Cellular and Molecular Mechanisms of Asbestos Carcinogenicity: Implications for Biopersistence

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Carcinogenic agents can influence the carcinogenic process either by mutating critical target genes or by increasing the number of cells at risk for mutations. Cytogenetic and molecular studies of asbestos-related cancers indicate that inactivation or loss of multiple tumor suppressor genes occurs during lung cancer development. Aneuploidy and other chromosomal changes induced by asbestos fibers may be involved in genetic alterations in asbestos-related cancers. Furthermore, asbestos fibers may influence the carcinogenic process by inducing cell proliferation, free radicals, or other promotional mechanisms. Therefore, asbestos fibers may act at multiple stages of the carcinogenic process by both genetic and epigenetic mechanisms. Biopersistence is undoubtedly important in fiber carcinogenicity. However, the time required for a fiber to remain in the lung to exert a cancer-related effect is difficult to specify. — Environ Health Perspect 102(Suppl 5):19–23 (1994)

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To understand the importance of biopersistence in fiber carcinogenesis, it is necessary to elucidate how and when fibers influence the carcinogenic process. Cancer is a chronic disease requiring a latency period of several decades. The necessity of this latency can be explained in large part by the multistep nature of the neoplastic process (*1–3*). There is now overwhelming evidence to support the concept that most, if not all, cancers develop as the consequence of mutations in critical genes (*1–3*). The number of mutations required for a given cancer is not known precisely and can be variable; however, many cancers have mutations in multiple genes; often 5 to 20 mutations in cancer-related genes are found in human tumors (*1–3*).

The critical target genes for cancers can be divided into two distinct classes—protooncogenes and tumor suppressor genes. Protooncogenes are activated by point mutations, chromosomal translocations, and gene amplification (*1–3*). Once activated or inappropriately expressed, these genes act as positive regulators of cell growth and invasion. Tumor suppressor genes, in contrast, are negative regulators of cancer cells. These genes must be inactivated, lost, or mutated for malignant properties of cancer cells to manifest (*1–3*). Mutational mechanisms for tumor suppressor genes in cancers include chromosome losses, gene deletions, and point mutations (*1–3*). Recent evidence strongly indicates that different steps in the multistep process of carcinogenesis are defined by mutational alterations of tumor suppressor genes (*2*). Common adult tumors in humans frequently have mutations in two to four different tumor suppressor genes, indicating multiple negative controls of malignant cells (*1–3*).

The most likely mechanism for acquisition of multiple mutations in the same cell is by clonal evolution (*4*). According to this model (Figure 1), cells with one critical mutation clonally expand. By increasing the number of cells with the first mutation, the probability of a second mutation in the same cells increases. Once a cell with two mutations arises, it will also clonally expand and thereby increase the probability of a third mutation within one of its progeny. This cell can then undergo further mutations by clonal evolution, allowing malignant development.

Carcinogenic agents can accelerate this process by several mechanisms at different stages. Neoplastic development can be enhanced by agents that mutate critical target genes, i.e., by either inactivation of tumor suppressor genes or activation of protooncogenes. Furthermore, agents that influence the rate of clonal expansion of cells will increase the number of cells at risk for mutations and thereby increase the rate of neoplastic progression and shorten the latency period for cancer development.

In the context of the multistep/multigene model of cancer, at what stages or steps do asbestos and other mineral fibers act, and what are the implications of these mechanisms for the role of biopersistence in carcinogenic potential of fibers? Examination of the molecular alterations in

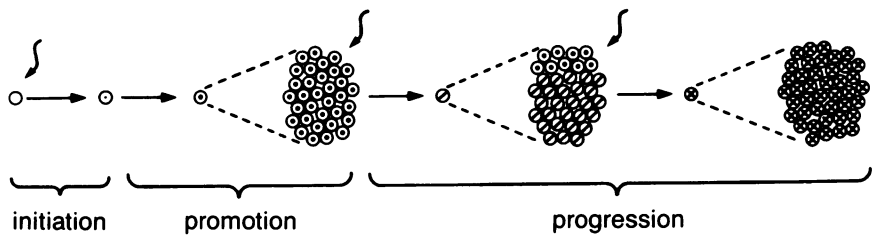

Figure 1. In this model of neoplastic development it is assumed that the heritable alterations of different genes occur as the consequence of chemically induced or spontaneous events.

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asbestos-related cancers (e.g., mesotheliomas) provides insight into the critical events in these neoplasms.

Cytogenetic analyses often provide the first clues as to the critical changes for a given tumor type. Nonrandom chromosome changes indicate chromosomal regions where critical target genes for cancers reside. Specific nonrandom chromosome alterations have been observed in human mesotheliomas (*5*). Of particular interest are alterations involving human chromosomes 1, 3, 5, 6, and 7 (Table 1). Deletions or rearrangements involving chromosome 1 have been reported in several studies involving human mesothelial cells (*5–14*); Gibaz et al. report that these aberrations occur with a frequency of >60% (*8*). A gene important for cellular senescence is located on human chromosome 1 (*15*). Alterations of this gene play a role in cellular immortalization and thus may be critical for development or progression of the neoplastic phenotype. Aberrations involving chromosome 1 frequently are observed in many other types of human cancer, including tumors of the reproductive organs, leukemias, breast cancer, colon carcinoma, melanoma, multiple endocrine neoplasia, and neuroblastoma (*15*).

A second commonly observed cytogenetic alteration in human mesothelioma cells is deletion or monosomy of chromosome 3 (*7,9,10,16*). A tumor suppressor gene involved in several human cancers, including lung cancer (*17*), renal cell carcinoma (*17*), and cervical carcinoma (*18*), is thought to be located on chromosome 3p. Alterations in this region of chromosome 3 observed in mesotheliomas suggest that this same tumor suppressor gene could be involved in mesothelioma development. Losses of tumor suppressor gene functions occur in several types of human cancer (*1–3*), but a direct role for this or any other tumor suppressor gene in the genesis of mesothelioma has yet to be demonstrated.

Extra copies (gains or polysomes) of chromosome 7 (*6,11*) have been observed frequently in human mesotheliomas suggesting that increased dosage of a gene on chromosome 7 may play a role in transformation of mesothelial cells. Chromosome 7 is the site of the protooncogene HER-1, which encodes the epidermal growth factor receptor (EGF-R) (*19*). Trisomy or polysomy of chromosome 7 has been observed in other types of human cancer (*20*), and it has been speculated that this could be responsible for altered expression of the EGF-R in melanoma cells (*20*). To date, the role of EGF-R expression in the development of mesothelioma has not been thoroughly investigated. In addition, platelet-derived growth factor-alpha (PDGF-α) also is located on human chromosome 7 (*21*), and a role for this growth factor in the development of mesothelioma has been suggested (*5*).

In a recent report, Pelin-Enlund and coworkers reported an excess of the short arm of chromosome 5 in 6 out of 7 human mesothelioma cell lines (*14*). In addition, that report cited monosomy of chromosome 13, the chromosome known to contain the human retinoblastoma (Rb) tumor suppressor gene (*3*). Loss of normal Rb gene function has been observed in human lung tumors (*3*), but no direct evidence exists for a role for this gene in mesothelioma development.

Deletion or loss of chromosome 6 recently was reported as the sole karyotypic abnormality in a mesothelioma (*22*), which suggests that loss of a gene on the long arm of this chromosome may be primary in mesotheliomas.

Losses of whole chromosomes or deletions of portions of specific chromosomes provide evidence for the loss of tumor suppressor genes in mesotheliomas, which is an important step in the genesis of most human tumors (*1–3*). Extra copies of specific chromosomes are also commonly observed in mesotheliomas; this finding suggests a possible dosage effect, such as amplification and overexpression of protooncogenes. Both normal and transformed mesothelial cells express and respond to a variety of growth factors and growth factor receptors (*5*), which can act as oncogenes if overexpressed or inappropriately expressed. However, a specific oncogene involved in mesotheliomas has not yet been identified (*5*).

Although asbestos and other mineral fibers do not induce gene mutations, they are active as chromosomal mutagens (*5,23,24*). Chromosomal gains and losses and chromosomal deletions are induced

Table 1. Frequent[a] nonrandom cytogenetic alterations associated with transformation of human mesothelial cells.

Chromosome	Monosomy or deletion	Reference polysomy	Rearrangement of structural abnormalities
1	Flejter et al. (*7*), Gibas et al. (*8*), Popescu et al. (*9*), Bello et al. (*10*)		Tiainen et al. (*11*), Mark (*12*), Versnel et al. (*13*), Pelin-Enlund et al. (*14*), Olofsson and Mark (*6*)
2	Olofsson and Mark (*6*)		Gibas et al. (*8*)
3	Flejter et al. (*7*), Bello et al. (*10*), Popescu et al. (*9*), Stenman et al. (*16*)		Gibas et al. (*8*), Mark (*12*), Decker et al. (*33*)
4	Versnel et al. (*13*), Olofsson and Mark (*6*)		
5	Bello et al. (*10*), Klominek et al. (*30*)	Pelin-Enlund et al. (*14*)	Versnel et al. (*13*), Ke et al. (*32*)
6	Bello et al. (*10*), Gibas et al. (*8*), Stenman et al. (*16*), Meloni et al. (*22*)		Versnel et al. (*13*), Ke et al. (*32*)
7	Popescu et al. (*9*)	Tiainen et al. (*11*), Olofsson and Mark (*6*)	Ke et al. (*32*)
9	Klominek et al. (*30*), Pelin-Enlund et al. (*14*)		Gibas et al. (*8*), Versnel et al. (*13*)
11	Lechner et al. (*31*)	Tiainen et al. (*11*)	Gibas et al. (*8*), Versnel et al. (*13*)
12			Versnel et al. (*13*), Decker et al. (*33*)
13	Pelin-Enlund et al. (*14*), Mark (*12*)		Versnel et al. (*13*), Ke et al. (*32*)
17	Olofsson and Mark (*6*)		Versnel et al. (*13*), Gibas et al. (*8*)
18	Flejter et al. (*7*), Olofsson and Mark (*6*)		
21	Olofsson and Mark (*6*), Lechner et al. (*31*)		
22	Flejter et al. (*7*), Tiainen et al. (*11*), Gibas et al. (*8*), Mark (*12*), Stenman et al. (*16*), Olofsson and Mark (*6*)		Versnel et al. (*13*), Versnel et al. (*13*)

[a] Reported in two or more studies.

following exposure of human and rodent cells, including mesothelial cells, to asbestos fibers.

A mechanism for asbestos-induced aneuploidy, i.e., losses and gains of individual chromosomes, has been proposed (*25,26*). In the first step, crocidolite asbestos fibers are taken up by the cells by phagocytosis within 24 hr after treatment (*25,26*); the intracellular fibers accumulate around the perinuclear region of the cells 24 to 48 hr after exposure. When the cells undergo mitosis, the physical presence of the fibers results in interference with chromosome segregation. Analysis of chrysotile-exposed cells in anaphase (*25*) reveals a large increase in the number of cells with abnormalities including lagging chromosomes, bridges, and sticky chromosomes. Asbestos fibers are observed in the mitotic cells and appear, in some cases, to interact directly with the chromosomes. Using ultrastructural analysis, Wang et al. (*27*) observed asbestos fibers apparently interacting with metaphase chromosomes after treatment of rat mesothelial cells in culture. It also has been proposed that the physical interaction of the asbestos fibers with the chromosomes or structural proteins of the spindle apparatus can cause mis-segregation of chromosomes during mitosis, resulting in aneuploidy (*25,26*). These findings provide a mechanism, at the chromosomal level, by which asbestos and other mineral fibers can induce cell transformation and cancer.

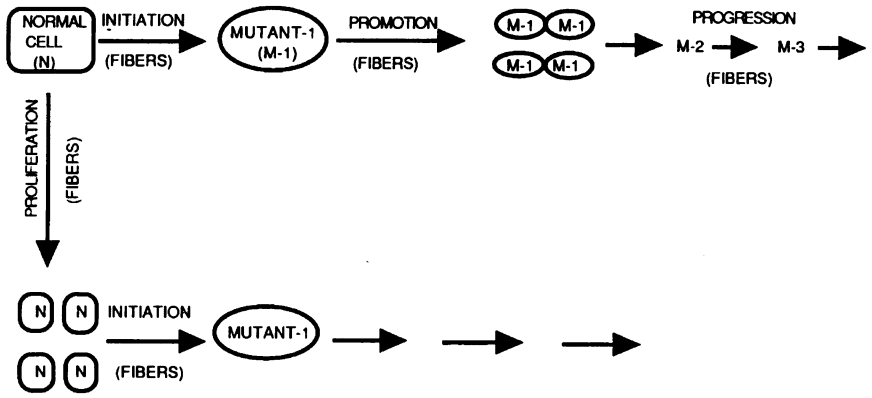

Figure 2. The multistep process of carcinogenesis results from multiple genetic changes required for a normal cell to evolve into a malignant cell. Asbestos fibers may initiate this process by a chromosomal mutation. However, additional mutations are required for clinically evident neoplasms. These secondary mutations are more likely to arise if the initiated (mutant-1) cells are stimulated to proliferate, for example, by fiber-related promotional mechanisms. Tumor promotion results in clonal expansion of the initiated cells, but preneoplastic cells must progress and acquire additional mutations. Asbestos fibers may influence this progression either by inducing chromosomal mutations in the intermediate cells or by stimulating cellular proliferation. Asbestos fibers also may stimulate proliferation of the normal cells thereby increasing the rate of either asbestos-induced or spontaneous mutations. Therefore, asbestos fibers can influence the carcinogenic process either at early or late stages by both genetic and epigenetic mechanisms. Synergism with other agents (e.g., cigarette smoke) can be explained by asbestos acting at one step and the synergistic carcinogen acting at a different step in this multistep process.

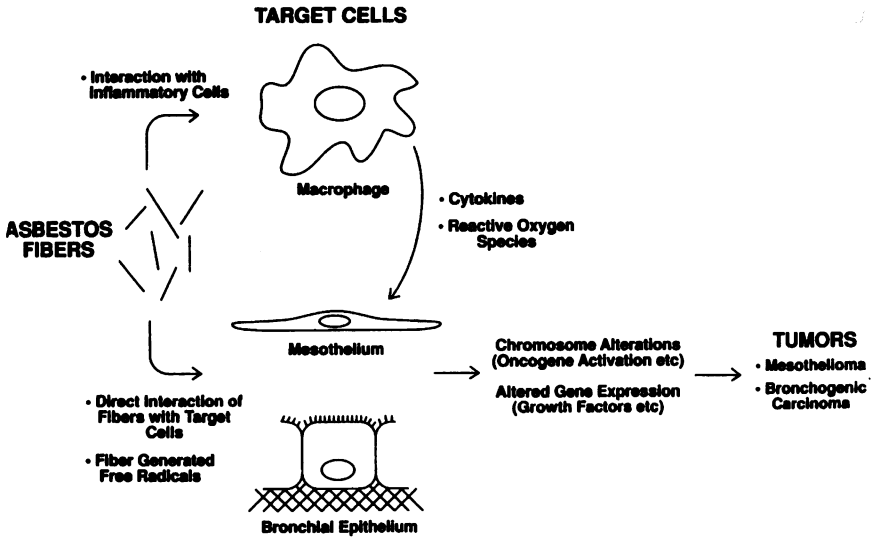

Figure 3. Pathways of asbestos carcinogenicity. Asbestos fibers may exert their carcinogenic effects on mesothelial and bronchial epithelial cells by direct and indirect mechanisms. Direct effects can occur following the physical interaction of fibers with target cells or by the generation of free radicals from the fiber source. Indirect effects arise following the interaction of fibers with inflammatory cells that produce mediators such as cytokines and various reactive oxygen species. As a result, target cells sustain genetic alterations that lead to the development of tumors. [Taken from Walker et al. (*5*) with permission.]

Asbestos can heritably transform cells in culture leading to neoplastic transformation (*5,28*). Furthermore, asbestos-induced cell transformation is dependent on fiber size as is asbestos carcinogenicity, *in vivo* (*28*). Long, thin fibers are more active than short, thick fibers. Chromosomal mutation induced by fibers is also fiber-size dependent, and this is the likely mechanism for asbestos-induced cell transformation (*29*).

Since asbestos fibers induce chromosomal mutations, including chromosome losses and deletions, and since nonrandom chromosomal losses and deletions are observed in asbestos-induced cancers, it is reasonable to assume these events are interrelated. Losses of tumor suppressor genes occur at different stages of the multistep process of carcinogenesis; thus, asbestos-induced deletions of these genes may occur early or late in the carcinogenic process.

In addition to mutational mechanisms involving chromosomal alterations, asbestos fibers may act on the carcinogenic process by stimulating cell proliferation (Figure 2). Asbestos fibers may stimulate proliferation of normal cells either by compensatory growth after cytotoxic injury or by stimulation of macrophages and inflammatory cells that release cytokines and growth factors (Figure 3) (*5*). Cell division is required for asbestos-induced chromosome mutations to occur. Increasing the rate of cell proliferation may also increase the number of cells with spontaneous mutations. Furthermore, asbestos- and fiber-induced cell proliferation could accelerate clonal expansion of initiated or preneoplastic cells, which would promote neoplastic development. Therefore, asbestos fibers could influence the carcinogenic

process at either early or late stages by both genetic and epigenetic mechanisms.

Biopersistence of fibers is undoubtedly important in their carcinogenic potency. However, the time required for a fiber to remain in the lung to exert a cancer-related effect is difficult to specify. Increasing biologically active exposures to critical target cells will increase the carcinogenic potency of a fiber. Biologically active exposures are determined by retention and clearance mechanisms, by durability of the fiber, by availability of the fibers for interaction with target cells, and by their reactivity. Fiber–target cell interactions are determined both by intrinsic properties of fibers and extrinsic chemical modifications of fibers in the tissue milieu. Biological masking of fibers by proteins and other biomolecules *in vivo* can inactivate even persistent fibers. The critical target cells for fiber carcinogenicity include the progenitor cells for the asbestos-related cancers, e.g., mesothelial and bronchial epithelial cells, as well as inflammatory cells that release cytokines and other modifiers of mesothelial and epithelial cells following fiber stimulation.

Given the multistep nature of the carcinogenic process and the multiple mechanisms by which fibers might influence it (*5*), it is very difficult to determine the degree of biopersistence necessary for fiber carcinogenicity. Longer exposures will increase the probability of a carcinogenic response, but very short exposures still may be significant. The mere presence of fibers in the lung, however, does not indicate biologically active exposures. Fibers in the lung at the time of disease detection may not be biologically active, and exposures to fibers that have not persisted to the point of disease manifestation may have been critical for early, preclinical stages.

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