenocarcinoma.	<sup>109</sup>
Fibronectin	None	Fibronectin is a glycoprotein involved in the extracellular matrix structure that plays a role in the generation of fibrotic tissue	<sup>110</sup>
Interleukin 6 and interleukin 8	IL-6 and IL-8	Members of a large family of cytokines that promote the development, differentiation and activation of lymphocytes and play an important role in the immune response	<sup>111,112</sup>

studied biomarkers of asbestos exposure are: the soluble mesothelin-related protein,<sup>103,104</sup> osteopontin,<sup>105</sup> fibulin-3 (Fb-3),<sup>106,107</sup> high mobility group box 1 protein (HMGB1),<sup>40,108</sup> aquaporin 1 (AQPI),<sup>109</sup> fibronectin,<sup>110</sup> (IL-6,<sup>111</sup> and IL-8<sup>112</sup> (Table 2). However, according to Ledda et al (2018),<sup>113</sup> none of the markers available today are sufficiently reliable to be used in the surveillance of subjects exposed to asbestos. Of note that, new biomarkers such as miRNAs have been recently introduced, which could be useful to monitor sensitivity to therapy and for prognostic purposes. Some examples of such miRNAs include miRNA-16-5p, miRNA-126-3p, miRNA-143-3p, miRNA-145-5p, miRNA-192-5p, miRNA-193a-3p, miRNA-200b-3p, miRNA-203a-3p, and miRNA-652-3p.<sup>114–118</sup>

## Epidemiology

According to global estimates, at least 107,000 people die every year from lung cancer, mesothelioma, and asbestosis as a result of occupational exposure to asbestos.<sup>5</sup> Almost 400 deaths have been attributed to non-professional exposure to asbestos. The number of asbestos-related diseases continues to rise, even in countries that banned the use of asbestos in the early 1990s. Due to the long latency periods associated with the diseases in question, suspending from now the use of asbestos will result in a decrease in the number of deaths related to asbestos alone after several decades.<sup>5</sup>

Even though asbestos has been banned in several countries around the world, in Colombia, limitations on the use of asbestos are few. In Colombia, asbestos consumption in 2015 was 5960 metric tons according to data published by the United States Geological Survey in 2018.<sup>119</sup> According to the Ministry of Social Protection, in Colombia there is only one exploitation of chrysotile asbestos, with an approximate production of 9,000 tons per year in recent years and 270,000 tons per year of asbestos-cement (10% asbestos+90% cement) registered in the 1980s.<sup>120</sup> There are no exact data of the other economic activities in which there is exposure to asbestos. However, the study group that, together with the Ministry, carried out the National Plan for the Prevention of Silicosis, Pneumoconiosis of the Coal Miner and Asbestosis 2010–2030, managed to detect – by surveying professional risk insurance companies (ARP) – 256 companies that develop 25 economic activities with asbestos use. In these companies, it was calculated that 7% of the workers (688 of 15,170) are exposed to asbestos.<sup>120</sup>

## Conclusions

Asbestos is one of the most important occupational carcinogens used in many industries around the world. The high pathogenicity of this mineral fiber is currently known and has been shown that exposure to it causes oxidative stress, fibrosis, chronic inflammation, direct damage to DNA, and mutagenesis, all of the above associated with the development

of lung diseases. Additionally, due to the long latency periods associated with diseases generated by asbestos exposure, the diagnosis of these is delayed, leading to a high percentage of deaths in people exposed. Taking into account the above, improving our knowledge about the mechanisms of cellular and molecular response to asbestos, could have a significant impact on our ability to determine susceptibility to exposure and to establish early diagnoses and more effective treatments.

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## Author contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work

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The authors report no conflicts of interest in this work.

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