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Multiple Mechanisms for the Carcinogenic Effects of Asbestos and Other Mineral Fibers

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Asbestos and other mineral fibers are carcinogenic to humans and animals but differ from many carcinogens in that they do not induce gene mutations. An understanding of these interesting human carcinogens, therefore, is an important problem in cancer research. Asbestos and other fibers induce predominately two types of cancers: mesotheliomas and bronchogenic carcinomas. Fiber size is an important factor in the carcinogenic activity of these substances as has been shown for mesothelioma induction. For bronchogenic carcinomas, but not for mesotheliomas, a synergistic effect of asbestos exposure and cigarette smoke has been observed in humans. The mechanisms by which fibers alone versus fibers in concert with other carcinogens induce cancers are probably distinct. In addition to fiber dimensions, fiber durability and surface properties of fibers are important properties affecting carcinogenicity. Evidence exists that asbestos is a complete carcinogen, an initiator and a promoter. Multiple mechanisms must be operative to explain the diverse effects of mineral fibers. Although asbestos is inactive as a gene mutagen, there is now clear evidence that it induces chromosomal mutations (aneuploidy and aberrations) in a wide variety of mammalian cells including mesothelial cells. Asbestos also induces transformation of cells in culture including mesothelial cells and fibroblasts. A mechanism for cell transformation, which is dependent on fiber dimension, has been proposed. The fibers are phagocytized by the cells and accumulate in the perinuclear region of the cells. When the cell undergoes mitosis, the physical presence of the fibers interferes with chromosome segregation and results in anaphase abnormalities. The transformed cells show aneuploidy and other chromosome abnormalities. These findings provide a mechanism at the chromosomal level by which asbestos and other mineral fibers might induce cell transformation and cancer. Identification of the critical target genes in asbestos carcinogenicity is required to understand this process, and recent progress in this area has been made. Results from several lines of investigation suggest that two distinct classes of genes, protooncogenes and tumor suppressor genes, are involved in the neoplastic process. In human mesothelioma, deletion of the short arm of chromosome 3 has been observed, which may result in the loss of a tumor suppressor gene on this chromosome. Recent results from our laboratory have also shown that an activated transforming oncogene exists in human mesotheliomas. Further molecular analysis of these cancers may help in understanding these neoplasms and the mechanisms of asbestos and other carcinogenic fibers.

Introduction

Asbestos and other mineral fibers are unusual and interesting carcinogens. They are ubiquitous environmental substances that are clearly carcinogenic to humans and to animals (*1,2*); but unlike most carcinogens, asbestos fibers are not electrophilic or DNA damaging (*3-5*). The mechanisms of action of this important class of carcinogens are little understood, although some of the important factors that contribute to the carcinogenicity of asbestos are known (Table 1).

Asbestos and other mineral fibers are known to induce predominantly two types of cancers in humans and animals: mesotheliomas and bronchogenic carcinomas (*1,2*). Stanton et al. (*6,7*) established that induction of mesotheliomas in animals depends strongly on fiber size. They showed that long (> 4 μm) and thin (< 0.25 μm diameter) fibers were much more carcinogenic than short and thick fibers. It is not clear whether the same fiber size dependence exists for the induction of bronchogenic carcinomas. A major factor in asbestos carcinogenicity of the lung is the synergism between asbestos and cigarette

Table 1. Important factors in carcinogenicity of asbestos and other mineral fibers.

Tumor	Factor
Mesotheliomas	Fiber size dependence
Bronchogenic carcinomas	Synergism with cigarette smoking

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Table 2. Fiber properties affecting carcinogenicity.

Fiber dimensions
Fiber durability
Surface properties of fibers

smoking (*8,9*). Asbestos is a complete carcinogen in the lung, but a multiplicative effect on lung cancers in humans is observed with cigarette smoke and asbestos exposure (*1,8–10*). To understand the action of asbestos in the lung, it is therefore important to elucidate how asbestos works alone as well as synergistically with cigarette smoking. Multiple mechanisms of action may be operative. The synergism observed with asbestos and cigarette smoking for lung cancers is not observed for mesotheliomas (*9,11*). Therefore, for different target cells and even the same target cells under different conditions, the mechanisms of action of asbestos may vary.

Several intrinsic properties of different asbestos and other mineral fibers may affect their carcinogenicity (Table 2). The importance of fiber dimensions has already been mentioned. Fiber durability also appears to be important (*2,3*). Some fibers are readily dissolved *in vivo* and therefore have a reduced biological activity (*3,12*). In addition to the physical state of the fibers, the physicochemical surface properties of the same fibers may be quite important (*2,3,13,14*). An interesting example is erionite, a zeolite mineral, which is a very potent inducer of mesotheliomas in humans and animals (*15–18*). The Stanton hypothesis of fiber dimension is operative for erionite fibers, i.e., short fibers are relatively inactive, but long fibers are far more potent than other fibers of comparable size (*16*). Therefore, additional properties of this mineral fiber must be important. Other types of fibers also exhibit activity that is fiber-size dependent but cannot be explained strictly on the basis of size. The generation of oxygen free radicals at the surface of certain fibers may offer an explanation for the importance of surface properties (*19,20*).

Asbestos and Multistage Carcinogenesis

One way to investigate the mechanism of tumor induction by asbestos and other mineral fibers is to determine the stage or stages in the multistep process of neoplastic development at which the fibers operate. A review of our understanding of asbestos in multistage carcinogenesis reveals that asbestos must have multiple mechanisms of action.

Evidence exists that asbestos is a complete carcinogen, an initiator and a promoter. The data implicating asbestos as a complete carcinogen and as an initiator are summarized in Table 3. Asbestos alone appears from epidemiological studies to be a complete carcinogen in humans, inducing mesotheliomas and lung cancers (*1,2*). In animals, asbestos is clearly a complete carcinogen, inducing the same types of tumors observed in humans (*1,10*). Epidemiological studies have shown that the incidence of mesotheliomas in exposed populations is independent of

Table 3. Evidence for complete carcinogenic and initiating activity of asbestos.

Asbestos is a known human carcinogen
Asbestos and other mineral fibers are complete carcinogens in animals by multiple routes of exposure including intrapleural and intraperitoneal injection, inhalation, and tracheal transplants
Incidence of mesotheliomas in exposed populations is independent of the age of first exposure, which is consistent with the hypothesis that asbestos affects an early stage in the carcinogenic process
Asbestos is inactive as a gene mutagen but induces chromosomal mutations (aneuploidy and aberrations) in a wide variety of mammalian cells
Asbestos induces transformation of human and rat mesothelial cells and hamster fibroblast cells in culture

age at first exposure, consistent with the hypothesis that asbestos affects an early stage in the carcinogenic process (*21,22*). On the other hand, it has been argued that asbestos must not act as an initiator because it lacks mutagenic activity, a property of most initiators. Although asbestos is not active as a gene mutagen in a variety of test systems, there is now clear evidence that it induces chromosomal mutations (aneuploidy and aberrations) in a wide variety of mammalian cells, including mesothelial cells (*3–5,23–31*). Finally, it has been shown that asbestos can induce transformation of cells in culture, including mesothelial cells and fibroblasts (*29,32–36*). Evidence exists with these model systems that the mechanism of asbestos-induced cell transformation involves a chromosomal mutation (*23,29,36*). Taken together, this evidence indicates that asbestos and other mineral fibers are complete carcinogens with tumor initiating activity for certain cancers.

Considerable evidence also exists for tumor-promoting activity associated with asbestos and other mineral fibers (Table 4). The marked synergism between asbestos exposure and smoking for the risk of lung cancer in humans has been cited as evidence for a tumor-promoting effect of asbestos (*2*). However, other explanations are possible for this effect: a) asbestos acts as an initiator and cigarette smoke as a promoter; b) asbestos acts as a tumor progressor for lung cancer rather than as a classical tumor promoter; or c) asbestos and the carcinogens in cigarette smoke act as cocarcinogens.

A cocarcinogenic effect of asbestos and polycyclic aromatic hydrocarbons has been demonstrated in animals (*1,2*). A promotionlike effect of asbestos has been demon-

Table 4. Evidence for tumor-promoting activity of asbestos and other mineral fibers.

Marked synergism exists between asbestos exposure and smoking for risk of lung cancer in humans
Rodents exposed by instillation to a combination of asbestos and chemical carcinogens such as polycyclic aromatic hydrocarbons have synergistic incidence of lung cancers (cocarcinogenic effect)
Asbestos enhances DMBA-induced carcinomas in heterotopic tracheal transplants in rats
Asbestos-induced changes in target tissues similar to those observed with tumor promoters such as hyperplasia, metaplasia, DNA synthesis, induction of ornithine decarboxylase, and stimulation of production of oxygen free radicals

strated for DMBA-induced carcinomas in heterotopic tracheal transplants in rats (*37*). However, in this system asbestos also has a complete carcinogenic activity. Asbestos induces changes in target tissues and in some cells in culture similar to those observed with phorbol ester tumor promotors (*19,20,38,39*). The mechanisms by which asbestos and other mineral fibers might act as tumor promoters are no doubt quite different from the clastogenic and carcinogenic effects described earlier. No single mechanism of action can be ascribed to asbestos, and like other potent carcinogens, these compounds exhibit multiple activities, many of which may contribute to carcinogenicity.

Further elucidation of the mechanism of action of mineral fibers, therefore, requires model systems in which a specific action can be studied. Several systems exist to study the cellular effects of asbestos (*40*). These have been used to study the action of asbestos as a possible tumor promoter and as an inducer of cell transformation.

Mechanisms of Asbestos-Induced Cell Transformation

Because asbestos and other mineral dusts were known to have toxic and chromosome damaging effects on cells in culture (*40*), several investigators were interested in whether these substances could induce cell transformation. Although asbestos is inactive as a gene mutagen in mammalian cells (*3–5*), it is able to induce heritable alterations in the growth properties of normal cells in culture resulting in neoplastic transformation of the treated cells (*3–5*).

Thomas Hesterberg, working in our laboratory, used an *in vitro* cell transformation system that employs early passage, diploid Syrian hamster embryo cells to address two questions concerning the mechanisms of asbestos carcinogenicity: the role of fiber dimension in the induction of cell transformation, and the role of genetic events in the heritable induction of cell transformation. The first question is important in determining the extent to which the induction of cell transformation resembles the induction of mesotheliomas *in vivo*, which is highly dependent on fiber size (*6,7*), while the second explores the mechanism of asbestos carcinogenicity. The lack of activity in most gene mutation assays predicts that either asbestos acts by an unusual genetic mechanism or by an epigenetic mechanism. Our results support the hypothesis that mineral dusts induce cell transformation via a chromosomal mutation.

We observed that chrysotile and crocidolite asbestos induced dose-dependent morphological transformation (Figs. 1 and 2) and neoplastic transformation of Syrian hamster embryo cells, which was indistinguishable from the cell transformation induced by other carcinogens such as benzo[a]pryrene (*32*). However, asbestos failed to induce gene mutations at two specific genetic loci in these cells (Table 5). Oshimura et al. (*23*) showed that the mineral fibers were active in inducing both numerical and structural chromosomal aberrations (Table 6).

Table 5. Specific locus mutation and toxicity of Syrian hamster embryo cells after treatment with transforming doses of asbestos and benzo[a]pyrene.

Treatment	Dose, $\mu g/cm^2$	Relative survival, %[a]	Mutation frequency[b]	
			Oua^r	*TG*^{r}
Control	0	100	$<10^{-6}$	$<10^{-6}$
Chrysotile	1.0	41	$<10^{-6}$	$<10^{-6}$
Chrysotile	2.0	28	$<10^{-6}$	$<10^{-6}$
Crocidolite	1.0	69	$<10^{-6}$	$<10^{-6}$
Crocidolite	2.0	41	$<10^{-6}$	$<10^{-6}$
Benzo[a]pyrene	1 $\mu g/mL$	69	$1.5 (\pm 0.7) \times 10^{-4}$	$1.0 (\pm 0.4) \times 10^{-4}$

[a]Measured immediately after treatment.
[b]Corrected for relative survival measured after mutant expression period. These data were reproduced from Oshimura et al. (*23*) with permission.

Table 6. Cytogenetic effects of 2 $\mu g/cm^2$ of various mineral dusts on Syrian hamster embryo cells *in vitro* (*23*).

Treatment	Transformation frequency[a]	Aneuploid cells[b] %	Chromosome aberrations[c]	Cells with micronuclei[d] %	Tetraploid cells[d,e] %	Binucleated cells %
Control	0	1.7	1	0.3	5	0.3
Chrysotile	6.2	12.5*	5	2.6*	33*	25.0*
Crocidolite	4.6	9*	4	1.1*	14*	11.2*
Fiberglass[f]	3.0	7*	4	3.0*	20*	18.4*
Milled fiberglass	0	2	1	0.5	6	0.4
Alpha quartz	0	3	1	0.5	5	0.3

[a]Cited from Hesterberg and Barrett (*32*). The transformation frequency was calculated by dividing the number of morphologically transformed colonies by the total number colonies examined × 1000.
[b]This represents the percentage of metaphases that contained a near-diploid number of chromosomes.
[c]Percentage of metaphases containing the following aberrations: chromatid breaks, isochromatid breaks, chromosome fragments, chromatid exchanges, or dicentric chromosomes.
[d]For each treatment group, 1000 cells were scored.
[e]Cells with a tetraploid (4N = 88) or near tetraploid (70–100) number of chromosomes.
[f]This fiberglass was obtained from Johns Manville (Code 100) and processed as described previously (*32*).
*Statistically significant from the control, $p < 0.5$, Fisher's exact test.

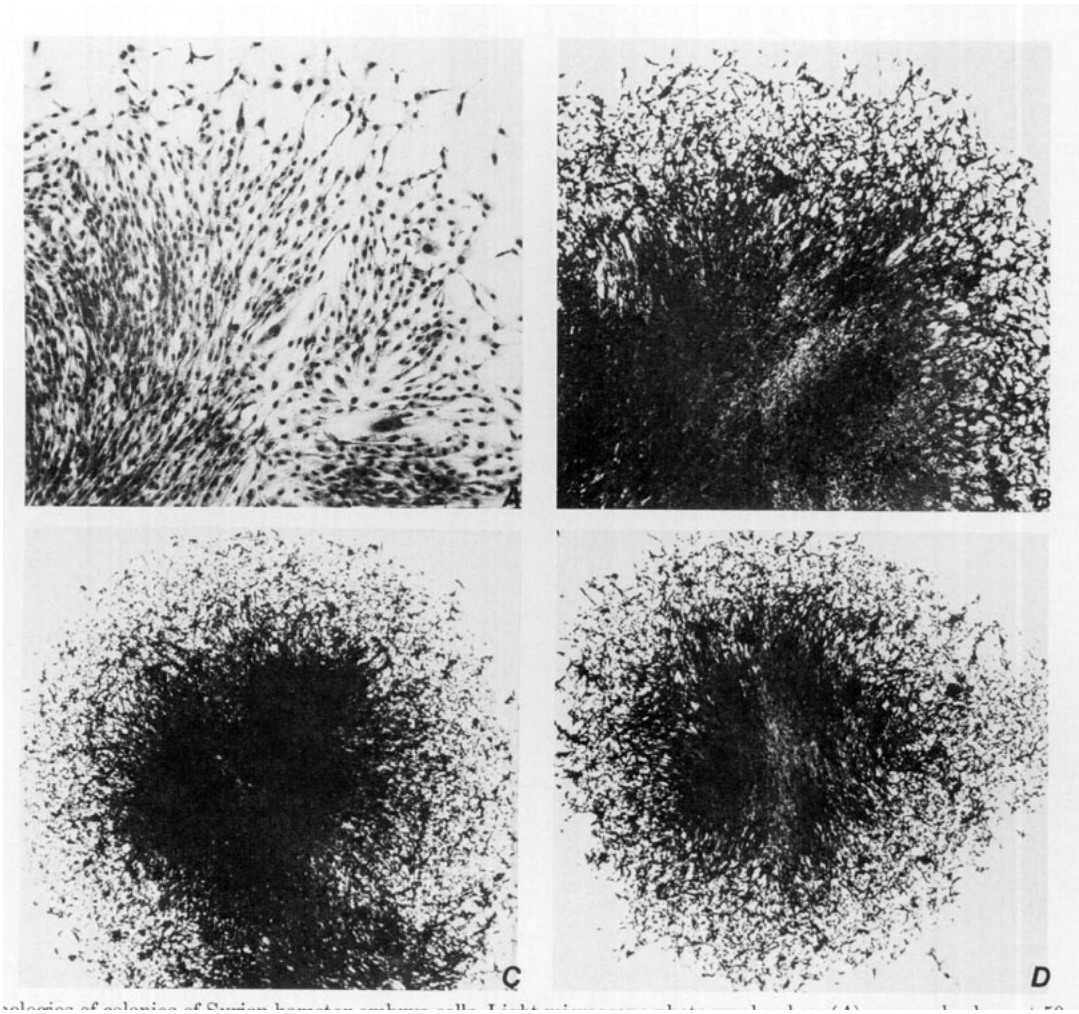

FIGURE 1. Morphologies of colonies of Syrian hamster embryo cells. Light microscope photographs show (*A*) a normal colony at 50 ×, (*B*) a chrysotile asbestos-transformed colony at 42 ×, (*C*) a crocidolite asbestos-transformed colony at 27 ×, and (*D*) a code 100 glass fiber-transformed colony at 27 × after fixation in absolute methanol and staining in 10% Giemsa 7 days after treatment.

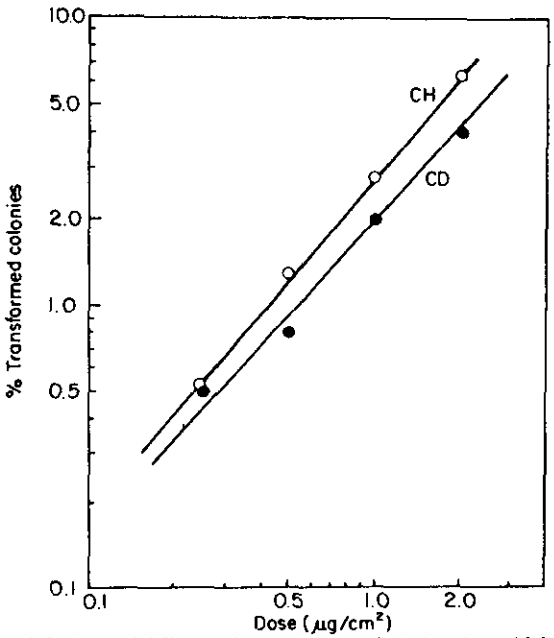

FIGURE 2. Effects of different doses of chrysotile (○) and crocidolite (●) asbestos on the transformation frequency of Syrian hamster embryo cells in culture. Reproduced from Hesterberg and Barrett (*32*) with *permission*.

Similar to the induction of mesotheliomas *in vivo*, cell transformation by mineral fibers was dependent on fiber size (Fig. 3). Transforming activity of the fibers was lost when the fibers were shortened to < 1 μm in length. Milling decreased the fiber length from 16.0 ± 1.7 μm before milling to 0.95 ± 0.12 μm after milling (*32*). The average diameter of the fibers (~ 0.18 μm) was unchanged by milling. This figure shows that milling completely eliminated the transforming ability of glass fibers, suggesting that fiber length is important in the induction of transformation. The relative potencies of mineral dusts in the induction of cell transformation *in vitro* is similar to their potencies in the induction of mesotheliomas *in vivo*. Thus, this cell transformation system provides a unique model for studying the mechanisms of mineral fiber tumorigenesis. The chromosome damage induced by fibers was likewise fiber-length dependent (Table 6).

Asbestos induces aneuploidy in the treated cells, causing losses and gains of individual chromosomes. We have proposed a mechanism for this type of genetic change (*23,33,41*). In collaboration with Arnold Brody, we showed that asbestos fibers are taken up by the cells within 24 hr after treatment by phagocytosis (*41*); the intracellular fibers accumulate around the perinuclear region of the

cells 24 to 48 hr after exposure (Fig. 4). When the cells undergo mitosis, the physical presence of the fibers results in interference with chromosome segregation. Analysis of anaphases in chrysotile-exposed cells (*42*) reveals a large increase in the number of cells with anaphase abnormalities, including lagging chromosomes, bridges, and sticky chromosomes (Fig. 5). Asbestos fibers are observed in the mitotic cells and appear, in some cases, to interact directly with the chromosomes. From these studies we propose that the physical interaction of asbestos fibers with the chromosomes or structural proteins of the spindle apparatus causes missegregation of chromosomes during mitosis, resulting in aneuploidy. These findings provide a mechanism, at the chromosomal level, by which asbestos and other mineral fibers might induce cell transformation and cancer (*23,33,41,42*). This hypothesis is supported by the finding of a nonrandom trisomy of chromosome 11 in asbestos-transformed Syrian hamster cells (*43*).

Lechner et al. (*29–31*) have shown that asbestos fibers alter the growth properties of normal human mesothelial cells, and this is associated with chromosomal changes in the treated cells. Patérour et al. (*35,36*) have also shown asbestos-induced transformation and chromosomal changes in rat mesothelial cells in culture. Thus, it appears that asbestos fibers can alter the growth properties of fibroblast and mesothelial cells in culture, resulting in neoplastic transformation of the cells. Asbestos fibers also induce chromosomal changes in the treated cells, and a chromosomal mutation is a likely mechanism for asbestos-induced cell transformation.

In each of the systems described, asbestos-induced neoplastic transformation is a multistep process. Asbestos treatment of the cells heritably alters their growth properties; these altered cells are preneoplastic and must undergo additional changes before they acquire neoplastic potential (*32*). This process has been studied in detail in our laboratory for asbestos and other carcinogen-induced neoplastic progression of Syrian hamster cells (*44*).

One pathway of neoplastic transformation is depicted in Figure 6. The earliest observable carcinogen-induced change is the morphological transformation shown in Figure 1. Normal Syrian hamster embryo cells have a flat morphology and grow in an orderly array in parallel, swirling patterns. If subcultured for 10 to 20 passages (30 to 40 population doublings), the normal Syrian hamster embryo cells enlarge and cease proliferation (termed cellular senescence). If exposed to a chemical carcinogen, colonies of cells (Fig. 1) are observed with a transformed morphology (criss-crossed growth with cells piling on top of each other, increased basophilia, and increased nuclear-cytoplasmic ratio). When isolated, some of these colonies escape cellular senescence and grow indefinitely (termed immortality). These altered cells are nontumorigenic but after further growth, new variant cells appear that grow in soft agar and produce tumors when injected into nude mice or syngeneic hamsters. The immortal cells have an increased propensity to develop into tumorigenic cells, and hence the immortal cell population is termed intermediate or preneoplastic (*45,46*).

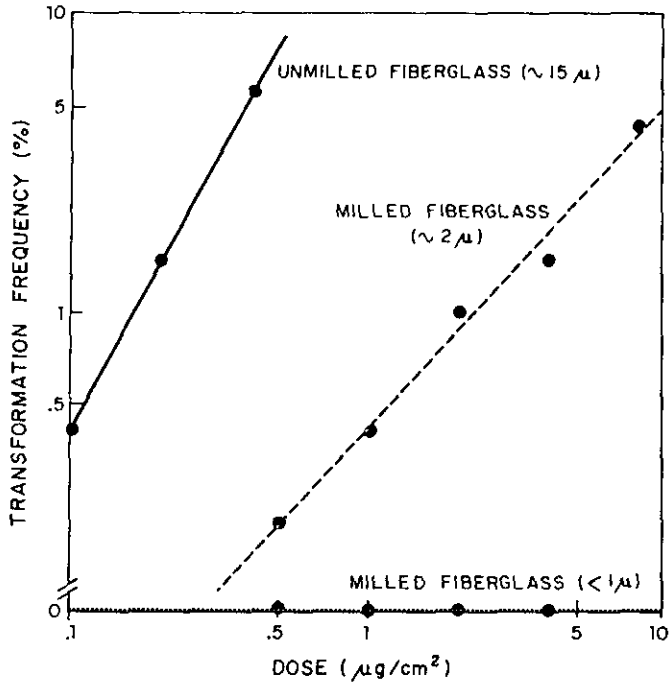

FIGURE 3. Effects of different doses of unmilled Code 100 glass fibers (O) or milled code 100 (●) on transformation frequency of SHE cells in culture. Reproduced from Hesterberg and Barrett (*32*) with permission.

Our studies indicate that following carcinogen treatment, neoplastic progression of these cells requires at least three heritable changes: induction of immortality, activation of a transforming oncogene, and inactivation of a tumor suppressor gene (*45,46*). The multistep nature of neoplastic transformation with chemical carcinogens is consistent with the findings that two cooperating oncogenes (e.g., *ras* plus *myc*) are required for neoplastic conversion of primary rat cells and with the hypothesis that these two oncogenes influence immortality and neoplastic conversion, respectively (*46*). In fact, analysis of asbestos-induced Syrian hamster tumor cell lines show that activated H-*ras* oncogenes are present in approximately 50% of the tumor-derived cell lines while the nontumorigenic immortalized cell lines lack the activated H-*ras* oncogene (*47*). Asbestos induces the first steps in the neoplastic process of these cells, i.e., morphological transformation and immortalization, and the H-*ras* gene mutation may occur many months later when the cells acquire tumorigenicity. Therefore, the H-*ras* gene mutation is not a direct result of the asbestos treatment, but it arises as a secondary, spontaneous change in the asbestos-induced preneoplastic cells. The *ras* genes are activated by point mutations and convert immortal preneoplastic cells to the tumorigenic state (*46*). Asbestos fibers fail to transform other preneoplastic cell lines, such as C3H10T½ cells (*48*), and this is consistent with the inability of the fibers to induce gene mutations. Asbestos fibers do induce early steps of transformation (i.e., immortalization) of normal, diploid cells, which result in

FIGURE 4. Scanning electron micrograph (*A*) and the corresponding backscatter electron image (*B*), showing the perinuclear accumulation of crocidolite asbestos fibers by a Syrian hamster embryo cell 24 hr after treatment with 1 $\mu g/cm^2$. N, nuclear region, which is demarcated by prominent nucleoli [asterisks (*)]; c, cytoplasm, × 1780. Reproduced from Hesterberg et al. (*41*) with permission.

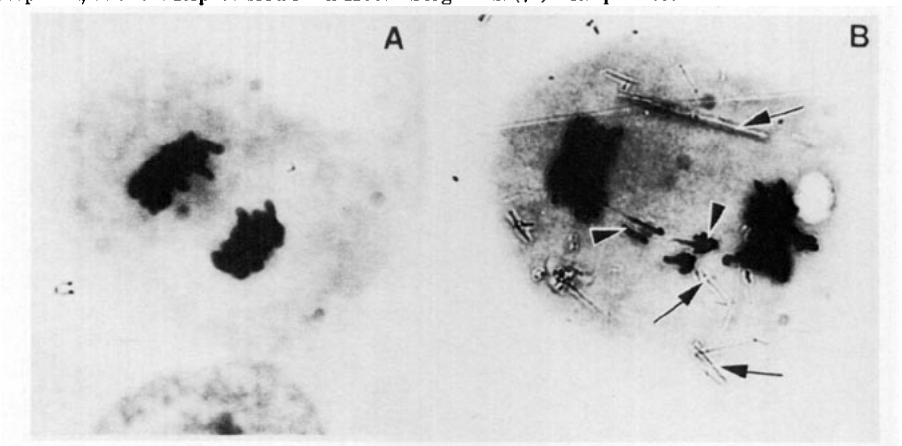

FIGURE 5. A normal (*A*) and an abnormal (*B*) anaphase from asbestos-treated Syrian hamster embryo cells. Note the asbestos fibers (arrows), some of which appear to be associated with displaced chromosomes (arrowheads) in the abnormal anaphase. Reproduced from Hesterberg and Barrett (*42*) with permission.

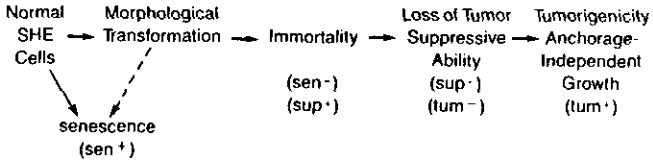

FIGURE 6. Pathway of neoplastic transformation of Syrian hamster embryo cells treated with asbestos or other carcinogens.

aneuploid cell lines. This is consistent with the ability of fibers to induce chromosomal mutations including aneuploidy (*23,26*).

In addition to activation of oncogenes such as *ras* and *myc*, there is evidence that a second class of genes is involved in the neoplastic transformation process. There is increasing evidence for the significance of tumor suppressor genes, also termed anti-oncogenes or recessive oncogenes. Tumor suppressor genes are normal cellular genes that act as negative regulators of tumor cell proliferation *in vivo* and must be lost or inactivated in neoplastic cells. In contrast protooncogenes are normal cellular genes that are activated by mutations to become oncogenes that act as positive proliferative signals for neoplastic cells (Table 7).

The importance of tumor suppressor genes is supported by several different lines of evidence (*46,49*), which include: a) suppression of tumorigenicity in cell hybrids; b) studies of genetic predisposition to cancer in humans and animals; c) nonrandom chromosome losses or deletions in specific tumors; d) loss of heterozygosity of specific chromosomal regions in tumors; and e) reversion of the malignant state by interactions of tumor cells with normal cells or by treatment of tumor cells with certain chemicals, growth factors, or differentiation-inducing substances.

We have shown that asbestos-induced immortal hamster cells at early passages retain a tumor suppressor gene function (*50*). At later passages, some of the cells lose this function, which can be measured by hybridization of the cells with tumor cells. From the later passages of the preneoplastic cells, subclones can be isolated that either retain tumor suppressor gene function (sup^-) or have lost this ability (sup^+). Sup^- variants are 1000-fold more susceptible to neoplastic transformation by transfection with the activated H-*ras* oncogene. These cells are also more susceptible to spontaneous and carcinogen-induced transformation, which supports the concept that loss of the gene is essential for neoplastic conversion (*45*).

There is evidence that loss of tumor suppressor genes and activation of oncogenes are both critical events in carcinogenesis. Asbestos and other mineral fibers are inactive as gene mutagens and cannot activate oncogenes by inducing point mutations. However, they may cause chromosome translocations or aneuploidy, resulting in increased oncogene expression. Likewise, suppressor genes are inactivated or lost by chromosome mutations, including chromosome loss and deletions, and asbestos fibers have been shown to induce these types of chromosomal aberrations in mesothelial and other cell types. Thus, the chromosomal mutations induced by carcinogenic fibers may contribute to multiple steps in the development of tumors caused by these agents.

Molecular Alterations in Human Mesotheliomas

One approach to eludicating the mechanisms of asbestos carcinogenicity is to define the critical molecular alterations in asbestos-induced cancers. As mesotheliomas are induced predominately, if not exclusively, as the consequence of asbestos exposure, these are ideal cancers for this analysis. Recently, two significant alterations in mesotheliomas have been reported that may shed light on the mechanisms of fiber carcinogenesis. Karyotypic analyses of human mesotheliomas (*51,52*) reveal multiple chromosome changes. Chromosomes 1, 2, 3, 6, 11, 17, and 22 are most frequently involved in either numerical or structural rearrangements. Chromosome mutations caused by asbestos may be involved in the induction of some of these changes. Among these chromosomal changes, deletions, inversions, or translocations of chromosome 3 are most frequent. The deletion or break points in different tumors are varied between 3p13 and p21. Interestingly, chromosome deletions and loss of heterozygosity of this chromosomal region frequently are observed in many lung cancers (*53,54*). It is possible that loss of a tumor suppressor gene in this chromosome region is a critical step in the carcinogenic process. We have recently established a highly tumorigenic mesothelioma cell line (Lamb and Barrett, unpublished), and attempts to suppress this tumor by introduction of a normal human chromosome 3 are in progress.

Table 7. Two classes of genes involved in carcinogenesis.

Protooncogenes	Tumor suppressor genes
Involved in cellular growth and differentiation	Function unknown but possibly involved in cellular growth and differentiation (negative regulators of cell growth?)
Family of genes exists	Family of genes exists
Must be activated (quantitatively or qualitatively in cancers	Must be inactivated or lost in cancers.
Mutational activation by point mutation, chromosome translocation or gene amplification	Mutational inactivation by chromosome loss, chromosome deletion, point mutation somatic recombination or gene conversion
Little evidence for involvement in hereditary cancers	Clear evidence for involvement in hereditary and nonhereditary cancers

In collaboration with Y. Suzuki and P. Chahinian at Mt. Sinai Hospital, we have also examined human mesotheliomas for activated transforming genes by the ability of DNA from these tumors to neoplastically transform NIH 3T3 cells (*58*). DNAs from three human mesotheliomas, diagnosed by histochemical and ultrastructural criteria, were isolated and cotransfected into NIH 3T3 cells by the calcium phosphate precipitation method along with a cosmid vector DNA encoding resistance to the antibiotic G418. Drug-resistant cells were selected, and when sufficient cells were obtained (2 weeks), the cultures were injected into nude mice. Cultures cotransfected with the cosmid vector and normal DNA were nontumorigenic for up to 16 weeks after injection; cultures treated with the mesothelioma DNAs formed tumors between 7 and 14 weeks. DNAs were isolated from nude mouse tumors and retransfected without additional cosmid vector. Secondary transfectants from two tumors were highly positive in the tumorigenicity assay with latency periods of 5 weeks. DNAs from the NIH 3T3 tumors were screened by Southern analyses for the presence of newly acquired sequences homologous to the H-*ras*, K-*ras*, or N-*ras* oncogenes, and hybridization was only observed for the endogenous mouse genes. However, the primary and secondary transfectants did contain human sequences detectable by hybridization to an *Alu* repetitive DNA probe. These results indicate that human mesotheliomas contain activated transforming genes that are not members of the *ras* gene family. The cosegregation of G418 resistance and tumorigenicity during secondary transfection suggests that the transforming genes and the cosmid vector are closely linked in at least two NIH 3T3 tumors. Further experiments to characterize and clone these genes by cosmid rescue are in progress. Identification of the molecular basis for the activation of this transforming gene or genes may yield new insights into cancer and asbestos carcinogenicity.

Conclusion

Asbestos and other mineral fibers induce multiple types of cancers and are likely to act by multiple mechanisms. Although asbestos fibers do not induce gene mutations, they are active inducers of chromosomal changes, which may affect either activation of protooncogenes or inactivation of tumor suppressor genes. The identification in human mesothelioma of activated transforming genes and the loss of a region on the short arm of chromosome 3 are indicative that protooncogenes and tumor suppressor genes are altered in these cancers, and it will be important in the future to understand the role of asbestos fibers in these alterations.

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REFERENCES

1. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 14, Asbestos. International Agency for Research on Cancer, Lyon, France, 1977.
2. National Research Council. Asbestiform Fibers: Nonoccupational Health Risks. National Academy Press, Washington, DC, 1984.
3. Jaurand, M. C. A particulate state carcinogenesis: Recent data on the mechanisms of action of fibres. IARC Monograph on Mineral Fibers in the Nonoccupational Environment. International Agency for Research on Cancer, Lyon, France, in press.
4. Chamberlain, M., and Tarmy, E. M. Asbestos and glass fibers in bacterial mutation tests. Mutat. Res. 43: 159–164 (1977).
5. Barrett, J. C. Relationship between mutagenesis and carcinogenesis. In: Mechanisms of Environmental Carcinogenesis: Role of Genetic and Epigenetic Changes, Vol. 1 (J. C. Barrett, Ed.), CRC Press, Boca Raton, FL, 1987, pp. 129–142.
6. Stanton, M. F., Layard, M., Tegeris, A., Miller, E., May, M., Morgan, E., and Smith, A. Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. J. Natl. Cancer Inst. 67: 965–975 (1981).
7. Harrington, J. S. Fiber carcinogenesis: Epidemiologic observations and the Stanton hypothesis. J. Natl. Cancer Inst. 67: 977–988 (1981).
8. Selikoff, I. J., Hammond, E. C., and Churg, J. Asbestos exposure, smoking, and neoplasia. JAMA 204: 101–110 (1968).
9. Hammond, E. C., Selikoff, I. J., and Seidman, H. Asbestos exposure, cigarette smoking and death rates. Ann. N.Y. Acad. Sci. 330: 473–490 (1979).
10. Wagner, J. C., Berry, G., Skidmore, J. W., and Timbrell, V. The effects of the inhalation of asbestos in rats. Br. J. Cancer 29: 252–269 (1974).
11. Peto, J., Seidman, H., and Selikoff, I. J. Mesothelioma mortality in asbestos workers: Implications for models of carcinogenesis and risk assessment. Br. J. Cancer 45: 124–135 (1982).
12. Morgan, A., and Holmes, A. The deposition of MMMF in the respiratory tract of the rat, their subsequent clearance, solubility *in vivo* and protein coating. In: Biological Effects of Man-Made Mineral Fibres. Proceedings of a WHO/IARC Conference, Vol. 2, 1984, pp. 1–17.
13. Harrington, J. S., Allison, A. C., and Badami, D. V. Mineral fibers: Chemical, physicochemical and biological properties. Adv. Pharmacol. Chemother. 12: 291–402 (1975).
14. Morgan, A., Davies, P., Wagner, J. C., Berry, G., and Holmes, A. The biological effects of magnesium-leached chrysotile asbestos. Br. J. Exp. Pathol. 58: 465–473 (1977).
15. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 42, Silica and Silicates. International Agency for Research on Cancer, Lyon, France, 1987, pp. 225–239.
16. Wagner, J. C., Skidmore, J. W., Hill, R. J., and Griffiths, D. M. Erionite exposure and mesotheliomas in rats. Br. J. Cancer 51: 727–730 (1985).
17. Suzuki, Y., and Kohyama, N. Malignant mesothelioma induced by asbestos and zeolite in the mouse peritoneal cavity. Environ. Res. 35: 277–292 (1984).
18. Spurney, K. R. Natural fibrous zeolites and their carcinogenicity—a review. Sci. Total Environ. 30: 147–166 (1983).
19. Mossman, B. T., and Marsh, J. P. Mechanisms of toxic injury by asbestos fibers: Role of oxygen free radicals. In: In Vitro Effects of Mineral Dusts. Third International Workshop, NATO ASI Series, Vol. G3 (E. G. Beck and J. Bignon, Eds.), Springer-Verlag, Berlin, 1985, pp. 66–74.
20. Mossman, B. T., Marsh, J. P., Doherty, J., Gilbert R., and Hill, S. Implication of active oxygen species as second messengers of asbestos toxicity. Drug Chem. Toxicol. 10: 157–180 (1987).
21. Peto, J., Seidman, H., and Selikoff, I. F. Mesothelioma mortality in asbestos workers: Implications for models of carcinogenesis and risk assessment. Br. J. Cancer 45: 124–135 (1982).
22. Peto, J., Henderson, B. E., and Pike, M. C. Trends in mesothelioma incidence in the United States and the forecast epidemic due to asbestos exposure during World War II. Quant. Occup. Cancer 9: 51–69 (1982).
23. Oshimura, M., Hesterberg, T. W., Tsutsui, T., and Barrett, J. C.

Correlation of asbestos-induced cytogenetic effects with cell transformation of Syrian hamster embryo cells in culture. Cancer Res. 44: 5017-5022 (1984).
24. Sincock, A. M., and Seabright, M. Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibers. Nature 257: 56-58 (1975).
25. Babu, K. A., Lakkad, B. C., Nigam, S. K., Bhatt, D. K., Karnik, A. B., Thakore, K. N., Kashyap, S. K., and Chatterjee, S. K. *In vitro* cytological and cytogenetic effects of an Indian variety of chrysotile asbestos. Environ. Res. 21: 416-422 (1980).
26. Valerio, F., DeFerran, M., Ottaggio, L., Repetto, E., and Santi, L. Cytogenetic effects of Rhodesian chrysotile on human lymphocytes *in vitro*. In: Biological Effects of Mineral Fibres, Vol. 1 (J. C. Wagner, Ed.), International Agency for Research on Cancer, Lyon, France, 1980, pp. 485- 489.
27. Lavappa, K. S., Fu, M. M., and Epstein, S. S. Cytogenetic studies on chrysotile asbestos. Environ. Res. 10: 165-173 (1975).
28. Jaurand, M. C., Kheuang, L., Magne, L., and Bignon, J. Chromosomal changes induced by chrysotile fibres or benzo-3,4-pyrene in rat pleural mesothelial cells. Mutat. Res. 169: 141-148 (1986).
29. Lechner, J. F., Tokiwa, T., LaVeck, M., Benedict, W. F., Banks-Schlegel, S., Yeager, H., Jr., Banerjee, A., and Harris, C. C. Asbestos-associated chromosomal changes in human mesothelial cells. Proc. Natl. Acad. Sci. (U.S.) 82: 3884-3888 (1985).
30. Linnainmaa, K., Gerwin, B., Pelin, K., Jantunen, K., La Veck, M., Lechner, J. F., and Harris, C. C. Asbestos-induced mesothelioma and chromosomal abnormalities in human mesothelial cells *in vitro*. In: Proceedings of the 3rd Joint U.S. Finnish NIOSH Science Symposium, 1986, pp. 119-122.
31. Lechner, J. F., Tokiwa, T., Yeager, H., Jr., and Harris, C. C. Asbestos-associated chromosomal changes in human mesothelial cells. In: In Vitro Effects of Mineral Dusts. Third International Workshop, NATO ASI Series, Vol. G3 (E. G. Beck and J. Bignon, Eds.), Springer-Verlag, Berlin, 1985, pp. 197-202.
32. Hesterberg, T. W., and Barrett, J. C. Dependence of asbestos- and mineral dust-induced transformation of mammalian cells in culture on fiber dimension. Cancer Res. 44: 2170-2180 (1984).
33. Hesterberg, T. W., Oshimura, M., and Barrett, J. C. Transformation of mammalian cells in culture by asbestos and other mineral dusts: A mechanism involving chromosomal mutation. In: In Vitro Effects of Mineral Dusts. Third International Workshop, NATO ASI Series, Vol. G3 (E. G. Beck and J. Bignon, Eds.), Springer-Verlag, Berlin, 1985, pp. 185-196.
34. Hesterberg, T. W., Brody, A. R., Oshimura, M., and Barrett, J. C. Asbestos and silica induce morphological transformation of mammalian cells in culture: A possible mechanism. In: Silica, Silicosis, and Cancer (D. F. Goldsmith, D. M. Winn, and Shy, C. M., Eds.), Praeger Press, New York, 1986, pp. 177-190.
35. Patérour, M. J., Renier, A., Bignon, J., and Jaurand, M. C. Induction of transformation in cultured rat pleural mesothelial cells by chrysotile fibres. In: In vitro Effects of Mineral Dusts. Third International Workshop, NATO ASI Series, Vol. G3 (E. G. Beck and J. Bignon, Eds.), Springer-Verlag, Berlin, 1985, pp. 203-207.
36. Patérour, M. J., Bignon, J., and Jaurand, M. C. In vitro transformation of rat pleural mesothelial cells by chrysotile and/or benzo-a-pyrene. Carcinogenesis 6: 523-529 (1985).
37. Topping, D. C., and Nettesheim, P. Two-stage carcinogenesis studies with asbestos in Fischer 344 rats. J. Natl. Cancer Inst. 3:627-30 (1980).
38. Mossman, B. T., and Craighead, J. E. Asbestos-induced epithelial changes in organ cultures of hamster trachea: Inhibition by retinyl methyl ether. Science 207: 311-13 (1980).
39. Mossman, B. T., Light, W. G., and Wei, E. T. Asbestos: Mechanisms of toxicity and carcinogenicity in the respiratory tract. Annu. Rev. Pharmacol. Toxicol. 23: 595-602 (1983).
40. Beck, E. G., and Bignon, J. *In Vitro* Effects of Mineral Dusts. Third International Workshop, NATO ASI Series. Springer-Verlag, Berlin, 1985.
41. Hesterberg, T. W., Butterick, C. J., Oshimura, M., Brody, A. R., and Barrett, J. C. Role of phagocytosis in Syrian hamster cell transformation and cytogenetic effects induced by asbestos and short and long glass fibers. Cancer Res. 46: 5795-5802 (1986).
42. Hesterberg, T. W., and Barrett, J. C. Induction by asbestos fibers of anaphase abnormalities: Mechanism for aneuploidy induction and possibly carcinogenesis. Carcinogenesis 6: 473-475 (1985).
43. Oshimura, M., Hesterberg, T. W., and Barrett, J. C. An early, nonrandom karyotypic change in immortal Syrian hamster cell lines transformed by asbestos: Trisomy of chromosome 11. Cancer Genet. Cytogenet. 22: 225-237 (1986).
44. Barrett, J. C., Hesterberg, T. W., Oshimura, M., and Tsutsui, T. Role of chemically induced mutagenic events in neoplastic transformation of Syrian hamster embryo cells. In: Carcinogenesis—A Comprehensive Survey: Mammalian Cell Transformation: Mechanisms of Carcinogenesis and Assays for Carcinogens, Vol. 9 (J. C. Barrett and R. W. Tennant, Eds.) Raven Press, New York, 1985, pp. 123-129.
45. Barrett, J. C., Oshimura, M., and Koi, M. Role of oncogenes and tumor suppressor genes in a multistep model of carcinogenesis. In: Critical Molecular Determinants of Carcinogenesis, Vol. 39 (F. F. Becker and T. J. Slaga, Eds.), University of Texas Press, Austin, TX, 1987, pp. 45-56.
46. Barrett, J. C., and Fletcher, W. F. Cellular and molecular mechanisms of multistep carcinogenesis in cell culture models. In: Mechanisms of Environmental Carcinogenesis: Multistep Models of Carcinogenesis, Vol. 2 (J. C. Barrett, Ed.), CRC Press, Boca Raton, FL, 1987, pp. 73-116.
47. Annab, L., Barrett, J. C., and Gilmer, T. M. Characterization of activated proto-oncogenes in chemically transformed Syrian hamster embryo cells. Proc. Am. Assoc. Cancer Res., in press.
48. Brown, R. C., Poole, A., and Fleming, G. T. A. The influence of asbestos dust on the oncogenic transformation of C3H10T 1/2 cells. Cancer Lett. 18: 221-227 (1983).
49. Klein, G. The approaching era of the tumor suppressor genes. Science 238: 1539-1545 (1987).
50. Koi, M., and Barrett, J. C. Loss of tumor-suppression function during chemically induced neoplastic progression of Syrian hamster embryo cells. Proc. Natl. Acad. Sci. (U.S.) 83: 5992-5996 (1986).
51. Gibas, Z., Li, F. P., Antman, K. H., Bernai, S., Stahel, R., and Sandberg, A. A. Chromosome changes in malignant mesothelioma. Cancer Genet. Cytogenet. 20: 191-201 (1986).
52. Popescu, N. C., Chahinian, A. P., and DiPaolo, J. A. Nonrandom chromosome alterations in human malignant mesothelioma. Cancer Res. 48: 142-147 (1988).
53. Kok, K., Osinga, J., Carritt, B., Davis, M. B., van der Hout, A. H., van der Veen, A. Y., Landsvater, R. M., de Leij. L. F. M. H., Berendsen, H. H., Postmus, P. E., Poppema, S., and Buys, C. M. C. M. Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. Nature 330: 578-581 (1987).
54. Whang-Peng, J., Kao-Shan, C. S., Lee, E. C., Bunn, P. A., Carney, D. N., Gazdar, A. T., and Minna, J. D. Specific chromosome defect associated with human small-cell lung cancer: Deletion 3p14-23. Science 215: 181-182 (1982).
55. Lamb, P., Wiseman, R., Ozawa, N., Suzuki, Y., Chahinian, P., and Barrett, J. C. Detection of transforming genes in human mesotheliomas. Proc. Am. Assoc. Cancer Res. 29: 565 (1988).