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Cellular and Molecular Parameters of Mesothelioma

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Abstract

Malignant mesotheliomas (MM) are neoplasms arising from mesothelial cells that line the body cavities, most commonly the pleural and peritoneal cavities. Although traditionally recognized as associated with occupational asbestos exposures, MMs can appear in individuals with no documented exposures to asbestos fibers, and emerging data suggest that genetic susceptibility and simian virus 40 (SV40) infections also facilitate the development of MMs. Both asbestos exposure and transfection of human mesothelial cells with SV40 large and small antigens (Tag, tag) cause genetic modifications and cell signaling events, most notably the induction of cell survival pathways and activation of receptors, and other proteins that favor the growth and establishment of MMs as well as their resistance to chemotherapy. Recent advances in high-throughput technologies documenting gene and protein expression in patients and animal models of MMs can now be validated in human MM tissue arrays. These have revealed expression profiles that allow more accurate diagnosis and prognosis of MMs. More importantly, serum proteomics has revealed two new candidates (osteopontin and serum mesothelin-related protein or SMRP) potentially useful in screening individuals for MMs. These mechanistic approaches offer new hope for early detection and treatment of these devastating tumors.

Keywords

asbestos; mesothelioma; cancer; SV40

Mesothelial cells are unusual in that they possess features of both mesenchymal and epithelial cells, and normally facilitate lubrication and movement of serosal surfaces [Mutsaers, 2004]. The processes involved in the initiation and development of malignant mesothelioma (MM), an aggressive tumor derived from mesothelial cells, are under intense investigation. The interest in this peculiar, phenotypically diverse cancer arises from the fact that its incidence is increasing worldwide, and patients generally die less than a year from initial diagnosis [Mossman and Gee, 1989; Robinson and Lake, 2005]. Thus, MM represents a great challenge to clinicians and cancer researchers due to its poor prognosis and marked resistance to current therapies.

MM is presently a worldwide problem [Bocchetta et al., 2001]. Although MM is a rare disease with an annual incidence in the USA of 2,000 to 3,000 cases, a steady rise in cases has been reported [Grondin and Sugarbaker, 1999] that may have recently plateaued [Weill et al., 2004]. In Europe, the incidence of MM has risen for decades and is expected to peak between the years 2010 and 2020 [Boutin et al., 1998]. Understanding the mechanisms of MM development and invasiveness, and elucidating potential biomarkers are intrinsic to prevention, screening, and effective therapies for MM. Here, we present recent data on the etiology and molecular pathogenesis of this tumor.

FACTORS CAUSING MALIGNANT MESOTHELIOMA

In the US the most important causal factor for the development of human mesothelioma is occupational exposure to asbestos, primarily the amphiboles, crocidolite, and amosite [Mossman and Gee, 1989; Mossman et al., 1990]. Asbestos is a naturally occurring group of fibers (defined as having a > or = 3:1 length to width ratio), each with its own unique structure and chemical composition. There are two subgroups: (1) the serpentine group, consisting of chrysotile [$\text{Mg}_6\text{Si}_4\text{O}_{10}(\text{OH})_8$]; and (2) the amphiboles, a group of rod-like fibers including crocidolite [$\text{Na}_2(\text{Fe}^{3+})_2(\text{Fe}^{2+})_3\text{Si}_8\text{O}_{22}(\text{OH})_2$], amosite [$(\text{Fe}, \text{Mg})_7\text{Si}_8\text{O}_{22}(\text{OH})_2$], tremolite [$\text{Ca}_2\text{Mg}_5\text{Si}_8\text{O}_{22}(\text{OH})_2$], anthophyllite [$(\text{Mg}, \text{Fe})_7\text{Si}_8\text{O}_{22}(\text{OH})_2$], and actinolite [$\text{Ca}_2(\text{Mg}, \text{Fe})_5\text{Si}_8\text{O}_{22}(\text{OH})_2$] [Guthrie and Mossman, 1993] (Fig. 1). Asbestos fibers are ubiquitous in certain geographic areas and become problematic to human health when they are inhaled during mining or use. Chrysotile is the most common type of asbestos used historically in over 2000 industrial products and found in US buildings and schools [Health Effects Institute-Asbestos Research, 1991]. Although the association between amphibole asbestos exposure and the development of MM is well documented [Brown et al., 1990], the carcinogenic potential of chrysotile asbestos alone or with negligible amphibole contamination remains controversial [Steenland and Stayner, 1997; Yano et al., 2001; Robinson and Lake, 2005]. Some researches suggested that chrysotile asbestos may produce MMs in man, but the number of cases is small and the required exposures large [Churg, 1988]. Recent studies have implicated tremolite fibers as the likely etiological factor in MM associated with Canadian chrysotile exposure [Churg et al., 1984; McDonald and McDonald, 1995, 1997; Roggli et al., 2002]. However, studies evaluating worker populations that are transient and may be exposed to different types of fibers over a lifetime are difficult to interpret.

The potential of asbestos fibers to cause cancer has been linked to their geometry, size, and chemical composition. Long (>5 μ) and thin (diameter <3 μ) fibers are a health concern [Vu and Lai, 1997] and have been found to cause MM, lung cancers, and fibrosis after inhalation and intrapleural or intraperitoneal administration to rodents [Health Effects Institute-Asbestos Research, 1991; Lesur et al., 1995]. In addition to size, the chemical composition of fibers plays an important role in determining the cytotoxicity, durability, biopersistence, and biodegradability of asbestos types [Guthrie and Mossman, 1993]. The greater durability of amphiboles compared to chrysotile appears to be one of the principal reasons for their greater carcinogenic potential in MM, as the latency period of MM is often 30-40 years from initial exposure to asbestos fibers [Mossman et al., 1990, 1996]. Amphibole fibers persist at sites of tumor development and may serve as stimuli for neoplastic growth of cells [Woodworth et al., 1983; Jaurand et al., 1984]. The persistence of amphibole fibers at the site of tumor development is important to understanding both tumor induction and promotion of MMs.

It is now believed that there may be factors other than asbestos that contribute to the initiation and progression of MMs. Some studies suggest that genetic factors play an important role in the etiology of MM in certain families with an unusual high incidence of the disease [Roushdy-Hammady et al., 2001; Huncharek, 2002]. The question of genetic susceptibility arises because only a small percentage of individuals exposed to asbestos or erionite fibers develop MM

[Emri et al., 2002; Carbone and Rdzanek, 2004]. Also, compelling multi-institutional studies suggest that the simian virus 40 (SV40) T-antigen (Tag) is present in a large percentage of human mesotheliomas. Approximately half of MMs in the US are positive for SV40 Tag [Carbone, 1999; Klein et al., 2002]. A causal link between SV40 and mesothelioma has also been strengthened by studies showing that SV40 sequences are selectively expressed in mesothelioma cells, and not in adjacent parenchymal cells or lung carcinomas [Shivapurkar et al., 1999]. Moreover, mechanistic work demonstrates that human mesothelial cells are uniquely susceptible to SV40 infection and malignant transformation [Bocchetta et al., 2000]. In these experiments, infection of normal human mesothelial cells by SV40 led to an extremely high rate of morphological transformation, for example, at least 1,000 times higher than other cell types infected with this virus and immortality. In addition, SV40 acts synergistically with asbestos to cause malignant transformation of human mesothelial cells [Bocchetta et al., 2000]. In recent epidemiologic studies, the risk of hazard ratio of developing MM due to asbestos exposure alone, or SV40 alone, was compared with the hazard ratio due to asbestos exposure plus SV40 infection [Porta et al., 2005]. Asbestos exposure alone was associated with the development of MMs, while the presence of SV40 alone was not. However, the combination of SV40 infection plus asbestos exposure revealed a risk of developing MM 27-fold higher than subjects with asbestos exposure alone. This study provides epidemiologic support for a possible co-carcinogenic effect between SV40 and asbestos exposure in the development of MM.

ADVANCES IN UNDERSTANDING THE CELLULAR AND MOLECULAR BIOLOGY OF MESOTHELIOMA

Recent advances in modern cellular and molecular biology have improved our understanding of the alterations that lead to the development and progression of MM.

Cytogenetics

Cytogenetic and molecular studies indicate that MM results from the accumulation of numerous somatic genetic events, mainly deletions. The occurrence of multiple, recurrent cytogenetic deletions suggest that loss and/or inactivation of tumor suppressor genes are critical to the development and progression of mesothelioma [Lee and Testa, 1999]. Deletions of specific regions in the short (p) arms of chromosomes 1, 3, and 9 and long (q) arms of 6, 13, 15, and 22 are repeatedly observed in MM, and loss of a copy of chromosome 22 is the most common numerical change seen [Murthy and Testa, 1999]. However, relatively little is known about critical genetic changes in the genesis of mesothelioma. Of the known cytogenetic changes, the most frequent is loss of *CDKN2A/ARF*, encoding the tumor suppressors p16^{INK4a} and p14^{ARF} at 9p21 (by homozygous deletion) [Cheng et al., 1994; Hirao et al., 2002; Altomare et al., 2005b], adversely affecting both Rb and p53 pathways, respectively. NF2 (Merlin), a tumor suppressor located at 22q12 (by an inactivating mutation coupled with allelic loss) is also frequently altered in mesotheliomas [Bianchi et al., 1995; Sekido et al., 1995; Lechner et al., 1997; Cheng et al., 1999]. Other conventional protooncogenes and tumor suppressor genes have been investigated including *NRAS* [Papp et al., 2001], *HRAS* and *KRAS* [Kitamura et al., 2002], and *TP53*, which encodes the tumor suppressor p53 [Mayall et al., 1999] but no consistently frequent mutations are found. The discovery of critical somatic gene alterations in MM and understanding how each of them contributes to the pathogenesis of this malignancy may ultimately lead to the design of more efficient preventive and therapeutic strategies. The identification of these somatic genetic changes should be facilitated by the recent development of various DNA microarray platforms, powerful new methods for high-resolution profiling of genomic imbalances. These techniques allows for rapid and reliable assessment of DNA copy number changes across the entire genome and could potentially lead to the identification of novel MM genes [Apostolou et al., 2005].

Tools for Revealing Growth Factors and Signaling Pathways Important in MM

Considerable advances have been made towards understanding the multiplicity of growth and angiogenic factors produced by MM using in vitro approaches [Mossman and Gruenert, 2002]. These cytokine pathways are important in the functional and phenotypic properties of MMs (Fig. 2). In vivo models of MM have been available for over 40 years, and they have provided solid evidence for the experimental induction of mesothelioma by asbestos, erionite, and SV40 [Saffiotti, 2005]. Although animal models have been valuable in testing carcinogenic potency of asbestos and other particulates in MM, a few investigators [Vaslet et al., 2002; Altomare et al., 2005a] have used animal models to address specific mechanisms and pathways in MM. Transgenic and knockout animals offer a valuable tool that has not been fully exploited in MM. For example, we recently showed that heterozygous *Nf2* knockout mice treated with asbestos recapitulate molecular features of the human disease counterpart including biallelic inactivation of *Nf2*, homozygous deletion of *Cdkn2a/Arf*, and activation of the Akt signal transduction pathway. Thus, this model could represent a faithful model for preclinical testing of novel therapeutic agents.

Cell Signaling by Asbestos in MM

Asbestos may act as both an initiator (genetically) and promoter (epigenetically) in the development of MMs [Mossman et al., 1990, 1996]. An important unresolved issue is whether asbestos fiber carcinogenicity occurs via direct interactions of asbestos fibers with mesothelial cells, through indirect mechanisms involving oxidative stress or both [Kamp et al., 1992; Shukla et al., 2003a]. A ramification of interaction of long (>5 μ) fibers with cells is frustrated phagocytosis and a prolonged oxidative burst [Hansen and Mossman, 1987]. The increased durability and high iron content of the amphiboles, crocidolite, and amosite, also may contribute to their higher carcinogenic potential via chronic generation of oxidants catalyzed by iron and/or surface reactions occurring on the fiber [Weitzman and Graceffa, 1984; Gulumian and van Wyk, 1987]. The cytotoxicity of crocidolite fibers in human lung carcinoma cells is directly linked to iron mobilization and is followed by increased ferritin synthesis, a perpetual feedback system for uptake of iron by cells [Chao et al., 1994; Fang and Aust, 1997].

Studies on animal models and cell cultures have confirmed that asbestos fibers generate reactive oxygen species (ROS) and reactive nitrogen species (RNS) [Mossman et al., 1990; Kamp et al., 1992; Shukla et al., 2003a]. These effects may be potentiated by inflammation associated with fiber exposures in vivo or by diminution of cellular reserves of glutathione or antioxidant enzymes [Kinnula, 1999; Shukla et al., 2004]. Asbestos fibers in vitro cause the production of DNA damage either via production of ROS or by direct damage to chromosomes after phagocytosis of fibers [Kamp et al., 1995; Lesur et al., 1995; Fung et al., 1997]. The consequences of such DNA damage could be the loss of tumor suppressor genes, activation of proto-oncogenes, or unregulated generation of growth factors through paracrine/autocrine mechanisms [Pass et al., 1996].

Asbestos either by direct interaction with growth factor receptors [Pache et al., 1998] or by oxidation of proteins, possibly phosphatases, causes stimulation of multiple cell signaling pathways linked to abnormal growth control in pulmonary epithelial cells, mesothelial cells, endothelial cells, and fibroblasts [Churg, 1996; Mossman and Churg, 1998; Ramos-Nino et al., 2002b]. Asbestos also activates redox-sensitive transcription factors such as NF- κ B [Janssen et al., 1995, 1997] and AP-1 [Heintz et al., 1993; Ramos-Nino et al., 2002b], which lead to increased cell survival, inflammation, and paradoxically, the upregulation of antioxidant enzymes such as manganese superoxide dismutase [Kinnula, 1999]. MnSOD is also overexpressed in asbestos-related mesotheliomas [Kahlos et al., 1999; Ramos-Nino et al., 2002a], rendering them highly resistant to oxidative stress in comparison to normal mesothelial

cells. Other studies have demonstrated overexpression of other enzymes related to oxidative stress, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (NOS2) [Marrogi et al., 2000; Edwards et al., 2002], and endothelial nitric oxide synthase (eNOS) [Soini et al., 2001] in MM. Thioredoxin, a small redox-active protein reduced by the selenoprotein thioredoxin reductase and NADPH, is associated in other models of cancer with cell growth and differentiation and is also overexpressed in MM cells [Sun et al., 2000]. This protein might be another factor governing the poor prognosis of MMs and their reduced responsiveness to conventional therapies. Overexpression of gamma-glutamylcysteine synthetase, a ratelimiting enzyme in glutathione-associated pathways, could also play an important role in the primary drug resistance of mesotheliomas [Jarvinen et al., 2002]. Catalytically active 5-lipoxygenase may also be involved in the regulation of proliferation and survival in MMs via a vascular endothelial growth factor (VEGF)-related circuit [Romano et al., 2001].

Tumor promotion was classically thought to be a proliferation-driven process. However, it is now recognized that neoplastic growth is an imbalance between apoptosis and proliferation. In support of this concept, a dynamic balance between apoptosis and cell proliferation is observed in mesothelial cells exposed to crocidolite asbestos [Goldberg et al., 1997]. Studies in vitro indicate that asbestos can induce apoptosis in mesothelial cells through formation of ROS [BeruBe et al., 1996; Broaddus et al., 1996] and mitochondrial pathways [Kamp et al., 1992; Shukla et al., 2003b,c; Panduri et al., 2003].

Epidermal Growth Factor Receptor (EGFR) and MMs

Studies in our group have found that the EGFR is an important initial target of asbestos fibers at the cell membrane. This growth factor is required for proliferation of human mesothelial cells, and is produced in an autocrine fashion in MM [Laveck et al., 1988].

Autophosphorylation [Zanella et al., 1996] and increased expression [Pache et al., 1998] of the EGFR occurs in mesothelial cells after in vitro exposures to asbestos. Moreover, aggregation and phosphorylation of the EGFR by long fibers initiates extracellular signal regulated kinase (ERK1/2) cell-signaling cascades linked to asbestos-induced injury and mitogenesis [Zanella et al., 1996; Pache et al., 1998]. Increased expression of EGFR in rat pleural mesothelial (RPM) cells correlates with the carcinogenicity of mineral fibers [Faux et al., 2000].

We have also shown that the EGFR is causally linked to activation of the mitogen activated protein kinase (MAPK) cascade and increased expression of the proto-oncogenes, *c-fos*, and *c-jun* in mesothelial cells [Heintz et al., 1993; Zanella et al., 1996]. Expression of both Fos and Jun family members (components of the transcription factor AP-1 complex) is required for transition through the G₁ phase and entry into the S phase of the cell cycle [Reddy and Mossman, 2002]. Most recently, ERK 1/2-induced activation by asbestos has been linked to the induction of *fra-1*, an important component of the AP-1 complex that is causally related to anchorage-independent growth in MM [Ramos-Nino et al., 2002b]. Microarray analyses also have shown increased expression of *fra-1* in rat and human mesotheliomas [Sandhu et al., 2000; Ramos-Nino et al., 2003].

PI3K/AKT Pathway in MM

A growing body of evidence suggests that the phosphatidylinositol 3-kinase (PI3-K/AKT) pathway plays an important role in human cancers, and numerous AKT substrates have been implicated in tumorigenesis [Bellacosa et al., 2005]. Activation of AKT triggers anti-apoptotic mechanisms, positively influences NF- κ B transcription, modulates angiogenesis, enhances telomerase activity, increases tumor invasion, and antagonizes cell-cycle arrest [Bellacosa et al., 2005]. We have demonstrated that the AKT pathway is frequently activated in MMs, and that inhibition of this pathway inhibits cell growth and increases sensitivity to conventional chemotherapeutic agents [Ramos-Nino et al., 2005; Altomare et al., 2005b].

Growth Factors and MM

Other potentially important key players in the initiation and progression of MM include TGF- α , which binds to the EGFR [Walker et al., 1995]; insulin-like growth factors (IGF) I and II that function as autocrine growth factor stimuli in normal mesothelial and MM cells [Lee et al., 1993; Rutten et al., 1995], and PDGF [Metheny-Barlow et al., 2001]. Increased levels of hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF), known growth factors for mesothelial cells, have been detected in pleural lavage fluids in rodents exposed to asbestos [Adamson and Bakowska, 2001]. The HGF receptor, c-Met, a proto-oncogene product whose activation leads to cell growth and altered morphogenesis, is activated in human MM cells [Cacciotti et al., 2001], and high expression levels of *fra-1*-dependent *c-met* have been detected in rat MM cells [Ramos-Nino et al., 2003].

Matrix Metalloproteinases (MMPs) and MM

MMs synthesize a wide array of matrix proteins, express receptors that bind these matrices, and secrete enzymes that have the capacity to degrade the extracellular matrix (ECM). For example, MMs exhibit elevated amounts of hyaluronan, and hyaluronan synthesis enhances cell proliferation, anchorage independent growth, and cell migration in a number of tumor types [Li and Heldin, 2001]. Using oligonucleotide microarray analysis, the hyaluronan receptor gene, *cd44*, is detected in high amounts in human and rat mesothelial cells exposed to asbestos and in MM cell lines where it may play a role in mesothelial cell motility and migration [Ramos-Nino et al., 2003]. Other ECM components such as fibrin via increased expression of tissue factor (TF) may play a role in pleural injury or establishment of MMs [Bajaj et al., 2000]. In a study on 16 patients in whom MMPs-1,-2,-3,-7, and -9, and tissue inhibitors of metalloproteinases (TIMP-1 and -2) were evaluated, MMP-1 and -2 were related directly to invasion and spread of MMs [Hirano et al., 2002].

Signaling by SV40 in MM

SV40-associated mesothelial cell transformation has been mainly attributed to the ability of SV40 Tag to inactivate the tumor suppressors, p53 [Carbone et al., 1997] and Rb [De Luca et al., 1997]. However, it is now clear that other events may be important in SV40-mediated co-carcinogenesis. For example, SV40-associated elaboration of growth factors by paracrine/autocrine mechanisms creates a favorable environment for MM development (Fig. 2). Some growth factors such as IGF-1, which is implicated in governing both growth rate and tumorigenicity of SV40-induced mesotheliomas [Pass et al., 1996, 1998], are induced by both asbestos and SV40 in MM cells. Also, SV40 small t antigen (tag) may stimulate ERK activity in MMs by binding to and inhibiting protein phosphatase 2A, a protein involved in dephosphorylation of many protein substrates. This may have relevance to carcinogenesis since co-expression of both SV40 Tag and tag are required for SV40-mediated human cell transformation [Rundell and Parakati, 2001].

Another pathway activated by both asbestos and SV40 is the PI3K/AKT pathway which is frequently activated in MM, particularly in SV40-positive MM specimens and cell lines [Cacciotti et al., 2005; Ramos-Nino et al., 2005]. HGF is another example of a SV40-induced growth factor in MMs. The HGF receptor, Met, a protooncogene product whose activation leads to cell growth and altered morphogenesis, is activated in SV40-positive human MM cells and is required for their growth in tissue culture [Cacciotti et al., 2001]. Moreover, when normal human mesothelial cells are transfected with full-length SV40 DNA, Met receptor activation is induced and associated with S-phase entry and alteration to a fibroblastoid morphology. Viral particles can infect adjacent mesothelial cells, perpetuating HGF-dependent Met activation. This work may be of special significance because high levels of HGF are detected in pleural effusions from patients with MM [Eagles et al., 1996]. It also suggests a mechanism whereby SV40-infected cells may propagate the growth of surrounding mesothelial cells.

Furthermore, SV40 infection induces release of VEGF from human mesothelial cells. VEGF is not only an autocrine growth factor for human MMs [Cacciotti et al., 2002], but a potent angiogenic factor necessary for vascularization and tumor growth. The establishment of a favorable tumor environment may be a contribution of SV40 infection to the development of MMs and one of several mechanisms whereby SV40 acts cooperatively with asbestos in the development of these malignancies.

EMERGING BIOMARKERS IN MM

Identification of biomarkers for MM might prove useful in screening at-risk populations, early diagnosis of MMs, and monitoring tumors in response to therapy. In recent studies, the detection of a soluble mesothelin-related protein (SMRP), in serum is encouraging [Robinson et al., 2005]. One study show that determination of SMRP in serum is a marker of MM with a sensitivity of 83% and specificity of 95% in 48 MM patients tested. Changes in serum SMRP levels paralleled clinical course and tumor size. Moreover, SMRP was elevated in 75% of patients at diagnosis, thus may prove useful for screening asbestos-exposed individuals for early detection of MM.

Another promising MM biomarker is osteopontin. In a recent publication [Pass et al., 2005], serum osteopontin levels were evaluated in three populations (190 patients): (1) subjects without cancer who were exposed to asbestos; (2) subjects without cancer who were not exposed to asbestos, and (3) patients with pleural MM who were exposed to asbestos. Results revealed that osteopontin levels could be used to distinguish individuals with exposure to asbestos who had MM. These results are exciting, as early detection of MMs may enable more effective therapies. Moreover, this research illustrates the importance of proteomics for the potential identification of MM-specific proteins for screening and diagnosis.

HIGH-THROUGHPUT TECHNOLOGIES IN MM RESEARCH

As high-throughput gene expression data on MM accumulates in animal models and on human tumors [Rihn et al., 2000; Sun et al., 2000; Kettunen et al., 2001, 2004; Mohr and Rihn, 2001; Gordon et al., 2002, 2003, 2005; Ramos-Nino et al., 2003; Singhal et al., 2003; Fox et al., 2004; Mohr et al., 2004; Pass et al., 2004; Gordon, 2005; Wali et al., 2005], genes that can be targeted to reverse functional changes and/or kill MMs will be revealed. These expression profiles also will potentially determine, in an unbiased manner, single genes or gene expression ratios for diagnosis or prognosis of MMs. Furthermore, these techniques will help investigate possible human susceptibility genes through single nucleotide polymorphism (SNP) arrays. As an example, pathological distinctions between MM and adenocarcinomas of the lung have been a challenging task. Recently, a study tested the fidelity of ratio-based diagnosis in 181 tissue samples. Validation of microarray data and ratio-based diagnosis was performed using calretinin/claudin-7 and VAC-b/TACSTD1 ratios, and the ratios correctly identified 95% and 99% of the two sets of tumors, respectively [Gordon et al., 2002, 2003]. In another study, MM cell lines that retained the ability to differentiate into either epithelial or fibroblast-like phenotypes of MM were used to identify the genes related to tumor cell differentiation using subtractive hybridization. Nine genes were found to be overexpressed in the epithelial sub-line, compared to two in the fibroblast-like phenotype. One of the genes expressed by the epithelial sub-line was thioredoxin, a small redox-active protein associated with cell growth and differentiation [Sun et al., 2000].

Recently, another study showed that gene expression profiles in pleural MMs can predict time to progression and survival patterns among two separate series of patients who underwent cytoreduction. This study showed with a 76%-95.2% range accuracy, using 27 classifiers, that it, was possible to predict actual time to progression and survival of patients with MM [Pass et al., 2004].

In an effort to establish possible targets for therapy, our laboratory has used microarray analysis and RNAi technology to elucidate the role of important genes in MM [Ramos-Nino et al., 2003]. We first characterized, using oligonucleotide microarray analysis, upregulated or downregulated gene expression in RPM cells and three rat MM cell lines. *Erk5*, *fra-1*, *c-met*, and *cd44*, among other genes were upregulated in MM cells. After confirming that *fra-1* mRNA was dramatically increased in asbestos-exposed RPM, and in human and rat MMs by real time-quantitative PCR (RT-QPCR), we selected candidate genes following patterns of *fra-1* expression. After confirmation of changes in mRNA levels using RT-QPCR, we then used RNAi technology to address the hypothesis that expression of some genes would be *fra-1* dependent. Knockout of *fra-1* revealed that expression of *c-met* and *cd44* genes encoding receptors were linked to *fra-1* expression in asbestos-treated mesothelial cells and MMs.

CD44 is the principal cell surface receptor for the ECM glycosaminoglycan, hyaluronan, which is elevated in MMs [Penno et al., 1995; Li and Heldin, 2001]. The binding of CD44 to hyaluronan mediates cell attachment and migration [Lewis et al., 2001]. An association between overexpression of CD44 or its alternative spliced variants and aggressiveness or metastasis of a variety of human tumors shows the importance of this protein in tumor invasiveness in vitro [Faassen et al., 1992; Thomas et al., 1992; Thomas, 1993] and in vivo models [Gunthert et al., 1991; Guo et al., 1994]. Our observation that *fra-1* expression upregulates *c-met* also has mechanistic implications in the development of MMs by asbestos and SV40, further supporting the concept that these diverse agents may act through similar cell signaling pathways (Fig. 2).

The use of human MM tissue arrays has also become an integral part of high-throughput molecular profiling of tumor specimens and these can now be used for rapid validation of results from genomic and proteomic arrays. Arrays from as many as 600 tumor specimens can be produced in a single paraffin block which allows screening by immunohistochemistry, fluorescence in situ hybridization, and RNA-RNA in situ hybridization [Wali and Pass, 2005]. An example of the use of tissue arrays in MM research was recently illustrated in a study showing frequent AKT phosphorylation in MMs [Altomare et al., 2005b].

CONCLUSIONS

As advances in cellular/ molecular technology and cell imaging progress, our knowledge of MM biology will expand dramatically. This information may be translated into the clinical arena to allow improvements in the screening, diagnosis, and therapy of MMs. Most importantly, deciphering the pathways that lead to development and maintenance of MMs will aid in designing targeted treatment regimens for this devastating disease.

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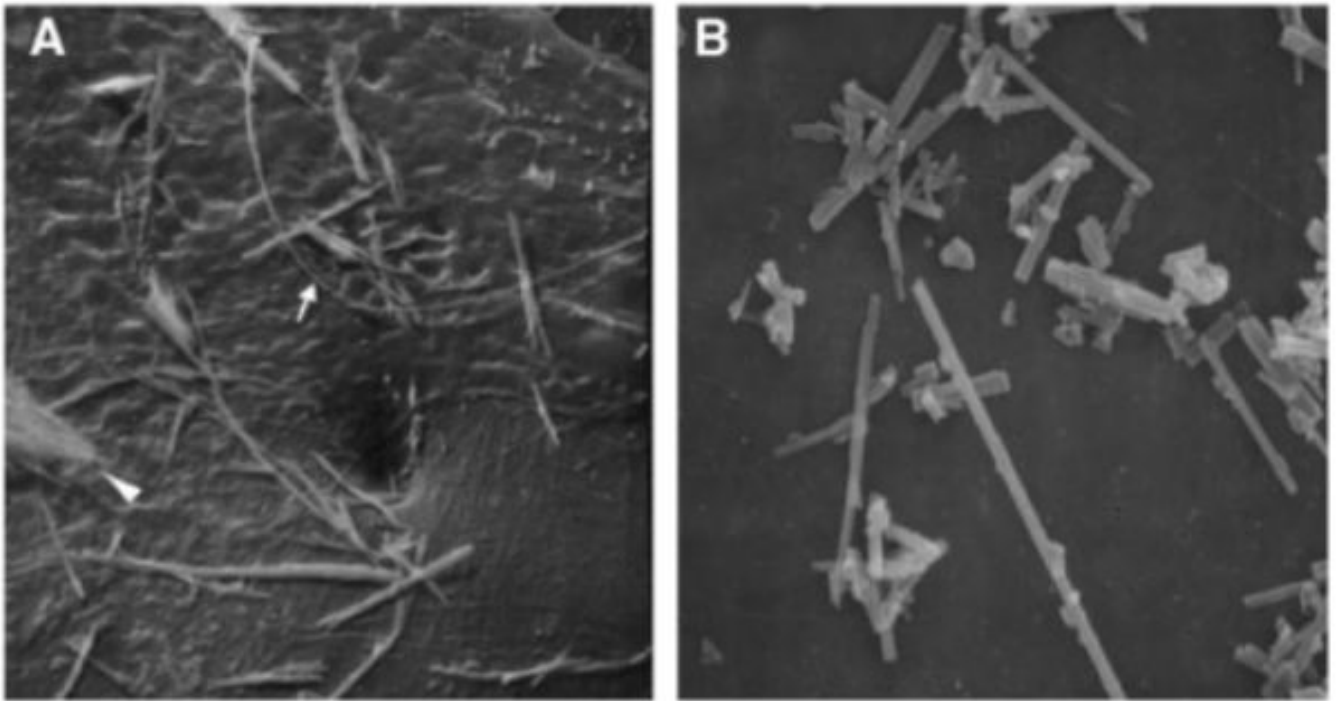


Fig. 1. Morphology of chrysotile (**A**) and crocidolite (**B**) asbestos fibers as illustrated by scanning electron microscopy. Arrow in A shows a long chrysotile fiber on the plasma membrane and arrowhead shows short fiber bundles.

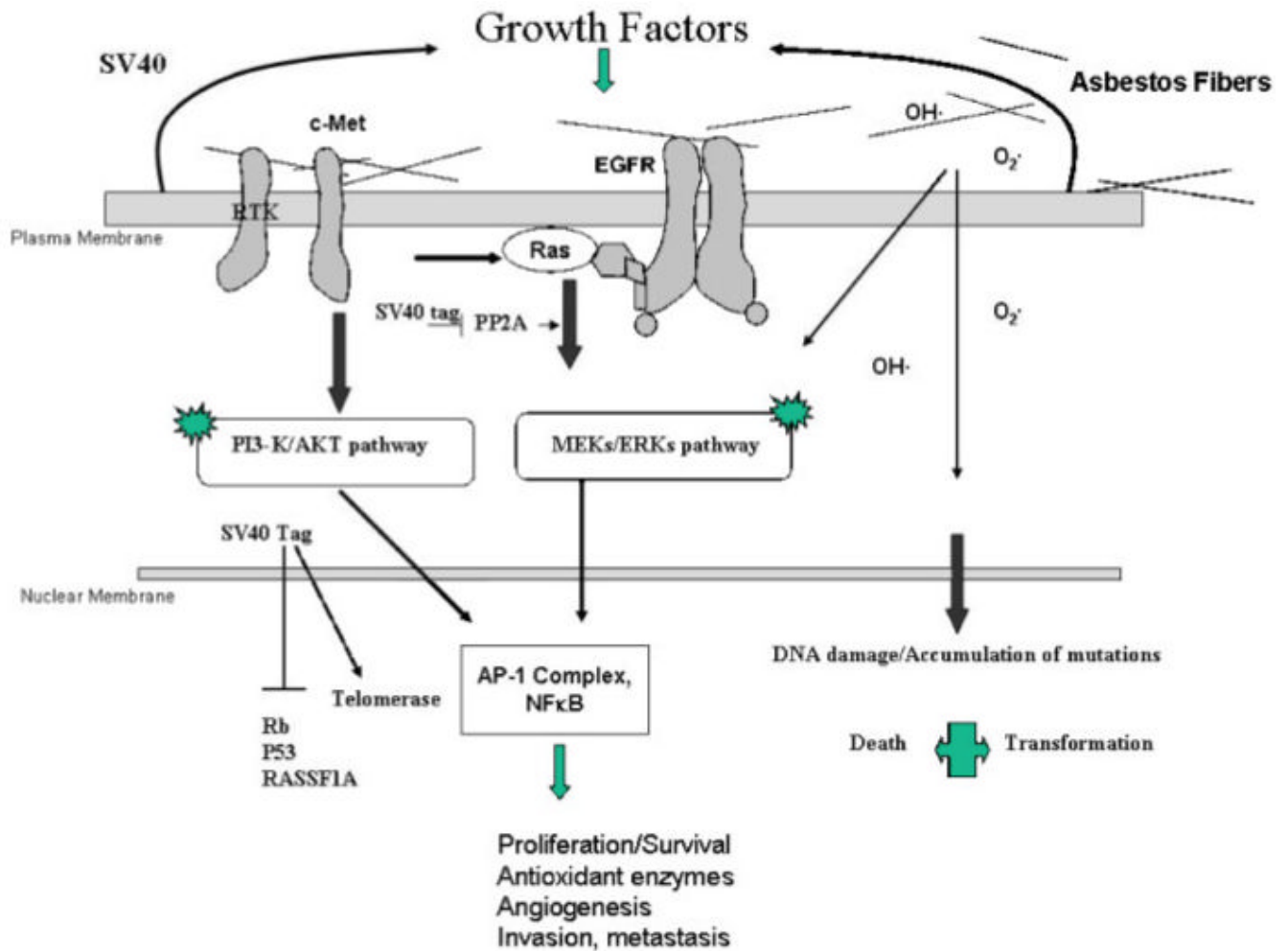


Fig. 2.

A schema indicating cell signaling pathways and outcomes in mesothelial cells that are triggered by asbestos fibers or associated with SV40 infection. SV40, Simian virus 40; SV40 tag, SV40 small t antigen; SV40 Tag, SV40 large T antigen; PI3K, phosphatidylinositol 3-kinase; MEK, mitogen activated protein (MAP)/ERK kinase; ERK, extracellular signal regulated protein kinase; Rb, retinoblastoma; AP-1, activator protein-1; NFκB, nuclear factor kappa-B; RASSF1A, ras association domain family 1A; RTK, receptor tyrosine kinases; EGFR, epidermal growth factor receptor; PP2A, protein serine/threonine phosphatase 2A.