MOLECULAR PATHWAYS

Targeting Mechanisms of Asbestos and Erionite Carcinogenesis in Mesothelioma

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Malignant mesothelioma is an aggressive malignancy related to asbestos and erionite exposure. AP-1 transcriptional activity and NF-kB signaling pathway have been linked to mesothelial cell transformation and tumor progression. HGF and c-Met are highly expressed in mesotheliomas. PI3K, AKT and the downstream mTOR are involved in cell growth and survival and are often found to be activated in mesothelioma. p16\textsuperscript{INK4a} and p14\textsuperscript{ARF} are frequently inactivated in human mesothelioma, and approximately 50% of mesotheliomas contain NF2 mutation. Molecular therapies aimed at interfering with these pathways have not improved the dismal prognosis of mesothelioma, except, possibly, for a small subset of patients that have benefit from certain therapies such as ranpirinase treatment. Recent studies have demonstrated the importance of asbestos-induced inflammation in the initiation and growth of mesothelioma; HMGB1 and Nalp3 inflammasome have been identified as key initiators of this process. Asbestos induces cell necrosis, causing the release of HMGB1 that, in turn, may activate Nalp3 inflammasome, a process that is enhanced by asbestos-induced production of ROS. HMGB1 and Nalp3 induce pro-inflammatory responses and lead to the secretion of IL-1β and TNF-α, and NF-κB activity, thereby promoting cell survival and tumor growth. Novel strategies that interfere with asbestos and erionite-mediated inflammation might prevent or delay the onset of mesothelioma in high-risk cohorts, including individuals genetically predisposed, and/or inhibit tumor growth. The very recent discovery that germline BAP1 mutations cause a new cancer syndrome characterized by mesothelioma, uveal melanoma and melanocytic tumors provides researchers with a novel target for prevention and early detection.
BACKGROUND

Asbestos is a non specific term that refers to six fibrous silicate minerals used commercially divided into two groups based upon the chemical composition and crystalline structures: serpentine and amphibole asbestos (1). Among serpentine minerals only chrysotile was used commercially. Chrysotile is a hydrated magnesium silicate and its stoichiometric chemical composition may be given as Mg$_3$Si$_2$O$_5$(OH)$_4$. Over 95% of asbestos used commercially in the world is chrysotile (1). The chemical composition of amphibole group asbestos minerals can vary widely. The commercially used asbestiform amphiboles are actinolite, tremolite, anthophyllite, amosite, and crocidolite. They are all hydrated silicates and have double tetrahedral chains with Si$_8$O$_{22}$ composition that extend along the c-axis. Amphiboles are distinguished from one another by the number of the cations Ca, Fe, Mg, and Na that they contain (1). Thus asbestos is a “commercial” not a scientific definition: There are about 396 fibrous minerals in nature: 390 of them are not called “asbestos’ —and they are not subject to restrictive regulations— because they had not been used commercially at the time regulations to control the use of some mineral fibers were implemented. The unintended consequence of this very confusing nomenclature is that it is often erroneously assumed that only “asbestos”, but not other mineral fibers, cause cancer. Erionite, for example, is one of these naturally occurring fibrous minerals. Exposure to erionite is less widespread but more potent than asbestos in causing mesothelioma (2, 3).

Exposure to asbestos and other fibrous minerals contributes to asbestosis, some lung cancers, and is the main cause of mesothelioma, a highly aggressive cancer that arises from mesothelial cells of the pleura, peritoneum and pericardium, with a median survival of a year from diagnosis (1). Presently, mesothelioma causes approximately 3,000 deaths/year in the US and an additional 5,000 deaths/year in Western Europe (1). Despite asbestos abatement efforts, mesothelioma rates have stabilized in the US and are estimated to increase by 5-10% per year in most European countries over the next 25 years (1). The continued use of asbestos in some
commercial products within the US and the difficulty of removing asbestos already in place present additional risks for mesothelioma and lung cancer that will persist into the foreseeable future. We can anticipate a dramatic increase in the incidence of mesothelioma and of other asbestos-associated malignancies in the third world, particularly in India, where the use of asbestos continues to increase exponentially and few, if any, precautions are taken (4).

Moreover, increased urban development may disturb outcrops of asbestos, erionite, or soil containing other types of carcinogenic mineral fibers, and thereby lead to more instances of exposure (1, 2, 5-9). Recent investigations uncovered exposure to erionite in North Dakota (ND) where over 300 miles of roads, playgrounds and driveways have been paved, mostly during the past 2 decades, with gravel-containing erionite. More erionite-exposure is suspected in nearby States. On cars and school buses transiting on ND roads the air-concentrations of erionite were equal or exceeded those found in some Turkish villages that experience a 6.5% mesothelioma mortality (9). A similar problem occurred in New Caledonia, where exposure to antigorite a type of serpentine mineral fiber that was used as road gravel, led to a mesothelioma epidemic (6).

Because the interval between initial asbestos or erionite exposure and diagnosis ranges from about 25 to 71 years (1, 9), there would be time to implement preventative therapies within exposed cohorts –including ND where most roads were paved with erionite-containing gravel in the recent past - if the precise mechanisms of asbestos carcinogenesis were identified and biomarkers of exposure and of mesothelioma were available to monitor the population at risk.

Pathogenesis of mesothelioma and mechanisms of asbestos carcinogenesis.

The observation that only a fraction (~5%) of workers exposed to high doses of asbestos for prolonged periods of time developed mesothelioma and the identification of clusters of mesothelioma cases within certain families suggested that genetics influences mineral fiber carcinogenesis (1). Studies of an epidemic of mesothelioma in Cappadocia and in the US demonstrated that the risk of developing mesothelioma was transmitted with an autosomal
dominant pattern in certain high families (2). Germline mutations of the BAP1 gene have now been linked to the high incidence of MM in some US families (10). Individuals with heterozygous BAP1 germline mutations are affected by a novel cancer syndrome characterized by very high risk of developing mesothelioma, uveal melanoma, and possibly additional cancers (10). Mesothelioma may become dominant in these families upon exposure to asbestos or erionite (10). The identification of BAP1 mutant carriers may be facilitated by the detection of melanocytic nevi as described by Wiesner et al (11) and confirmed by genetic testing. In addition to familiar cases, BAP1 somatic mutations have been identified in 25% of sporadic mesotheliomas (10, 12) and in 84% of metastasizing uveal melanomas (13) but appear to be relatively rare in other cancers (14, 15). BAP1 is a deubiquitinating enzyme that has tumor suppressor activity (14, 15). BAP1 seems to regulate deubiquitination during the DNA damage response and the cell cycle, thus influencing S-phase progression, cell necrosis and apoptosis (14, 15). Mutations that abolish the deubiquitinating activity of BAP1 and/or its nuclear localization abolish BAP1 tumor suppressor activity (15). The exact target of BAP1 remains unclear. Ventii et al proposed that expression of BAP1 induces early exit out of G1 causing an accumulation of DNA damage and cell death (15). Along those lines, our hypothesis is that by influencing DNA damage/repair, BAP1 may help prevent environmental carcinogenesis caused by asbestos/erionite or UV light, thus explaining the very high incidence of mesothelioma, uveal melanoma and melanocytic tumors—rather than other cancer types not environmentally related—among BAP1 germline mutant carriers.

DNA damage may be directly caused through mechanical interference of asbestos fibers with chromosome segregation during mitosis (16), or, more likely, indirectly through asbestos-related induction of mesothelial cells and macrophages to generate mutagenic reactive oxygen (ROS) and nitrogen (iNOS) species, both of which are mutagenic in vitro (17, 18). The generation of oxidants by macrophages as they attempt to digest asbestos fibers may trigger
the activation of several signaling pathways. Among them, mitogen activated protein kinases (MAPK) signaling and the resulting activator protein 1 (AP-1) transcriptional activity has been linked to mesothelial cell transformation (19). Other minerals, such as crystalline silica, also elicit ROS and iNOS production from lung macrophages but do not cause mesothelioma (20). Thus, the capacity of asbestos to induce ROS and iNOS is only one of multiple factors that contribute to asbestos carcinogenesis.

In addition to triggering MAPK signaling, asbestos may directly initiate AP-1 activity through epidermal growth factor receptor (EGFR) and downstream pathways (19). Like EGFR signaling, hepatocyte growth/scatter factor (HGF) and its receptor tyrosine kinase c-Met leads to cell proliferation, as well as cell motility, upon their activation. Both HGF and c-Met are highly expressed in most mesotheliomas, especially those that are SV40-positive (21). HGF activation is mediated through the phosphatidylinositol-3-kinase (PI3K)/MEK5/Fos-related antigen 1 (Fra-1) feedback pathway (19). PI3K, AKT and the downstream mTOR are involved in cell growth and survival and are often found to be activated in mesothelioma (22).

P16\textsuperscript{INK4a} and p14\textsuperscript{ARF} are frequently inactivated in mesothelioma (23-25), and approximately 50% of mesotheliomas contain missense or nonsense mutations in the neurofibromatosis type 2 (NF2) gene (26, 27). Mice that were homozygous null for the ARF tumor suppressor rapidly developed mesothelioma upon exposure to asbestos, while mice heterozygous for ARF were also susceptible to mesothelioma and consistently exhibited biallelic inactivation of ARF (28, 29).

Chronic Inflammation and Mesothelioma.

Inflammation is the hallmark of asbestos deposition in tissue and contributes to asbestos carcinogenesis (1, 30, 31). The inflammatory infiltrate into areas of asbestos exposure consists largely of phagocytic macrophages that internalize asbestos and release numerous cytokines and mutagenic ROS (32). Among the cytokines secreted, tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and
IL-1β have been convincingly linked to asbestos-related carcinogenesis (31, 33). Both IL-1β and TNF-α enhanced erionite fiber-induced transformation of the immortalized non-tumorigenic human mesothelial cell line MeT-5A (33). Caspase-1 activation promotes the secretion of IL-1β, and requires the assembly of high molecular weight complexes known as inflammasomes (34). The Nalp3 inflammasome, the best characterized of these complexes, is activated by a wide variety of stimuli that includes exogenous pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) (34, 35). Asbestos induces cell necrosis, causing the release of High-mobility group protein B1 (HMGB1) (36), a typical DAMP and a key mediator of inflammation (37) that, in turn, can trigger activation of Nalp3 inflammasome and subsequent IL-1β secretion, a process that is enhanced by asbestos-induced production of ROS (38, 39). Nalp3-deficient mice exhibited decreased pulmonary pathology in response to silica and asbestos exposure compared to wild-type controls (38).

HMGB1 is actively secreted by reactive macrophages and other inflammatory cells, as well as by HM upon asbestos exposure (36). HMGB1 is a critical regulator in the initiation of asbestos-mediated inflammation leading to the release of TNF-α and subsequent NF-κB signaling (36). TNF-α is a central mediator in asbestos-, silica- and bleomycin-induced models of pulmonary fibrogenesis (40). Asbestos-exposed TNF-α receptor knockout mice did not develop the fibro-proliferative lesions found in asbestos-exposed wild-type mice (41). TNF-α-induced NF-κB signaling was shown to be the critical link between inflammation and carcinogenesis in multiple cancer models, including mesothelioma (31). Asbestos-mediated TNF-α signaling induces the activation of NF-κB-dependent mechanisms thus promoting the survival of HM following asbestos exposure (31). This allows HM that accumulated asbestos-induced genetic damage to survive, divide and propagate genetic aberrations in pre-malignant cells that can give rise to a malignant clone.
In healthy cells, HMGB1 is primarily found in the nucleus, where it stabilizes chromatin and plays multiple roles in DNA transcription, replication and recombination. During programmed necrosis, HMGB1 translocates from the nucleus to the cytosol and to the extracellular space, where it binds several pro-inflammatory molecules and triggers the inflammatory responses that distinguish this type of cell death from apoptosis. These findings provide mechanistic links between asbestos-induced cell death, chronic inflammation, and mesothelioma. Secreted HMGB1 stimulates RAGE, TLR2 and TLR4 (the three main HMGB1 receptors) expressed on neighboring macrophages and induces the release of several inflammatory cytokines, including TNF-α and IL-1β. In addition, HMGB1 enhances the activity of NF-κB, which promotes tumor formation, progression, and metastasis (42). An investigation of targeting extracellular HMGB1 as a novel strategy for mesothelioma prevention and/or therapy is currently ongoing (43). As shown in Figure 1, we hypothesize that HMGB1 functions as a “master switch” that initiates a series of inflammatory responses leading to malignant transformation of asbestos- or erionite-damaged HM.

CLINICAL–TRANSLATIONAL ADVANCES

Molecular therapies.

While increased expression of EGFR has been noted in human mesothelioma, a Phase II clinical trial of the EGFR signaling inhibitor gefitinib (Iressa) yielded disappointing results (44). Because receptor tyrosine kinases (RTKs) are frequently activated in mesothelioma, investigators tested the possible benefits of small molecule RTK inhibitors, including erlotinib (Tarceva) and imatinib (Gleevec) (Figure 2). However, the results of such studies to date have not been promising (45, 46). The accumulation of cytoplasmic β-catenin, a downstream component of the Wnt signaling pathway, in the majority of human mesotheliomas indicates that Wnt signaling is abnormally activated (47). Moreover, the disheveled proteins, also downstream...
of Wnt, are often over-expressed in mesothelioma and siRNA knockdown of disheveled suppressed mesothelioma growth (48). These data suggest that agents targeting components of the Wnt signaling pathway could benefit mesothelioma patients. The inverse correlation between VEGF serum levels and mesothelioma patient survival (49) suggested that VEGF signaling contributes to mesothelioma. However, a phase II clinical trial of the humanized anti-VEGF monoclonal antibody Bevacizumab plus erlotinib (Figure 2) in mesothelioma patients yielded no clinical benefits (50). PI3K, AKT and the downstream mTOR are often found to be activated in mesothelioma, and inhibition of mTOR using rapamycin enhances the apoptosis of mesothelioma cells in vitro (22), which suggest that mTOR may serve as a target for mesothelioma therapies (Figure 2). NF-κB signaling pathway is critical for the pathogenesis of mesothelioma. Therapies aimed at inhibiting NF-κB activity may benefit a small subset (10%) of patients (51-53).

Molecular therapies that target aspects of tumor immunity may also exert a significant impact on the course of mesothelioma, as the altered microenvironment impacts the ability of the immune system to mount anti-tumor responses. Sterman and Albelda have led several clinical trials examining the effects of intrapleural delivery of type I interferon-encoded adenoviruses. These trials demonstrated that high local concentrations of IFN-α or IFN-β were well tolerated and induced strong cellular and humoral anti-tumor immune responses, leading to tumor cell death (54). Some mesothelioma patients experienced prolonged survivals.

In summary, molecular therapies have not impacted the average survival of mesothelioma patients, although in several of these trials 5-10% of patients have responded and have experienced prolonged survivals. Since the results of clinical trials normally look at “averages”, there is no significant benefit could be detected for any therapy when this benefit occurs in a small fraction of patients. Thus the challenge ahead of us is to identify the subset of patients that will respond to a given type of therapy.
Targeting asbestos induced inflammation to prevent or treat mesothelioma.

Chronic inflammation has been associated with an increased risk of developing numerous cancers. Accordingly, daily treatment with aspirin for 5 or more years reduced tumor burden in several common malignancies (55). Animal experiments support a beneficial role for anti-inflammatory therapies in mesothelioma (56); thus, we hypothesize that prolonged aspirin treatment may help reduce the incidence of mesothelioma and other asbestos-related malignancies among high-risk cohorts that have either a lengthy history of exposure and/or genetic predisposition.

Based on recent findings, it is tempting to speculate that HMGB1 and the Nalp3 inflammasome act as critical initiators of chronic inflammation in asbestos- and erionite-exposed individuals, with the secretion of IL-1β and TNF-α acting as the key downstream driving force. HMGB1, Nalp3, TNF-α and IL-1β can therefore all serve as potential targets for inhibitors of asbestos-induced inflammation leading to mesothelioma. Indeed, Hamada et al. showed that patients with idiopathic pulmonary fibrosis exhibited elevated levels of HMGB1 in the bronchoalveolar lavage fluid (BALF), and that treatment with an anti-HMGB1 antibody prevented bleomycin-induced lung fibrosis in mice (57). As several solid tumors in addition to mesothelioma display elevated levels of HMGB1, including melanoma, prostate, pancreatic, breast and gastrointestinal cancers (42), therapies that seek to block HMGB1 signaling would likely prove effective in other cancer types in addition to mesothelioma.

Treatment with an IL-1 receptor antagonist can protect mice from developing fibrosis upon exposure to bleomycin or silica (58); and in a murine models of bleomycin- or silica-induced pulmonary fibrosis, infusion with the human recombinant soluble TNF receptor rsTNFR-β was effective not only in preventing the development of pulmonary fibrosis but also in the treatment of established fibrosis (40). Similar results were observed using anti-TNF-α antibodies
Specific FDA-approved reagents that inhibit these molecules are available. Anakinra, an IL-1 receptor antagonist, is used in therapies for patients with autoimmune diseases and gout. Remicade (Infliximab), a chimeric human-mouse anti-TNF-α, and Enbrel (Etanercept), a soluble TNF receptor fusion protein, have both been used to treat patients with rheumatoid arthritis and other diseases such as plaque psoriasis and ankylosing spondylitis. Glyburide, the most widely used sulfonylurea drug for type 2 diabetes in the US, inhibits the Nalp3 inflammasome (60). Specific molecules that target the activity of HMGB1 are anti-HMGB1 and anti-RAGE antibodies, recombinant HMG Box A, and ethyl pyruvate, an inhibitor of HMGB1 secretion (42) (Figure 2).

Early detection of mesothelioma is associated with improved clinical outcomes (1). The finding that significantly higher serum levels of HMGB1 were detected in asbestos-exposed individuals compared to cohorts of smokers with histologically proven bronchial inflammation and dysplasia (36) suggests that HMGB1 may be a potential maker of exposure to carcinogenic mineral fibers. Moreover, soluble mesothelin-related peptides (SMRP) is a candidate marker for the early detection of mesothelioma (i.e., before the appearance of clinical symptoms). Soon, with the support of the early detection research network of the NCI, we will begin a prospective three-year clinical trial of 400 high-risk individuals from high-risk mesothelioma villages in Turkey to investigate the validity of SMRP and HMGB1 as early detection markers.

SUMMARY

Because the latency period from initial asbestos or erionite exposure to disease progression is often decades long (1), novel therapies that prevent or delay carcinogenesis in exposed individuals would make a substantial impact in decreasing mesothelioma mortality. In light of our recent increased understanding that asbestos carcinogenesis is linked to chronic inflammation we can design multiple strategies to target inflammation in asbestos- and erionite-exposed
individuals. Clinical and translational research that focuses on these strategies has the potential to reduce the impact of the carcinogenic effect of asbestos and erionite exposure. Moreover, genetic testing for BAP1 mutations should help us identify among exposed cohorts of those genetically susceptible individuals that have the highest risk of developing mesothelioma (10). These individuals could be targeted for early detection, for example, by monitoring HMGB1, SMRP or other biomarkers. Strategies that seek to prevent carcinogenesis in asbestos/erionite-exposed, high-risk individuals would have the most wide-reaching impact on the incidence of this deadly cancer.
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FIGURE LEGENDS

Fig. 1. Working hypothesis of mesothelioma carcinogenesis. Asbestos causes necrotic HM death, leading to the release of HMGB1 into the extracellular space. HMGB1 elicits macrophage accumulation and triggers the inflammatory response and TNF-α secretion. TNF-α activates the NF-κB pathway, which increases survival of asbestos-damaged HM. This allows key genetic alterations to accumulate within HM that sustain asbestos-induced DNA damage that lead to the initiation of mesothelioma.

Fig. 2. Potential targets and strategies for mesothelioma therapy that have been proposed based on recent studies.
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