PROSPECTS
Journal of Cellular Biochemistry 115:1–7 (2014)

Journal of Cellular Biochemistry

Malignant Mesothelioma: Development to Therapy

Joyce K. Thompson, Catherine M. Westbom, and Arti Shukla*

Pathology Department, University of Vermont, College of Medicine, Burlington, Vermont

ABSTRACT

Malignant mesothelioma (MM) is an aggressive cancer of the mesothelium caused by asbestos. Asbestos use has been reduced but not completely stopped. In addition, natural or man-made disasters will continue to dislodge asbestos from old buildings into the atmosphere and as long as respirable asbestos is available, MM will continue to be a threat. Due to the long latency period of MM development, it would still take decades to eradicate this disease if asbestos was completely removed from our lives today. Therefore, there is a need for researchers and clinicians to work together to understand this deadly disease and find a solution for early diagnosis and treatment. This article focuses on developmental mechanisms as well as current therapies available for MM. J. Cell. Biochem. 115: 1–7, 2014.

KEY WORDS: MALIGNANT MESOTHELIOMA; ASBESTOS; MESOTHELIUM; INFLAMMATION

Malignant mesothelioma (MM) is a cancer of the mesothelial lining of the body caused by asbestos. In the US, asbestos was used for various purposes including building materials until the 1970s, suggesting that old buildings still have asbestos. Asbestos fibers are friable when damaged, leading to smaller fibers which can become airborne when disturbed. This is of particular interest because asbestos is only harmful when airborne fibers are inhaled. Natural disasters like tornados and man-made disasters such as the terrorist attacks on September 11, 2001 can rip apart old buildings resulting in airborne asbestos. There are various types of asbestos fibers, all of which are proven carcinogens in human beings. Respirable asbestos fibers, once inhaled, can cause serious damage to the lung, resulting in lung fibrosis (asbestosis) or MM. Asbestos fibers have also been shown to reach the pleural cavity and directly injure mesothelial cells. The injury to mesothelial cells may result in activation of inflammatory pathways including inflammasomes and the release of inflammatory cytokines. By autocrine and/or paracrine pathways, these cytokines and other signaling molecules can cause mesothelial to fibroblastic transformation (MFT) and finally transformed mesothelial cells can give rise to MM (Fig. 1).

In the present prospect, we discuss in detail how asbestos fibers can reach the mesothelial cells and cause their transformation, resulting in MM development. In the latter part of this review, we touch upon proposed and current biomarkers and therapies for the diagnosis and treatment of MM.

ASBESTOS

Asbestos is the commercial name for a group of six naturally occurring silicate mineral fibers (actinolite, anthophyllite, chrysotile, cummingtonite-grunerite (amosite), crocidolite, and tremolite) that each demonstrate longitudinal parting into very thin fibrils. Prior to the 1970s, asbestos was a globally popular construction material due to its resistance to heat, fire, electricity, and chemical damage. Unfortunately, with the increase of asbestos use came an increase in asbestos related diseases such as asbestosis and MM. Crocidolite (also called blue asbestos) is regarded as the most carcinogenic of asbestos types in part because of its durable nature and rod-like shape [Guthrie and Mossman, 1993]. Once lodged in the lungs, asbestos fibers move to locations such as the pleura by unconfirmed mechanisms and cannot be naturally expelled from the body. It is theorized that the asbestos could be redistributed to the body cavities in two ways: by fibers physically moving to the outside of the lung tissue and being picked up by the pleura and/or through fibers being picked up by the lymphatic and/or blood systems in an attempt to clear the foreign material [Cugell and Kamp, 2004].

The molecular pathogenesis of MM is still an elusive multifactorial event involving multiple mechanisms. Many aspects of asbestos fibers; such as the length and shape of the fiber, durability, chemical composition and biopersistance promote disease, and carcinogenesis [Shukla et al., 2003]. Both local and more distant responses are involved in the disease progression of mesothelioma. Local to

Grant sponsor: NIH; Grant number: 1RO1 ES021110; Grant sponsor: Mesothelioma Applied Research Foundation (MARF); Grant sponsor: Pathology Department (COM UVM).

*Correspondence to: Arti Shukla, PhD, Associate Professor, Pathology Department, University of Vermont, College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405. E-mail: arti.shukla@uvm.edu

Manuscript Received: 1 August 2013; Manuscript Accepted: 5 August 2013

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 19 August 2013

DOI 10.1002/jcb.24642 • © 2013 Wiley Periodicals, Inc.

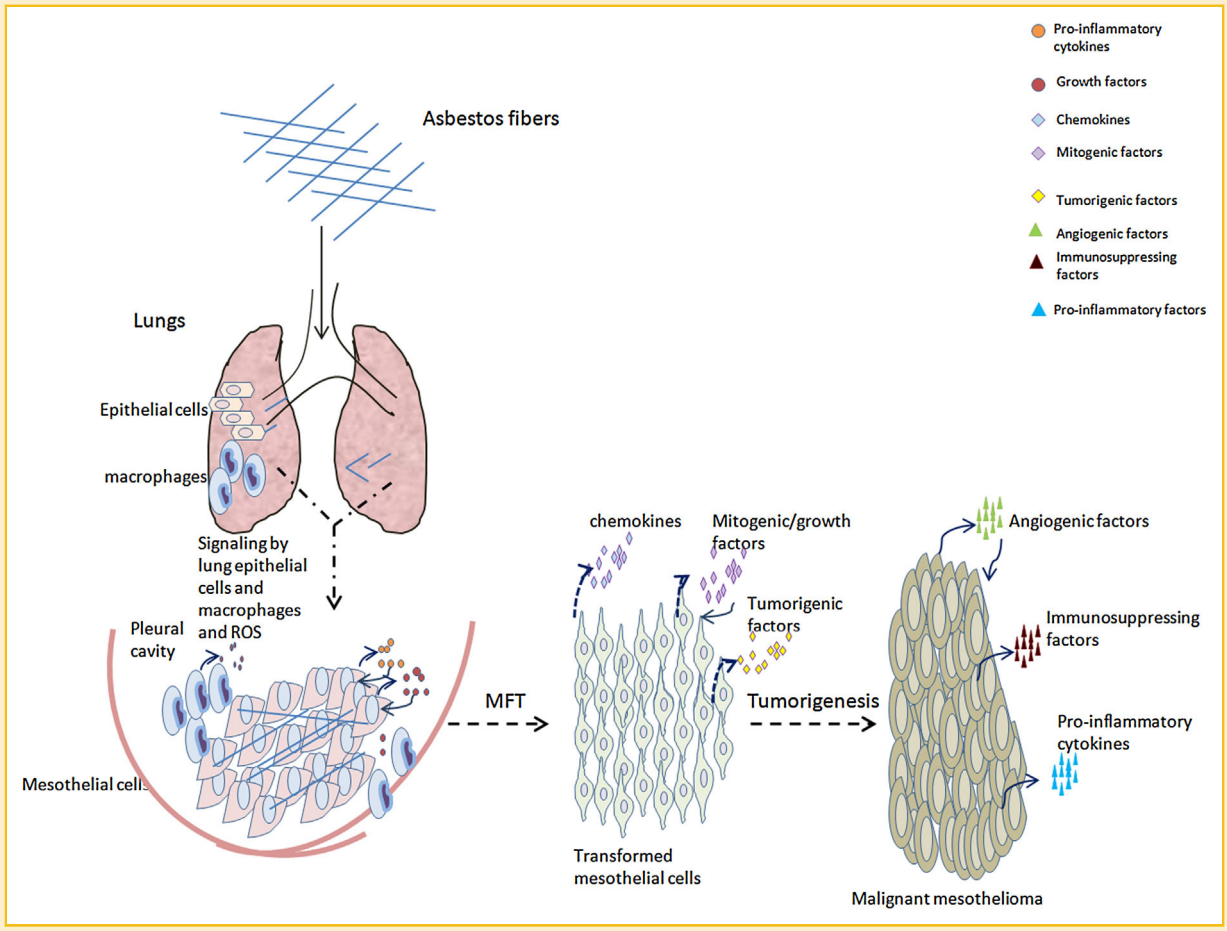

Fig. 1. A schema showing development of malignant mesothelioma (MM) in response to asbestos exposure. Inhaled asbestos fibers first encounter tracheal and lung epithelial cells as well as alveolar macrophages which attempt to clear the fibers. ROS, inflammatory mediators as well as signaling pathways that are activated in response to the fibers may be involved in sending signals to the pleura to initiate transformation of pleural mesothelial cells. Asbestos fibers may also be carried into the pleural cavity by direct transfer or by the lymphatic system where they may come into direct contact with the mesothelial cells. Chronic inflammation and growth factor signaling in combination with factors thus released by the mesothelial cells and pleural macrophages may lead to the initiation of transformation (mesothelial to fibroblast transition (MFT)) events that eventually lead to tumorigenesis and development of malignant mesothelioma.

asbestos deposits, phagocytic cells attempt to phagocytose the asbestos fibers, which leads to frustrated phagocytosis due to the high aspect ratio (length to diameter ratio) of the fibers. In conjunction with this process of frustrated phagocytosis, reactive oxygen species (ROS) are generated leading to increased growth factor signaling, proliferation, and signal transduction pathway stimulation in response to local tissue damage [Mossman et al., 2013]. One can imagine how this multifaceted environment of tissue damage, growth, and survival creates an environment that promotes cell transformation.

MESOTHELIAL CELLS

Asbestos exposure and injury to mesothelial cells results in MM development. The mesothelial cell, a specialized type of cell that makes up the protective layer of tissue called the mesothelium, is mostly flat and thin [Mossman et al., 2013]. The primary function of the mesothelial cell is to form a protective monolayer over the internal organs, thereby providing a non-adhesive surface supporting organ movement aided by production of a lubricating fluid. Mesothelial cells are functionally diverse cells with multiple functions and properties. The importance of these cells in reference to normal organ function is supported by the fact that injury to the mesothelium can cause organs to adhere to the serosal wall consequently leading to the restriction of movement within the affected cavity [Mutsaers, 2004]. Examples of this would be the restriction of lung movement, breathing capabilities and occasionally, cardiac function caused by injury to the pleural mesothelium, such as is seen in late stage MM.

In addition to the barrier functions of the mesothelial cell, these cells are also involved in the transportation of fluid and cells across serosal cavities, antigen presentation, immune surveillance, cytokine and chemokine production, inflammation, wound healing, coagulation, fibrinolysis, and tumor cell adhesion [Mutsaers, 2004; Yung and Chan, 2007]. Once believed to only provide a barrier to protect the inner organs; the mesothelial cell has proved to be a cell with an astounding number of capabilities that could provide information on more

advanced treatment for mesothelial diseases such as MM. Mesothelial cells are capable of initiating cell proliferation, wound repair, differentiation, migration, and inflammation via the release of molecular mediators. Some of these mediators include cytokines, chemokines, growth factors, and matrix components [Mutsaers, 2004].

The mesothelial cells secrete a lubricant consisting of glycosaminoglycans and phosphatidylcholine to facilitate smooth organ movement and further protect against invading organisms and abrasive damage [Mutsaers, 2004]. The lubrication secreted by mesothelial cells has also been linked to cancer prevention. To this effect, hyaluronan, a glycosaminoglycan secreted by mesothelial cells, has been shown to prevent ovarian tumor cell attachment to peritoneal mesothelial cells [Jones et al., 1995].

EFFECT OF ASBESTOS EXPOSURE ON MESOTHELIAL CELLS

The result of asbestos exposure of the lung in inhalation studies has shown that asbestos induces an acute inflammatory response locally around fibers. This acute inflammatory response includes the release of pro-inflammatory cytokines, macrophage and neutrophil recruitment, airway epithelial cell proliferation, and later mesothelial cell proliferation [Mossman et al., 2011]. As discussed previously, asbestos is capable of moving from the lung to the pleura to affect the mesothelial cells that lay there. It has been shown that long asbestos fibers are more durable than shorter fibers and can cause chronic inflammation and repeated injury to pleural mesothelial cells [Moalli et al., 1987]. It is believed that this chronic inflammation from asbestos exposure would predispose local mesothelial cells to carcinogenesis. Recent supporting data from Xu et al. [2012] demonstrated the presence of crocidolite asbestos fibers, administered by intrapulmonary spraying, in macrophages of the pleural cavity lavage fluid. In this study crocidolite asbestos exposure induced hyperplastic proliferative lesions of the visceral mesothelium as well as abundant inflammatory cell infiltration. This suggests that inflammatory reactions in the lung and pleural cavity were responsible for the proliferative lesions seen in the pleural mesothelium.

Although basic mechanisms of how asbestos exposure leads to mesothelial cell proliferation/transformation and development of MM is not clear, several in vitro studies by our group and others have shed light on the possible mechanisms involved in the process. Using microarray, gene or protein pathway arrays, we and others have reported a special signature of gene expression in mesothelial cells exposed to asbestos [Nymark et al., 2007]. Wang et al. [2011] found about 21 proteins/phosphoproteins that were dysregulated, mostly associated with EGFR/ERK and PI3K/AKT pathways. Our group on the other hand, used microarray technology and human mesothelial cells exposed to either crocidolite asbestos or Libby six-mix. Libby six-mix caused alteration in several genes, the most prominent being superoxide dismutase (SOD). In addition to up-regulating SOD, Libby six-mix also caused increased production of oxidants and a transient decrease in reduced glutathione (GSH) [Hillegass et al., 2010], suggesting that Libby six-mix affects human mesothelial cells by altering their oxidative environment. Yet another study from our group demonstrated that crocidolite asbestos exposure can cause an altered profile of genes in human peritoneal mesothelial cells. With crocidolite asbestos activating transcription factor (ATF3) 3, a cyclic AMP response element binding protein (CREB) family member was the highest expressing gene [Shukla et al., 2009a]. Furthermore, down-regulation of ATF3 by siRNA (small interfering RNA) caused a significant decrease in secreted levels of pro-inflammatory cytokines (IL-1β, IL-13, G-CSF) and growth factors (VEGF and PDGF-BB). Findings from this study again emphasize the role of asbestos-induced inflammation in mesothelial cell injury and subsequent carcinogenesis. Another member of this family, CREB1 was also activated by asbestos in human mesothelial cells, and human MM cells and tumors showed constitutive activation of CREB [Shukla et al., 2009b]. Detailed work using siRNA to silence CREB1 revealed its role as a pro-survival protein acting via Bcl2 up-regulation. Follow-up in vivo studies from our group confirmed that CREB1 regulates MM tumor growth predominantly by regulating inflammation (unpublished data).

Another signaling pathway that is studied extensively by our group is extracellular signal regulated kinases (ERKs). ERKs are modulated by asbestos in mesothelial cells and may be responsible for causing MMs. Crocidolite asbestos exposure of telomerase immortalized human mesothelial cells (LP9) and SV40 transformed human mesothelial cells (MET5A) caused activation of ERK1/2 via epidermal growth factor receptor (EGFR). Furthermore, silencing of ERK1, 2 or AKT by siRNA demonstrated that asbestos-induced cell death is ERK1/2 dependent in both cell lines [Shukla et al., 2011]. It is also noted in this study that MET5A cells were more resistant to asbestos-induced toxicity than LP9 cells. The increased resistance to asbestos in MET5A cells can be attributed to elevated levels of calretinin, as elevated calretinin levels strongly correlate to enhanced asbestos resistance [Henzi et al., 2009]. Recently, we have shown another ERK, ERK5 to be activated by asbestos in human mesothelial cells and may play a role in the development of MM [Shukla et al., 2013].

While altering molecular expression and activation in mesothelial cells, asbestos also produces a significant amount of cell death. Mesothelial cell death by asbestos has been shown to involve a regulated form of necrosis that causes the release of high-mobility group box 1 (HMGB1), (an inflammatory protein usually located in the nucleus) into the extracellular space. Mesothelial cells as well as macrophages secrete tumor necrosis factor alpha (TNF-α) in response to HMGB1-induced inflammation, activating NF-κB [Yang et al., 2010]. NF-κB is part of a survival pathway that allows some of the mesothelial cells exposed to asbestos to survive and potentially transform into MM cells [Yang et al., 2006]. Our unpublished data further show that asbestos-induced HMGB1 secretion from human mesothelial cells is NLRP3 (NOD like receptor protein 3) inflammasome dependent. This study is also first to demonstrate that asbestos can prime and activate NLRP3 inflammasomes in mesothelial cells, resulting in IL-1β and IL-18 release which may be responsible for transforming mesothelial cells in an autocrine manner. With this short review of current pathways involved in mesothelial cell exposure to asbestos it becomes clear that the mesothelial cell is diverse and complicated in its capacity to react to harm. Understanding the mechanisms of transformation of mesothelial cells by asbestos may provide enlightenment of the possible pathways

responsible for development of MM and should be considered as potential targets.

MESOTHELIOMA

MM is an asbestos-associated malignancy of mesothelial cells that is typically diagnosed at a late stage with a poor prognosis (median survival: 9–13 months) [Robinson et al., 2005]. Work related asbestos exposure is the major cause of MM [Mossman et al., 1990]. Most commonly, MM arises in the pleural area of the mesothelium surrounding the lungs, but it can also infrequently develop in the mesothelium of the peritoneum, pericardium and tunica vaginalis. After initial exposure to asbestos, MM development can take 20–60 years to manifest. The evidence that work-related is a direct and major cause of MM is overwhelming starting with Wagner et al. [1960] and continuing today with countless studies containing supporting evidence.

It remains unclear why asbestos exposure leads to MM in certain individuals, while others do not develop the devastating disease. Although 70–80% of MMs are caused by work-related exposure to asbestos, only about 5% of those exposed to asbestos develop MM [Gazdar and Carbone, 2003]. These observations are indicative of an individual characteristic pre-disposing certain populations to become susceptible to MM development when exposed to asbestos. Genetic factors can play a role in the development and progression of MM as depicted in a study on the population in parts of Cappadocia, Turkey exposed to erionite (a fiber found in the volcanic rock of this area that shares many similar properties with crocidolite asbestos). Erionite related MM reached epidemic proportions, but only in families that passed on a predisposition to MM by erionite exposure in an autosomal-dominant manner [Carbone et al., 2007]. More recent studies have shown that a significant number of sporadic MM cases exhibit somatic BRCA 1 associated protein (BAP1) mutations while cases of familial MM were found to have germline BAP1 mutations [Bott et al., 2011; Testa et al., 2011]. In the case of the individuals with familial MM, BAP1 germline mutations appeared to predispose them to develop MM after exposure to very low levels of asbestos found in construction materials in their homes [Testa et al., 2011]. Together, these studies indicate that genetic factors such as BAP1 mutations contribute to a predisposition to develop MM after asbestos exposure.

The annual incidence rate of MM in the United States is approximately 3,300 cases per year [Teta et al., 2008]. In the UK, the continually rising peak is projected to begin its descent in 2015. Japan, on the other hand, is not projected to peak until the year 2027; according to a recent study looking at occupational exposure to asbestos [Myojin et al., 2012]. Numbers of MM diagnoses in Japan began to climb in the year 2000 due to late asbestos use restriction in addition to a lack of proper ventilation and protective equipment to protect from asbestos exposure. Like the US, Japan has also not banned the use of asbestos entirely, only restricted its use, thus not eliminating future risk. Large areas of the world remain that have not restricted the use of asbestos and fail to provide appropriate protective equipment.

Asbestos use has by and large caused this worldwide problem of asbestos-related diseases that continues today. The WHO estimates that 125 million people worldwide are currently exposed to asbestos in the workplace [World Health Organization, 2012]. Even in locations where asbestos is banned, people continue to be at risk for asbestos exposure. Older buildings that still contain asbestos are a potential hazard to local populations and rescue workers. History shows us that, in cases of natural disasters or explosions, asbestos from buildings can become airborne, contaminating the air with asbestos fibers that can be inhaled by individuals in the surrounding area. One such example is the fall of the twin towers in the United States. Rescue workers and the local population were exposed to asbestos from the air as well as fibers that had settled to the ground from damaged construction material. The asbestos-exposed population is now at risk for developing MM and other asbestos-related diseases. Factors that predispose specific individuals but not others to MM require further study to be elucidated. Such studies may help provide new chemotherapeutic targets to improve the treatment of MM and increase the median survival after diagnosis.

CURRENT MM TREATMENT OPTIONS

MM is refractory to most treatment options currently available and its diagnosis also poses a great challenge to pathologists [Allen, 2013]. Current treatment options include surgical resection of the tumor (e.g., radical pleurectomy [RP] or extrapleural pneumonectomy [EPP]) if operable, chemotherapy and radiotherapy as single agents or as part of a multimodal approach [Mossman et al., 2013]. These options do not improve survival substantially. Additionally, small molecule inhibitors of growth factor signaling pathways such as EGFR and mitogen activated protein kinase kinase (MEK) among others, are also being investigated for use as chemotherapy for MM. Targeted delivery of chemotherapeutics as well as immunotherapeutics are also in development for the management of MM [Mossman et al., 2013].

MM is often diagnosed in the late stages when the disease is too advanced for current therapies. This unfortunately leaves palliative care as the only option. While EPP facilitates complete resection of all diseased tissue from the pleural cavity, the number of MM patients that qualify to undergo this radical surgery is limited by their cardiopulmonary function test outcomes [Bolukbas et al., 2013]. In EPP, resection of the affected lung en bloc with the pericardium and parietal pleural tissue is conducted to achieve complete removal of visible tumor mass [Tilleman et al., 2009]. However, because it is difficult to assess whether all malignant cells have been removed and MM has a history of local reoccurrence even after surgery, EPP and RP are now combined with chemotherapy or radiotherapy to improve the survival rate and extend time to recurrence [Tilleman et al., 2009; Bolukbas et al., 2013]. Chemotherapeutics that are approved for treatment of MM include permetrexed and cisplatin [Hazarika et al., 2004; Vogelzang, 2005]. On the other hand, protease inhibitors like bortezomib and monoclonal antibodies against vascular endothelial growth factor (VEGF) are in phase II trials as part of combination therapies with cisplatin and permetrexed [Dowell et al., 2012; O'Brien et al., 2013]. Unfortunately, VEGF inhibitors have failed to improve survival [Dowell et al., 2012] while therapies including bortezomib may have some promise [O'Brien et al., 2013]. The inability of the above mentioned treatment options to improve survival after

diagnosis with MM beyond 12 months combined with the lack of biomarkers that enable the early detection of MM necessitates the urgent need for the discovery of more effective treatment modalities and biomarkers.

Preclinical studies and trials are being conducted to develop better management options for MM. These include the study of antigens over-expressed in MM and other cancers, like mesothelin and podoplanin. Over-expression of podoplanin and mesothelin has been shown to be important for adhesion and viability of MM cell lines [Hassan et al., 2004; Abe et al., 2013]. Mesothelin (MSLN) is a differentiation antigen that is expressed on the surface of normal mesothelial cells and over-expressed in MM, pancreatic and ovarian cancer. MSLN has been exploited as a means of targeting MM and other MSLN over-expressing cancers through the conjugation of the MSLN antibody to an immunotoxin [Tang et al., 2013; Weldon et al., 2013], as well as for targeted drug delivery and immunotherapy. Recent work from our group has demonstrated a targeted drug delivery method for the delivery of doxorubicin (Dox) using acid prepared mesoporous silica particles (APMS) loaded with Dox and functionalized with mesothelin antibodies that target to tumor cells in in vivo studies thereby reducing the toxicity of doxorubicin [Macura et al., 2012]. Mesothelin itself has a pro-proliferative effect on MMs and knocking it down with siMSLN decrease the viability of MM cell lines [Wang et al., 2012].

An additional antigen being investigated as a target for the development of MM immunotherapy is podoplanin. Podoplanin was first discovered in podocytes and has been shown to be over-expressed in some MM cell lines with increased invasive phenotypes [Yamaki et al., 2013]. By treating MM cell lines with high expression of podoplanin with the human–rat antibody chimera, NZ-8, Abe et al. [2013] were able to demonstrate increased antibody dependent cellular cytotoxicity (ADCC) as well as increased complement dependent cytotoxicity in vitro specific to cells over-expressing podoplanin only [Abe et al., 2013]. The ADCC observed in the MM cell lines was also shown to be mediated by human mononuclear cells as opposed to rat NK cells in the case of the rat antibody NZ-1 in vivo using subcutaneous SCID mice models of MM [Abe et al., 2013].

While antibody mediated therapies are being developed and may prove effective with low side effects, such therapies will only be effective for MM patients whose tumors over-express the requisite antigens. As such, other targets that are more common to the majority of MM tumors are needed. Targeting signaling pathways essential to the survival/proliferation of MM tumors would also help improve treatment outcomes, especially when used as part of a multi-treatment modality [Heintz et al., 2010]. Small molecule inhibitors against EGFR, PI3K, or MEK have been shown to reduce tumor size in preclinical studies when used together [Kryeziu et al., 2013]. Combination treatment therapies will help combat the refractory nature of MM tumors and delay the development of resistance since multiple targets are being utilized [Miyoshi et al., 2012]. Our recent work has demonstrated the significant role of ERK5 in MM tumorigenesis and projected it as a potential therapeutic target [Shukla et al., 2013]. Furthermore, knockdown and inhibition of ERK 1 and 2 has also been demonstrated to reduce tumor growth rates in mouse tumor xenograft studies while increasing the sensitivity of different MM cell lines to Dox [Shukla et al., 2010]. The transcription factor, CREB1, has also been found to be constitutively activated in human MM cells and tumors where it conferred protection from apoptotic cell death as evidenced by an increase in Dox-induced cell death after silencing of CREB1 with siCREB [Shukla et al., 2009b]. Therefore, identifying and employing inhibitors of CREB as part of a multimodal therapeutic approach to the treatment of MM could help improve survival outcomes. The receptor tyrosine kinase Eph receptor B4 (EphB4), important for a variety of developmental processes and over-expressed in MM [Xia et al., 2005], has recently been shown to be a potential therapeutic target for treating MM [Liu et al., 2013]. In that study, Liu et al. [2013] showed that the EphB4 inhibitor, sEphB4-HAS, used alone or in combination with the VEGF inhibitor, Bevacizumab, was effective at reducing tumor volume, angiogenesis and proliferation of cells in subcutaneous mouse xenograft models of human sarcomatoid MM (H2373 MM cells).

Another way of improving the early diagnosis of MM would be the discovery of a panel of biomarkers. Validated biomarkers would enable the early detection of MM in patients with the hope that early detection would facilitate complete resection of small tumors and more effective treatment of responsive early stage MM tumors. The few biomarkers available are still being validated and are yet to be proven as concrete prognostic and detection tools for MM. Mesothelin, the most studied MM biomarker to date, is found circulating in the serum of MM patients and recent studies indicate that the soluble mesothelin related peptide (SMRP) detected in pleural effusions is effective at distinguishing MM from other pleural effusion causes and holds promise as a marker for monitoring disease progress/treatment efficacy [Filiberti et al., 2013; Pantazopoulos et al., 2013]. One other gene product of the MSLN gene, megakaryocyte potentiating factor (also known as N-ERC) has been shown to be equally as effective as MSLN/SMRP at distinguishing MM pleural effusions from non-malignant and other pleural effusions [Hollevoet et al., 2010]. Although MSLN and the other mesothelin related proteins are highly specific for MM, they are not over-expressed by the poorly differentiated sarcomatoid MM subtype [Hollevoet et al., 2010]. This limits their use as biomarkers for all MM subtypes. To this end, other biomarkers that are widely expressed in all or most MM subtypes are under investigation.

Osteopontin (OPN), a widely expressed cancer antigen, has been studied as a potential diagnostic tool for the early detection of MM. OPN is a cell surface sialoprotein that is involved in bone matrix formation and tumor invasion among other functions [reviewed in Wang and Denhardt, 2008]. OPN is over-expressed in a number of cancers including MM, lung and breast cancer [Felten et al., 2013; Hartung and Weber, 2013]. Due to the presence of a thrombin cleavage site on OPN, however, variable results have been obtained when testing serum from MM patients [reviewed in Pass and Carbone, 2009]. Moreover, HMGB1, an inflammatory protein that also plays a role in transcription, proliferation and DNA repair is found to be elevated in the sera of MM patients and has been shown to be of great diagnostic value for both malignant pleural and peritoneal mesotheliomas [Jube et al., 2012]. As HMGB1 is considered to be a general marker of tissue injury and inflammation, its specificity for MM needs to be validated. Recently, Pass et al. showed that Fibulin-3, a basement membrane protein of mesenchymal origin [Giltay et al., 1999], is a specific biomarker of MM in blood and pleural effusion

samples from MM patients and matched controls. A blinded validation study also confirmed the specificity of this biomarker [Pass et al., 2012]. However, since the discovery of Fibulin-3 as a biomarker for the detection MM is fairly recent, more rigorous validation studies will have to be carried out to confirm its use as a detection and prognostic tool for MM.

CONCLUSION

Mesothelioma is a devastating disease that has been causally linked to asbestos exposure. Although effort by several groups is in progress to understand this disease better so that more efficient therapies can be designed, there is still work to be done. The difficulty in diagnosing MM early and determining who is at risk of developing MM after asbestos exposure has hampered attempts at improving survival beyond 12 months after diagnoses. With the help of coordinated teams of researchers and physicians further progress in understanding this deadly disease and designing effective therapies can be achieved.

REFERENCES

Abe S, Morita Y, Kaneko MK, Hanibuchi M, Tsujimoto Y, Goto H, Kakiuchi S, Aono Y, Huang J, Sato S, Kishuku M, Taniguchi Y, Azuma M, Kawazoe K, Sekido Y, Yano S, Akiyama S, Sone S, Minakuchi K, Kato Y, Nishioka Y. 2013. A novel targeting therapy of malignant mesothelioma using anti-podoplanin antibody. J Immunol 190(12):6239–6249.

Allen TC. 2013. Accurate diagnosis of mesothelioma: More important than ever. Arch Pathol Lab Med 137(5):601–602.

Bolukbas S, Eberlein M, Fisseler-Eckhoff A, Schirren J. 2013. Radical pleurectomy and chemoradiation for malignant pleural mesothelioma: The outcome of incomplete resections. Lung Cancer 81(2):241-246.

Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, Creaney J, Lake RA, Zakowski MF, Reva B, Sander C, Delsite R, Powell S, Zhou Q, Shen R, Olshen A, Rusch V, Ladanyi M. 2011. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet 43(7):668–672.

Carbone M, Emri S, Dogan AU, Steele I, Tuncer M, Pass HI, Baris YI. 2007. A mesothelioma epidemic in Cappadocia: Scientific developments and unexpected social outcomes. Nat Rev Cancer 7(2):147–154.

Cugell DW, Kamp DW. 2004. Asbestos and the pleura: A review. Chest 125 (3):1103–1117.

Dowell JE, Dunphy FR, Taub RN, Gerber DE, Ngov L, Yan J, Xie Y, Kindler HL. 2012. A multicenter phase II study of cisplatin, pemetrexed, and bevacizumab in patients with advanced malignant mesothelioma. Lung Cancer 77(3): 567–571.

Felten MK, Khatab K, Knoll L, Schettgen T, Muller-Berndorff H, Kraus T. 2013. Changes of mesothelin and osteopontin levels over time in formerly asbestos-exposed power industry workers. Int Arch Occup Environ Health.

Filiberti R, Parodi S, Libener R, Ivaldi GP, Canessa PA, Ugolini D, Bobbio B, Marroni P. 2013. Diagnostic value of mesothelin in pleural fluids: Comparison with CYFRA 21-1. and CEA. Med Oncol 30(2):543.

Gazdar AF, Carbone M. 2003. Molecular pathogenesis of malignant mesothelioma and its relationship to simian virus 40. Clin Lung Cancer 5 (3):177–181.

Giltay R, Timpl R, Kostka G. 1999. Sequence, recombinant expression and tissue localization of two novel extracellular matrix proteins, fibulin-3 and fibulin-4. Matrix Biol 18(5):469–480.

Guthrie GD, Jr, Mossman BT editor. 1993. Health effects of mineral dusts, reviews in mineralogy, Vol. 28 (Series editor: Paul H. Ribbe), Washington, D.C.: Mineralogical Society of America. pp. 1–584.

Hartung F, Weber GF. 2013. RNA blood levels of osteopontin splice variants are cancer markers. SpringerPlus 2(1):110.

Hassan R, Bera T, Pastan I. 2004. Mesothelin: A new target for immunotherapy. Clin Cancer Res 10(12Pt 1):3937–3942.

Hazarika M, White RM, Johnson JR, Pazdur R. 2004. FDA drug approval summaries: Pemetrexed (Alimta). Oncologist 9(5):482–488.

Heintz NH, Janssen-Heininger YM, Mossman BT. 2010. Asbestos, lung cancers, and mesotheliomas: From molecular approaches to targeting tumor survival pathways. Am J Respir Cell Mol Biol 42(2):133–139.

Henzi T, Blum WV, Pfefferli M, Kawecki TJ, Salicio V, Schwaller B. 2009. SV40-induced expression of calretinin protects mesothelial cells from asbestos cytotoxicity and may be a key factor contributing to mesothelioma pathogenesis. Am J Pathol 174(6):2324–2336.

Hillegass JM, Shukla A, MacPherson MB, Lathrop SA, Alexeeva V, Perkins TN, van der Vliet A, Vacek PM, Gunter ME, Mossman BT. 2010. Mechanisms of oxidative stress and alterations in gene expression by Libby six-mix in human mesothelial cells. Part Fibre Toxicol 7:26.

Hollevoet K, Nackaerts K, Thimpont J, Germonpre P, Bosquee L, De Vuyst P, Legrand C, Kellen E, Kishi Y, Delanghe JR, van Meerbeeck JP. 2010. Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. Am J Respir Crit Care Med 181(6):620–625.

Jones LM, Gardner MJ, Catterall JB, Turner GA. 1995. Hyaluronic acid secreted by mesothelial cells: A natural barrier to ovarian cancer cell adhesion. Clin Exp Metastasis 13(5):373–380.

Jube S, Rivera ZS, Bianchi ME, Powers A, Wang E, Pagano I, Pass HI, Gaudino G, Carbone M, Yang H. 2012. Cancer cell secretion of the DAMP protein HMGB1 supports progression in malignant mesothelioma. Cancer Res 72(13): 3290–3301.

Kryeziu K, Jungwirth U, Hoda MA, Ferk F, Knasmuller S, Karnthaler-Benbakka C, Kowol CR, Berger W, Heffeter P. 2013. Synergistic anticancer activity of arsenic trioxide with erlotinib is based on inhibition of EGFR-mediated DNA double-strand break repair. Mol Cancer Ther 12(6):1073–1084.

Liu R, Ferguson BD, Zhou Y, Naga K, Salgia R, Gill PS, Krasnoperov V. 2013. EphB4 as a therapeutic target in mesothelioma. BMC Cancer 13:269.

Macura SL, Hillegass JM, Steinbacher JL, MacPherson MB, Shukla A, Beuschel SL, Perkins TN, Butnor KJ, Lathrop MJ, Sayan M, Hekmatyar K, Taatjes DJ, Kauppinen RA, Landry CC, Mossman BT. 2012. A multifunctional mesothelin antibody-tagged microparticle targets human mesotheliomas. J Histochem Cytochem 60(9):658–674.

Miyoshi S, Hamada H, Hamaguchi N, Kato A, Katayama H, Irifune K, Ito R, Miyazaki T, Okura T, Higaki J. 2012. Antitumor activity of MEK and PI3K inhibitors against malignant pleural mesothelioma cells in vitro and in vivo. Int J Oncol 41(2):449–456.

Moalli PA, MacDonald JL, Goodglick LA, Kane AB. 1987. Acute injury and regeneration of the mesothelium in response to asbestos fibers. Am J Pathol 128(3):426–445.

Mossman BT, Bignon J, Corn M, Seaton A, Gee JB. 1990. Asbestos: Scientific developments and implications for public policy. Science 247(4940):294–301.

Mossman BT, Lippmann M, Hesterberg TW, Kelsey KT, Barchowsky A, Bonner JC. 2011. Pulmonary endpoints (lung carcinomas and asbestosis) following inhalation exposure to asbestos. J Toxicol Environ Health B Crit Rev 14(1–4): 76–121.

Mossman BT, Shukla A, Heintz NH, Verschraegen CF, Thomas A, Hassan R. 2013. New insights into understanding the mechanisms, pathogenesis, and management of malignant mesotheliomas. Am J Pathol 182(4):1065–1077.

Mutsaers SE. 2004. The mesothelial cell. Int J Biochem Cell Biol 36(1):9–16.

Myojin T, Azuma K, Okumura J, Uchiyama I. 2012. Future trends of mesothelioma mortality in Japan based on a risk function. Ind Health 50 (3):197–204.

Nymark P, Lindholm PM, Korpela MV, Lahti L, Ruosaari S, Kaski S, Hollmen J, Anttila S, Kinnula VL, Knuutila S. 2007. Gene expression profiles in asbestos-exposed epithelial and mesothelial lung cell lines. BMC Genomics 8. doi:10.1186/1471-2164-8-62

O'Brien ME, Gaafar RM, Popat S, Grossi F, Price A, Talbot DC, Cufer T, Ottensmeier C, Danson S, Pallis A, Hasan B, Van Meerbeeck JP, Baas P. 2013. Phase II study of first-line bortezomib and cisplatin in malignant pleural mesothelioma and prospective validation of progression free survival rate as a primary end-point for mesothelioma clinical trials (European Organisation for Research and Treatment of Cancer 08052). Eur J Cancer 49(13):2815–2822.

Pantazopoulos I, Boura P, Xanthos T, Syrigos K. 2013. Effectiveness of mesothelin family proteins and osteopontin for malignant mesothelioma. Eur Respir J 41(3):706–715.

Pass HI, Carbone M. 2009. Current status of screening for malignant pleural mesothelioma. Semin Thorac Cardiovasc Surg 21(2):97–104.

Pass HI, Levin SM, Harbut MR, Melamed J, Chiriboga L, Donington J, Huflejt M, Carbone M, Chia D, Goodglick L, Goodman GE, Thornquist MD, Liu G, de Perrot M, Tsao MS, Goparaju C. 2012. Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. N Engl J Med 367(15):1417–1427.

Robinson BW, Musk AW, Lake RA. 2005. Malignant mesothelioma. Lancet 366 (9483):397–408.

Shukla A, Gulumian M, Hei TK, Kamp D, Rahman Q, Mossman BT. 2003. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. Free Radic Biol Med 34(9):1117–1129.

Shukla A, MacPherson MB, Hillegass J, Ramos-Nino ME, Alexeeva V, Vacek PM, Bond JP, Pass HI, Steele C, Mossman BT. 2009a. Alterations in gene expression in human mesothelial cells correlate with mineral pathogenicity. Am J Respir Cell Mol Biol 41(1):114–123.

Shukla A, Bosenberg MW, MacPherson MB, Butnor KJ, Heintz NH, Pass HI, Carbone M, Testa JR, Mossman BT. 2009b. Activated cAMP response element binding protein is overexpressed in human mesotheliomas and inhibits apoptosis. Am J Pathol 175(5):2197–2206.

Shukla A, Hillegass JM, MacPherson MB, Beuschel SL, Vacek PM, Pass HI, Carbone M, Testa JR, Mossman BT. 2010. Blocking of ERK1 and ERK2 sensitizes human mesothelioma cells to doxorubicin. Mol Cancer 9:314.

Shukla A, Barrett TF, MacPherson MB, Hillegass JM, Fukagawa NK, Swain WA, O'Byrne KJ, Testa JR, Pass HI, Faux SP, Mossman BT. 2011. An extracellular signal-regulated kinase 2 survival pathway mediates resistance of human mesothelioma cells to asbestos-induced injury. Am J Respir Cell Mol Biol 45(5):906–914.

Shukla A, Miller JM, Cason C, Sayan M, MacPherson MB, Beuschel SL, Hillegass J, Vacek PM, Pass HI, Mossman BT. 2013. Extracellular signal-regulated kinase 5: A potential therapeutic target for malignant mesotheliomas. Clin Cancer Res 19(8):2071–2083.

Tang Z, Feng M, Gao W, Phung Y, Chen W, Chaudhary A, St Croix B, Qian M, Dimitrov DS, Ho M. 2013. A human single-domain antibody elicits potent antitumor activity by targeting an epitope in mesothelin close to the cancer cell surface. Mol Cancer Ther 12(4):416–426.

Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, Cox NJ, Dogan AU, Pass HI, Trusa S, Hesdorffer M, Nasu M, Powers A, Rivera Z, Comertpay S, Tanji M, Gaudino G, Yang H, Carbone M. 2011. Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 43(10):1022–1025.

Teta MJ, Mink PJ, Lau E, Sceurman BK, Foster ED. 2008. US mesothelioma patterns 1973–2002: Indicators of change and insights into background rates. Eur J Cancer Prev 17(6):525–534.

Tilleman TR, Richards WG, Zellos L, Johnson BE, Jaklitsch MT, Mueller J, Yeap BY, Mujoomdar AA, Ducko CT, Bueno R, Sugarbaker DJ. 2009. Extrapleural pneumonectomy followed by intracavitary intraoperative hyperthermic cisplatin with pharmacologic cytoprotection for treatment of malignant pleural mesothelioma: A phase II prospective study. J Thorac Cardiovasc Surg 138(2):405–411.

Vogelzang NJ. 2005. Standard therapy for the treatment of malignant pleural mesothelioma. Lung Cancer 50(Suppl1):S23–S24.

Wagner JC, Sleggs CA, Marchand P. 1960. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med 17:260–271.

Wang KX, Denhardt DT. 2008. Osteopontin: Role in immune regulation and stress responses. Cytokine Growth Factor Rev 19(5–6):333–345.

Wang H, Gillis A, Zhao C, Lee E, Wu J, Zhang F, Ye F, Zhang DY. 2011. Crocidolite asbestos-induced signal pathway dysregulation in mesothelial cells. Mutat Res 723(2):171–176.

Wang K, Xiang L, Zhang J, Beers R, Walker DA, Onda M, Hassan R, Pastan I. 2012. Inhibition of mesothelin as a novel strategy for targeting cancer cells. PLoS ONE 7(4):e33214.

Weldon JE, Xiang L, Zhang J, Beers R, Walker DA, Onda M, Hassan R, Pastan I. 2013. A recombinant immunotoxin against the tumor-associated antigen mesothelin reengineered for high activity, low off-target toxicity, and reduced antigenicity. Mol Cancer Ther 12(1):48–57.

World Health Organization. 2012. History of fighting against the toxic substance, asbestos. Available from: http://www.asbestos.com/asbestos/who.php. Accessed on November 26, 2012.

Xia G, Kumar SR, Masood R, Koss M, Templeman C, Quinn D, Zhu S, Reddy R, Krasnoperov V, Gill PS. 2005. Up-regulation of EphB4 in mesothelioma and its biological significance. Clin Cancer Res 11(12):4305–4315.

Xu J, Futakuchi M, Shimizu H, Alexander DB, Yanagihara K, Fukamachi K, Suzui M, Kanno J, Hirose A, Ogata A, Sakamoto Y, Nakae D, Omori T, Tsuda H. 2012. Multi-walled carbon nanotubes translocate into the pleural cavity and induce visceral mesothelial proliferation in rats. Cancer Sci 103(12):2045–2050.

Yamaki E, Yajima T, Kosaka T, Mogi A, Tanaka S, Kuwano H. 2013. Podoplanin overexpression in human mesothelioma cell lines enhances the tumorigenic phenotype. Oncol Rep 29(3):932–940.

Yang H, Bocchetta M, Kroczynska B, Elmishad AG, Chen Y, Liu Z, Bubici C, Mossman BT, Pass HI, Testa JR, Franzoso G, Carbone M. 2006. TNF-alpha inhibits asbestos-induced cytotoxicity via a NF-kappaB-dependent pathway,a possible mechanism for asbestos-induced oncogenesis. Proc Natl Acad Sci USA 103(27):10397–10402.

Yang H, Rivera Z, Jube S, Nasu M, Bertino P, Goparaju C, Franzoso G, Lotze MT, Krausz T, Pass HI, Bianchi ME, Carbone M. 2010. Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release,resultant inflammation. Proc Natl Acad Sci USA 107 (28):12611–12616.

Yung S, Chan TM. 2007. Mesothelial cells. Perit Dial Int 27:(Suppl 2):S110-S115.