INVITED REVIEW ARTICLE

MECHANISMS OF ASBESTOS-INDUCED CARCINOGENESIS

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ABSTRACT

Respiratory exposure to asbestos fibers has been associated with diffuse malignant mesothelioma (DMM) in humans. Despite advancements in the molecular analyses of human DMM and the development of animal models, the carcinogenic mechanisms of the disease remain unclear. There are basically three hypotheses regarding the pathogenesis of asbestos-induced DMM, which may be summarized as follows: (1) the "oxidative stress theory" is based on the fact that phagocytic cells that engulf asbestos fibers produce large amounts of free radicals due to their inability to digest the fibers, and epidemiological studies indicating that iron-containing asbestos fibers appear more carcinogenic; (2) the "chromosome tangling theory" postulates that asbestos fibers damage chromosomes when cells divide; and (3) the "theory of adsorption of many specific proteins as well as carcinogenic molecules" states that asbestos fibers *in vivo* concentrate proteins or chemicals including the components of cigarette smoke. Elucidation of the major mechanisms underlying DMM would be helpful for the development of novel strategies to prevent DMM induction in people who have already been exposed to asbestos.

Key Words: Asbestos, Mesothelioma, Iron, Oxidative stress

INTRODUCTION

Asbestos fibers have been heavily used in industry since World War II to the present because of their durability, heat-resistance, and low cost.[1,2] However, in 1987, the IARC designated asbestos fibers as a Group I (definite) carcinogen for humans (http://monographs.iarc.fr/ENG/Classification/crthgr01.php), and asbestos fibers were banned in many Western countries in the 1990's.[1,2] In June of 2005, asbestos-associated deaths suddenly attracted widespread attention in Japan when it was reported that over the past 26 years, 79 factory workers using asbestos died from a rare asbestos-associated cancer called diffuse malignant mesothelioma (DMM).[3] Furthermore, people who lived near those factories also suffered from the same fatal disease.[4]

The characteristics of DMM are as follows: (1) it is associated with repeated asbestos exposure,[5,6] (2) once diagnosed, prognosis is overwhelmingly poor,[7] and (3) it takes 30 to 40 years after the start of asbestos exposure for DMM to occur.[5,6] Currently, approximately 1,000 and 3,000 patients are diagnosed each year with DMM in Japan and the United States, respectively. It is expected that the incidence of DMM in Japan will peak in 2025 with a cumulative 100,000

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deaths predicted from this neoplasm.[8] This article briefly reviews the history, epidemiology, and genomic analysis of human DMM, and discusses the possible molecular mechanisms underlying asbestos-induced carcinogenesis.
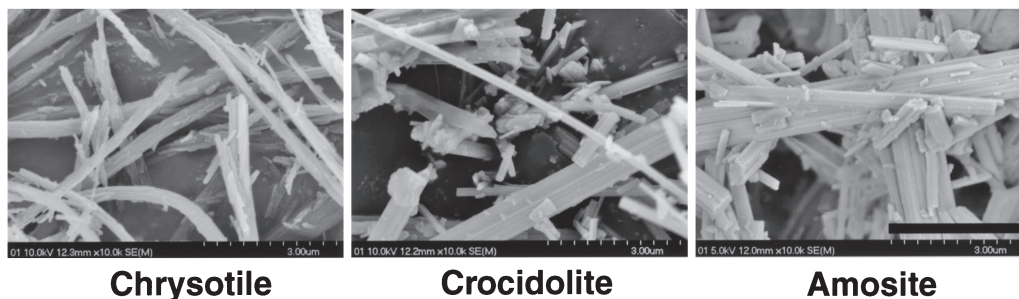
Asbestos fibers

Asbestos is a naturally occurring mineral conventionally divided into two mineralogic groups. The amphiboles include crocidolite (blue asbestos), amosite (brown asbestos), tremolite, anthophyllite, and actinolite. Among the amphiboles, only crocidolite and amosite have widespread commercial utilization. The noncommercial amphiboles (the most commonly occurring and widely distributed amphibole asbestos mineral group) are primarily significant as contaminants of other minerals, such as chrysotile.[9] The second group of asbestos minerals is the serpentine group, of which chrysotile (white asbestos) is the sole variety.

The amphibole and serpentine minerals occur both as asbestiform (fibrous) and nonasbestiform (massive) varieties of identical chemical composition. Amphibole crystallization is believed to occur initially as the massive form under conditions of moderate temperature and pressure, with transformation into the fibrous form occurring when the unstable massive form is submitted to rock stresses. Similarly, serpentine minerals first crystallize as the massive form, with chrysotile being subsequently formed by recrystallization.[10]

Chrysotile is a hydrated magnesium silicate with the chemical composition indicated in Table 1. Individual fibrils of chrysotile have diameters of 20–40 nm. Crushing of chrysotile ore produces fiber bundles consisting of variable numbers of aggregated individual fibrils. These

Table 1 Composition and characteristics of asbestos fibers

Name	Composition	Source	Morphology
Chrysotile	$Mg_6Si_4O_{10}(OH)_8$	U.S. and Canada	Curly, pliable
Crocidolite	$Na_2(Fe^{3+})_2(Fe^{2+})_3Si_8O_{22}(OH)_2$	South Africa Western Australia	Rodlike, durable
Amosite	$(Fe, Mg)_7Si_8O_{22}(OH)_2$	South Africa	Rodlike, durable
Anthophyllite	$(Mg, Fe)_7Si_8O_{22}(OH)_2$	Finland	Rodlike, durable
Tremolite	$Ca_2Mg_5Si_8O_{22}(OH)_2$	Exists in some deposits of Canadian chrysotile	Rodlike, durable
Actinolite	$Ca_2(Mg, Fe)_5Si_8O_{22}(OH)_2$	Not mined	Rodlike, durable

Chrysotile **Crocidolite** **Amosite**

Fig. 1 Scanning electron micrographs contrast curved fibers of chrysotile asbestos (left) with straight fibers of crocidolite (center) and amosite (right; bar=3 μm). Asbestos fibers are from UICC.

fibers have varying lengths that may exceed 100 μm. Typically, chrysotile fibers exhibit a curved, curly, or wavy morphology, which is most apparent in fiber bundles exceeding 10 μm in length (Fig. 1). In addition, the ends of chrysotile fiber bundles often exhibit a splayed appearance because of the separation of individual fibrillar units. This curly morphology influences the interceptive deposition of chrysotile fibers, which in turn, affects the depth of penetration into the lower respiratory tract. Inhalational studies in rats have shown that substantial numbers of chrysotile fibers 5 μm or greater in length can penetrate into the lung periphery.[11,12]

The amphiboles are a group of hydrated silicates with a wide range of cation substitutions within the silicate backbone of the crystal structure. The predicted chemical formulas of the asbestiform varieties of amphibole minerals are summarized in Table 1. The diameters of individual fibers vary considerably with substantial overlap among members of the amphibole group. However, crocidolite generally has the finest fiber diameters (Fig. 1). Amosite fibers are on average somewhat thicker, and the noncommercial amphibole fibers tend to be the coarsest.

History and epidemiology

The discovery of this rare tumor known as diffuse malignant mesothelioma (DMM), and the subsequent controversies that arose about its causation by specific forms of commercial asbestos is a long and complex story. During the late 19th and early 20th centuries, there was a general consensus that some sarcomas arose from the pleura when there was no evidence of a primary tumor elsewhere. There are three major histological types of DMM in the present classification, namely, epithelioid, sarcomatoid, and biphasic.[13] Miller and Wynn[14] were the first to recognize that a peritoneal neoplasm was able to present both epithelial and fibroblastic characteristics because of the embryologic relationship of these cells to the mesoderm.

In 1924, Robertson's article[15] on endothelioma of the pleura was probably the most thorough review of the literature then available. At the time of the article's publication, endotheliomas or primary pleural malignancies were certainly rare. In 1940, Ewing, who raised the question of the influence of chronic irritation and inflammation in causing connective tissue changes in the pleura, speculated that some cases of pleural malignancies were associated with tuberculosis.[16] Many of the previously reported cases showed evidence of coexistent tuberculosis. The trauma and chronic inflammation as a cause of pleural transformation were reviewed by Ewing. During the 1940's and 50's, in the confusion about whether mesothelioma was truly a separate clinical entity, there were five different opinions as to the source of the tumor: (1) an aberrant nest of lung epithelium turned malignant within the lining of the pleura; (2) the endothelial lining of the subpleural lymphatics was the source of the tumor, hence the name endothelioma; (3) the tumor arose from the pleural capillary endothelium or endothelial lining of the subpleural lymphatics, or both; (4) the tumor originated from the mesothelial lining of the pleura itself, or was a mesothelial-derived tumor or a mesothelioma; (5) such tumors of epithelial origin always arise from a primary tumor elsewhere.

Amidst confusion over the concept and origin of the tumor, early reports began to filter in that some patients with asbestosis developed an unusual form of pleural malignancy. The first was by Wedler, who reported the results of 29 autopsies on asbestos workers in Germany.[17] Four had bronchial cancers and two others had a malignant pleural growth. He commented about his own impression that the incidence of cancer, which was 20% for malignant tumors in this population, was much too high to have occurred by chance. The seminal year for establishing the association between asbestos exposure and mesothelioma was 1960, when Wagner *et al.* published a paper entitled "Diffuse malignant mesothelioma and asbestos exposure in North-western Cape Province."[5] The paper was very controversial at that time, since it described 33 cases of DMM with exposure to only one type of asbestos, the so-called Cape Blue asbestos

mined in the asbestos hills west of Kimberly in the northwest Cape Province of South Africa. He also reported that the tumor was rarely seen elsewhere in South Africa. In 1962, Wagner was able to produce mesothelial tumors of the pleura by direct implantation of asbestos dust in laboratory animals.[18] The association between asbestos exposure and diffuse abdominal tumors was established in the English literature by Enticknap and Smither in 1964.[19]

Of particular interest epidemiologically was the case control study of Newhouse and Thompson.[20] They diagnosed 83 patients with mesothelioma in association with a Cape Blue asbestos factory that had opened in London in 1913. There were 27 peritoneal tumors and 56 pleural tumors. The factory had used only Cape crocidolite exclusively until 1926, when small amounts of amosite and chrysotile were added. Particularly distressing was the discovery of 36 patients with no known employment or domestic exposure to asbestos. Eleven of these patients lived within a half mile of the asbestos factory, suggesting neighborhood exposure. A paradigm shift occurred. By 1970, it was generally accepted that low-level exposure to northwest Cape Blue crocidolite was capable of causing mesothelioma. By 1966, the import of crocidolite asbestos was voluntarily abandoned in England, and new asbestos regulations accepting the relationship between asbestos and mesothelioma were adopted in 1969. The history of the early years of the discovery of mesothelioma is an example of how slowly the medical community accepts new discoveries.[21] Acceptance was partly delayed by the lack of specific mesothelial cell markers such as are available today to ensure proper diagnosis (calretinin, podoplanin, WT1, cytokeratin, *etc.*). Since then, extensive epidemiological data have accumulated.[22] The general current consensus is that the risk of mesothelioma and lung cancer is greatest with crocidolite, less with amosite, and apparently less with chrysotile. With regard to amosite and chrysotile, there appears to be a higher risk in manufacturing than in mining and milling.

Genomic analysis of human diffuse malignant mesothelioma

Carcinogenesis is a multi-stage process, consisting of genetic and epigenetic alterations. It is generally accepted that virtually all neoplasms undergo certain genetic changes, whether they are large deletions, amplifications or just a point mutation. Mesothelioma tumors accumulate a spectrum of acquired genetic lesions during the molecular pathogenesis leading to overt cancer. Perhaps reflecting the unique history of asbestos exposure routinely seen in mesothelioma patients, many of the well characterized mutations found in other cancers such as *p53* and *ras* family alterations are not common features in DMM.[23] Nonetheless, a variety of well-defined molecular abnormalities have been identified in the majority of DMM cases. The lack of a heritable model for mesothelioma, such as that observed in breast and colon cancers, has fueled the genetic studies of asbestos-induced mesothelioma. Chromosome banding techniques have revealed that most DMMs have complex karyotypes.[24,25] The karyotypes of 39 DMMs repeatedly exhibited extensive aneuploidy and structural rearrangements of various chromosomes, in particular the short arms of chromosomes 1, 3, and 9, and the long arm of chromosome 6. The loss of one copy of chromosome 22 is the single most consistent numerical change observed in DMMs.[26] Here, I mention three tumor suppressor genes that are mutated in the majority of human DMM cases.

Multiple lines of research have revealed that among the most commonly acquired genetic abnormalities in cancer is the loss of G1 to S checkpoint control. When mesothelioma cell lines and tumors were examined for the presence of $p16^{INK4A}$ gene product, a cyclin-dependent kinase inihibitor, it was found to be absent in all cases.[27] Furthermore, a non-deletional loss of $p16^{INK4A}$ gene expression via epigenetic mechanisms of the genes at the 9p21 locus is less common in mesothelioma than in other cancers.[28,29] Potentially, a loss of genetic material at the 9p21 locus leads to the loss of cell cycle regulation through both the pRb and p53 pathways.[30]

Subsequent positional cloning isolated the *NF2* gene as the targeted gene whose loss of

function accounts for this clinical syndrome. The 70-kd *NF2* gene product is a moesin ezrin radixin-like protein that maps to 22q11-q13.1 and has also been called both Merlin and Schwannomin. *NF2* is associated with a high frequency of the loss of chromosome 22, and inactivating mutations are frequently observed in this gene.[31,32] Recently, it was reported that YAP1 is involved in mesothelial cell growth; the transcriptional co-activator activity of YAP1 is functionally inhibited by Merlin through phosphorylation and cytoplasmic retention of YAP1.[33]

Mutations of the *WT1* gene in mesothelioma were noted after the rather striking finding that the WT1 protein is routinely expressed in normal mesothelium as well as in urogenital tissues. Furthermore, DMMs were generally found to express elevated levels of WT1 protein, as detected by immunohistochemistry. The presence of nuclear staining for WT1 has been reported in 75% to 100% of mesothelioma tumors and cell lines examined to date.[34]

Animal models of diffuse malignant mesothelioma
It is well established that inhalational, intraperitoneal and intrapleural exposure to asbestos fibers causes mesothelioma and/or lung cancer.[35-38] This has been reviewed in detail elsewhere.[1,21] A macroscopic appearance of rat peritoneal mesothelioma induced by crocidolite is shown in Fig. 2. DMM was successfully induced also by repeated intraperitoneal injections of ferric saccharate[39] that is deposited in the peritoneum. In contrast, the repeated intraperitoneal administration of ferric nitrilotriacetate, an iron chelate soluble at neutral pH, has been found to induce oxidative stress in the renal proximal tubules, ultimately leading to a high incidence of renal cell carcinoma.[40-42]
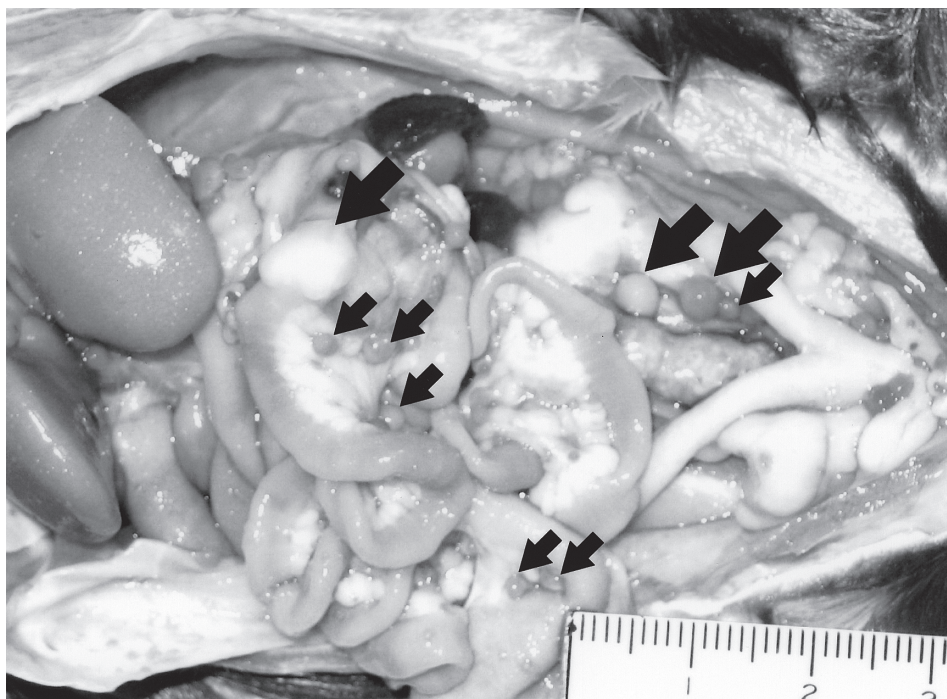
Fig. 2 Peritoneal diffuse malignant mesothelioma induced by intraperitoneal injection of crocidolite in a female rat. Arrows: various size of grape-like tumors are observed in mesentery.

Mechanisms of carcinogenesis

The strong association between asbestos exposure and malignant mesothelioma has been widely accepted since 1960. Although asbestos is the primary etiologic agent for this tumor, a certain number of patients who develop mesothelioma have no known asbestos exposure. Radiation,[43] nonasbestos mineral fibers, organic chemicals, chronic inflammation,[44] and simian virus 40 (SV40) exposure have also been suggested as risk factors for mesothelioma in humans. Although the "SV40 oncogenic virus contamination theory" (polio vaccination) does not appear to be a major factor, at least in Japan,[45,46] there is evidence that this oncogenic polyomavirus can play a variety of promotional roles in the carcinogenic processes of mesothelioma.[47]

Currently, there are three hypotheses regarding the pathogenesis of asbestos-induced DMM, which may be summarized as follows: (1) the "oxidative stress theory"[48] is based on the fact that asbestos fibers are foreign bodies, and that epidemiological studies show asbestos fibers containing iron (a transitional metal which catalyzes free radical generation) to be more carcinogenic;[49] (2) the "chromosome tangling theory" postulates that asbestos fibers damage chromosomes when cells divide;[50] and (3) the "theory of adsorption of many specific proteins as well as carcinogenic molecules" states that asbestos *in vivo* contains chemicals including components of cigarette smoke.[48]

Here each hypothesis is described in greater detail, and include my own interpretations (Fig. 3). In the "oxidative stress theory," there are two distinct players, namely, endogenous iron in asbestos fibers and phagocytes. Regarding the former, surface iron can catalyze the production of reactive oxygen species. Recently, my laboratory has shown, using several different methods, that amosite and crocidolite are good catalysts for free-radical generation.[51] In the pathologic diagnosis of asbestos exposure, the presence of asbestos (ferruginous) bodies is an important hall-mark. Indeed, excess iron is carcinogenic not only to animals, but also to humans.[41,42,52] In the latter case, "frustrated macrophages engulfing overly long asbestos fibers" have been frequently shown. It is well established that chronic inflammation is a risk factor for carcinogenesis.[53] It is interesting to note here that one of the major target genes in ferric nitrilotriacetate-induced renal cell carcinoma is *p16INK4A* arising from homozygous deletion or methylation of its promoter region.[54] I believe that this is not a coincidence, and that the genetic locus of *p16INK4A* is some-how susceptible to oxidative stress by iron. Additionally, the mesodermal origin of target cells in both carcinogeneses may be associated. My laboratory is interested in whether there are any genomic loci susceptible to oxidative stress. We are currently working on this hypothesis under the concept of oxygenomics.[42,55] This idea is important for the elucidation of the carcinogenic mechanism of human DMM according to the "oxidative stress theory."

It is somewhat more difficult to understand the "chromosome tangling hypothesis." We recently found that asbestos fibers including crocidolite are actively taken up by several different kinds of cultured cells.[51] Furthermore, those fibers enter both the cytoplasm and the nucleus. In this situation, asbestos fibers may tangle with chromosomes when cells divide. Whether there is a specificity of tangling for any chromosomal region is the next question to be addressed.

Finally, as for the "adsorption theory," it is well known that the surface of asbestos fibers have a high affinity for certain proteins and molecules. This appears to be at least partly as-sociated with positive or negative charges on the asbestos surface. MacCorkle *et al.* reported an interesting finding that cytoskeletal and cell cycle proteins have high affinities for CCD-18 cells.[56] However, it remains unclear how an asbestos (ferruginous) body is produced following exposure to asbestos. Recently, we found that, although chrysotile does not contain iron as a constituent, it concentrates iron in the surrounding tissue, probably via hemolysis.[51] It is well known that it takes 30 to 40 years from the start of asbestos exposure for DMM to manifest itself. This probably includes the time needed for the asbestos fibers to reach the parietal pleura
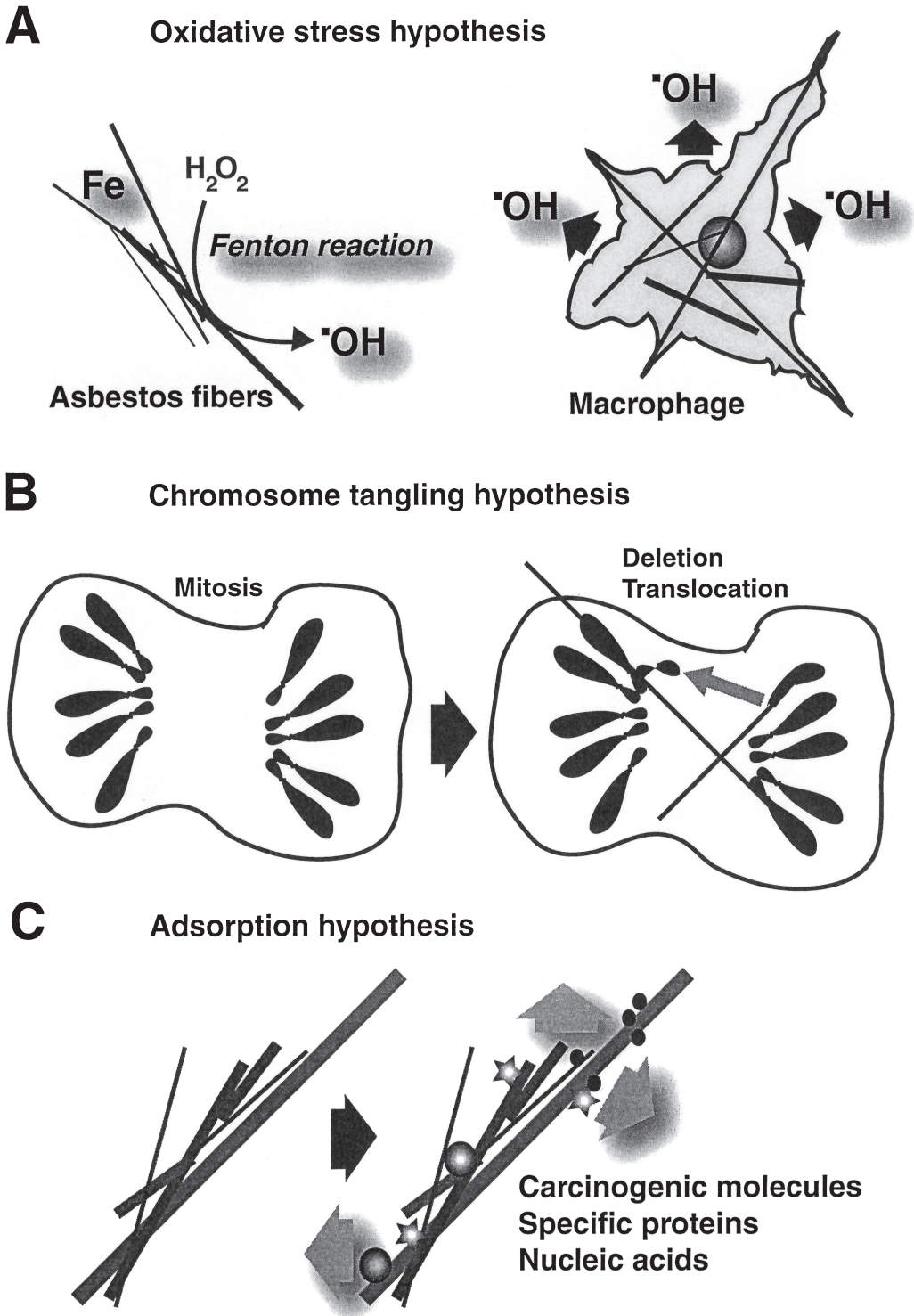
A **Oxidative stress hypothesis**

Fe

H_2O_2

Fenton reaction

$^{\cdot}OH$

Asbestos fibers

$^{\cdot}OH$

$^{\cdot}OH$

$^{\cdot}OH$

Macrophage

B **Chromosome tangling hypothesis**

Mitosis

Deletion
Translocation

C **Adsorption hypothesis**

Carcinogenic molecules
Specific proteins
Nucleic acids

Fig. 3 Possible mechanisms of asbestos-induced carcinogenesis.

according to the negative pressure of the pleural cavity.

Concluding remarks

The molecular mechanism of asbestos-induced carcinogenesis is complex. We believe that catalytic iron, whether endogenously or by other mechanisms, plays an important role in this carcinogenesis. These observations suggest that the modulation of iron status is one of the measures for the prevention of DMM in those already exposed to asbestos. The shape, size, and adsorbing nature of the fibers also appear to be critically important. Recently, doubts have arisen concerning the safety of commercially available carbon nanotubes,[57,58] which may possess the same carcinogenicity as asbestos fibers because of their similar characteristics. Ample care has to be taken to prevent a tragedy similar to the one caused by asbestos exposure.

ACKNOWLDGMENTS

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