Chapter 12
Biomarkers of Response to Asbestos Exposure

Clementina Mesaros, Liwei Weng, and Ian A. Blair

Abstract Asbestos-related diseases (ARDs) resulting from exposure to asbestos include lung cancer and malignant mesothelioma (MM). This has significant health and economic implications that have been well documented. The 20–40-year latency periods of ARDs and their low incidence rates in the general population make preventative strategies and early treatment extremely challenging. The availability of well-validated diagnostic biomarkers of asbestos exposure would greatly facilitate both prevention and early treatment strategies. In this chapter, we have summarized the state of knowledge on biomarkers of response to asbestos exposure and highlighted recent advances, including the discovery of new specific biomarker based on the posttranslational modifications of the high mobility group box 1 (HMGB1) protein. Asbestos is inhaled and trapped primarily in lung tissue and so can only be detected in bronchoalveolar lavage fluid. This makes direct exposure assessments very difficult. In contrast, biomarkers of response, which reflect a change in biologic function in response to asbestos exposure, have proved to be more useful. MM is the major biological response to asbestos that can be readily monitored, and numerous studies have used this disease as confirmation of a prior asbestos exposure. There is some new evidence that an increase in serum nonacetylated HMGB1 can serve as a biological response biomarker of asbestos exposure; whereas acetylated serum HMGB1 is associated with progression to MM. Finally, we discuss the potential merit of combined use of a multiplexed serum lipid biomarker panel with serum protein biomarkers.

Keywords Malignant mesothelioma • Diagnostic • Mesothelin • Osteopontin • Fibulin-3 • High mobility group box 1 • Acetylation • Slow off-rate modified aptamers • Mass spectrometry

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12.1 Biomarkers of Malignant Mesothelioma (MM) and Other Asbestos-Related Diseases (ARDs)

Studies with animal models (Guo et al. 2014; Xu et al. 2014; Kalra et al. 2015) together with genetic (Testa et al. 2011; Carbone et al. 2013; Cheung et al. 2013; Carbone et al. 2015; Ohar et al. 2016) and epidemiological research (Becklake 1976; Lemen et al. 1980; Britton 2002; Stayner et al. 2013; Prazakova et al. 2014) have shown unequivocally that asbestos exposure can lead to ARDs such as MM and lung cancer as well as pulmonary fibrosis. MM is a heterogeneous, aggressive cancer that is mainly observed on the serosal surfaces of the pleura and to a lesser extent in the peritoneum as well as much less commonly in the lining of the testes and pericardium (Montjoy et al. 2009). Surgery can result in long-term benefit for early stage MMs, where there is a better chance that most or all of the cancer can be removed (Lang-Lazdunski 2014). Unfortunately, treatment of later stages of MM is not curative, and so the focus is more on palliative care (Sterman and Albelda 2005). The National Institute for Occupational Safety and Health analysis of the annual cause of death records for 1999–2005 (the most recent years available) revealed that there were 18,068 deaths from MM in the USA, with the number of reported MM deaths increasing from 2482 in 1999 to 2704 in 2005 (Bang et al. 2009). However, the annual death rate was stable at 14.1/million in 1999 and 14.0/million in 2005. Asbestos is still mined in Brazil, Canada, China, Kazakhstan, Russia, and Zimbabwe even though its use is restricted in many Western countries (Frank and Joshi 2014). Therefore, prolonged latency periods from exposure to diagnosis coupled with the ongoing mining and use of asbestos have led to the increasing prevalence of this deadly disease worldwide (Linton et al. 2012; Stayner et al. 2013). Although the occupational exposure to asbestos has declined in the USA through restrictions on its use, there are risks for nonoccupational environmental exposures at old mining sites such as Libby, Montana (Peipins et al. 2003), and old asbestos industrial manufacturing sites such as the BoRit superfund site in Ambler, PA (http://www.boritcag.org).

Early detection of ARDs, when surgical removal of tumors is more successful, provides an attractive therapeutic approach. However, most clinical cases result from exposures that occurred decades earlier (Selikoff et al. 1980; Reid et al. 2014). This means that the discovery and validation of biomarkers of response to asbestos exposure would greatly facilitate the detection of asbestos-exposed individuals prior to the onset of ARDs (Mesaros et al. 2015). This would allow the exposed individuals to be more closely monitored for any sign of disease and allow treatment before progression to inoperable cancer occurs. In addition, biological response biomarker assays conducted in populations living proximal to a site contaminated with asbestos (such as the BoRit site in Ambler) would allow the efficacy of site remediation to be assessed.

12.2 Asbestos Exposure and Biomarkers of Oxidative Stress

There is significant experimental evidence to suggest that oxidative stress is involved in the cellular response to asbestos exposure (Fung et al. 1997; Swain et al. 2004; Schurkes et al. 2004) and in the etiology of asbestos-induced pulmonary fibrosis (Cheresh et al. 2015; Marczynski et al. 2000b). Isoprostanes (Milne et al. 2005) and 8-oxo-2′-deoxyguanosine (dGuo) (Mesaros et al. 2012) are widely used as biomarkers of oxidative stress, although isoprostanes are the only rigorously validated biomarkers of oxidative stress (Kadiiska et al. 2005). Several studies have explored the use of isoprostanes (Pelclova et al. 2008) or 8-oxo-dGuo (Marczynski et al. 2000a; Valavanidis et al. 2009; Hanaoka et al. 1993; Yoshida et al. 2001; Pilger and Rudiger 2006; Chew and Toyokuni 2015) as biomarkers of asbestos exposure. Regrettably, neither of these biomarkers can distinguish asbestos exposure from other causes of oxidative stress such as cigarette smoking (Navarro-Compan et al. 2013; Mesaros et al. 2012; Ellegaard and Poulsen 2016) and atherosclerosis (Victor et al. 2009; Serban and Dragan 2014; Schulze and Lee, 2005; Armstrong et al. 2011; Peluso et al. 2012).

Inhaled asbestos fibers, which are typically longer (typically >5 μm) than they are wide (typically <2 μm diameter) (Boulanger et al. 2014), infiltrate the lung, reach the pleural surface, and are engulfed by phagocytic cells – primarily macrophages (Pooley 1972). It has been suggested that macrophages exposed to asbestos undergo frustrated phagocytosis of the elongated fibers. This process then causes chronic production of reactive oxygen species (ROS), activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, and cytokine release (Ramos-Nino et al. 2006). DNA damage caused by this ROS production (and subsequent mutagenesis) is one mechanism that could be involved in MM carcinogenesis (Mossman et al. 2013). This could occur through the direct action of ROS on DNA or through ROS-derived lipid peroxide-mediated DNA damage (Blair 2008).

Asbestos is also known to activate the nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome, which promotes the release of interleukin (IL)-1β and IL-18 (Hillegass et al. 2013). Only a small proportion of patients who have been exposed to asbestos develop MM. Consequently, the disease is thought to result from an interaction of genetic and environmental factors. For example, cyclin-dependent kinase inhibitor 4a (p16INK4a) and ARF tumor suppressor (p14ARF) genes are frequently inactivated somatically in MMs, and approximately 50% of MM tumors exhibit inactivation of the neurofibromin type 2 gene (*NF2*) due to a combination of somatic nonsense or missense mutations and loss of the remaining wild-type allele (Cheng et al. 1994; Bianchi et al. 1995; Altomare et al. 2011; Sekido 2013; de Assis et al. 2014). In addition, mice with germline heterozygous inactivating mutations of these genes develop MM at an accelerated rate compared to genetically normal littermates when exposed to asbestos through intraperitoneal injections. Moreover,

germline and sporadic mutations of the tumor suppressor, breast cancer susceptibility (BRCA)-1 associated protein-1 (BAP1) gene, results in a predisposition for MM (Testa et al. 2011; Carbone et al. 2013; Cheung et al. 2013).

12.3 Biomarkers of Response to Asbestos Exposure

Asbestos fibers are not normally present in urine or plasma but they can appear in bronchoalveolar lavage (BAL) fluid. Quantification of asbestos fibers in BAL provides a qualitative/categorical approach to exposure assessment but has not been useful as a predictive biomarker (Sartorelli et al. 2007). This means that it is necessary to analyze biomarkers of response to asbestos fibers rather than directly quantifying the numbers of fibers. Before being implemented as a diagnostics test in clinical settings, biological response biomarkers must first be fully characterized and validated in large sample sets. Candidate biomarkers of exposure to asbestos often lack diagnostic utility because of poor sensitivity and/or inadequate specificity through confounding exposures or individual variability. Additional problems can arise from bioanalytical issues such as pre-analytic stability, inconsistent sample preparation, inconsistent sample processing, or inadequate technology. This means that robust and reproducible bioanalytical assay methodology is required for accurate and reproducible biomarker analysis. Rigorously validated assays for response biomarkers of exposure to asbestos could serve a critical role in early detection of ARDs and in monitoring novel approaches to treatment.

The major route of exposure to asbestos is thought to be through inhalation although there is now some evidence that asbestos can be transported in water (Wu et al. 2015), so it could potentially also be absorbed orally. Inhaled fibers are transported throughout the respiratory system, penetrate pleural cells in the lining of the lung, and induce oxidative stress as noted above. Proteins involved in the immune response, cell proliferation, and the generation of fibrotic tissue are showing promise as potential biomarkers of asbestos exposure. Other factors likely to impact risk of ARDs include common environmental variables, germline genetic factors (Testa et al. 2011), and differential expression of genes that interact with such variables (Hillegass et al. 2010).

We have reviewed the current status of biomarkers of response of human populations exposed to asbestos. The review is focused primarily on MM biomarkers as this is a disease that arises primarily from asbestos exposure. Validation of the more promising MM biomarkers in larger population studies will facilitate early detection of the disease, improve preventative measures, and help assess the efficacy of novel therapies.

12.4 Mesothelin as a Biomarker of MM

Mesothelin is a 40-kDa glycoprotein, which results from proteolytic cleavage of a 69-kDa mesothelin precursor, and is overexpressed in several types of cancer including MM. The soluble form, soluble mesothelin-related protein (SMRP),

sometimes known as soluble mesothelin-related peptide, has emerged as a potential biomarker in serum or urine for the early detection of MM (Creaney et al. 2010a; Creaney et al. 2010b). A number of enzyme-linked immunosorbent assay (ELISA) kits have been developed to analyze SMRP including the MESOMARK serum assay, which is a US Food and Drug Administration (FDA)-approved biomarker for use as an aid in the monitoring of patients with epithelioid and biphasic MM (Table 12.1) (Beyer et al. 2007). A meta-analysis of 30 studies revealed a mean sensitivity of 66% and specificity 97% for SMRP as a serum biomarker of MM when compared with healthy controls (Fig. 12.1) (Cui et al. 2014).

A study conducted in Australia with a sandwich ELISA using two monoclonal antibodies showed increased levels of SMRP in MM patients when compared with healthy controls (Robinson et al. 2003). However, the small sample size did not permit adequate statistical power. A study on the occupational exposure to asbestos in the Czech Republic showed that subjects who had been exposed to asbestos with benign lung disease had higher serum levels of SMRP than normal subjects. Individuals with benign disease had lower serum SMRP levels than subjects with MM (Jakubec et al. 2015). A Turkish biomarker study analyzed serum SMRP in 24 patients with MM from naturally occurring asbestos, 279 subjects with pleural plaques, 123 healthy exposed, and 120 control subjects. This study revealed that serum SMRP had a sensitivity of 63% and specificity of only 74% for detecting the MM patients in this population (Bayram et al. 2014).

Several other studies have found higher levels of serum SMRP in asbestos-exposed individuals than unexposed controls (Pass et al. 2008; Rodriguez Portal et al.

Table 12.1 Response biomarkers of asbestos exposure

Asbestos biomarker	Abbrev	Description	Refrences
Soluble mesothelin-related peptide or soluble mesothelin-related protein	SMRP	Mesothelin and SMRP are 40-kDa glycoproteins from proteolytic cleavage of the 69-kDa mesothelin precursor protein	Robinson et al. (2003)
Osteopontin	None	A 32-kDa integrin-binding protein involved in tumorigenesis, progression, and metastasis	Pass et al. (2005)
Fibulin-3	None	Fibulin-3 is a 57-kDa protein that belongs to a family of extracellular proteins expressed in the basement membranes of blood vessels	Pass et al. (2012)
Nonacetylated high mobility group box 1	HMGB1	HMGB1 is a 30-kDa chromatin protein. The unmodified protein has a nuclear location	Tabata et al. (2013a)
Acetylated high mobility group box 1	Acetylated HMGB1	Lysine hyperacetylation within two nuclear localization signals (Fig. 12.4) causes translocation of HMGB1 into the cytosol	Napolitano et al. (2016)
Proteomic biomarkers	None	A panel of 13 high abundance serum proteins identified by Slow Off-rate Modified Aptamer (SOMAmer) technology	Ostroff et al. (2012)

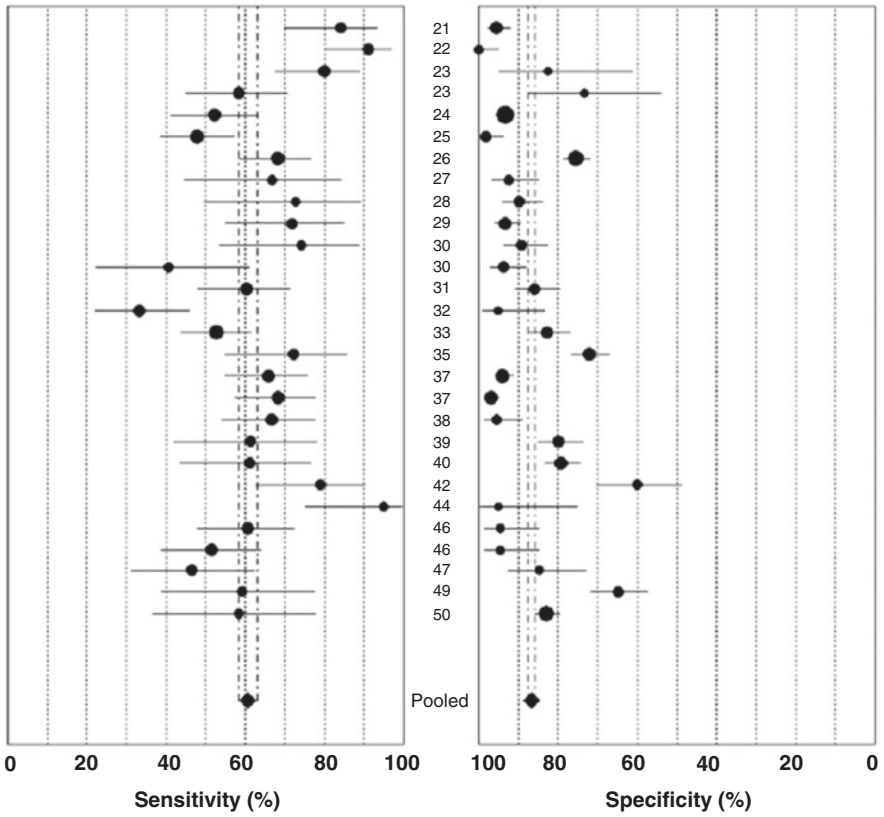

Fig. 12.1 Forest plots of estimates of sensitivity and specificity for soluble mesothelin family proteins in serum for diagnosing malignant pleural mesothelioma. The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars are 95% CIs. Numbers indicate the reference numbers of studies cited in the original reference list. Modified with permission from Cui et al. (2014)

2009; Marini et al. 2011). One of these studies (Marini et al. 2011) also examined the correlation between mesothelin (SMRP) levels and frequency of micronuclei in blood. A statistically significant positive correlation of the SMRP levels with the frequency of mononuclei in the mononucleated lymphocytes was observed.

Another Australian study measured serum SMRP in a cohort of 514 asbestos-exposed subjects in which the severity of ARDs was assessed separately (Park et al. 2012). The serum SMRP level in the population with compensable ARDs was positively associated with disability assessment. Conversely, the mean SMRP level in healthy asbestos-exposed subjects was significantly lower than those with pleural plaques and in subjects with ARDs who received compensation. The authors concluded that serum SMRP levels correlated with the severity of compensable ARDs and that serum SMRP could potentially be applied to monitor the progress of ARDs.

One known potential problem apart from the variable sensitivity and specificity of serum SMRP assays (de Assis et al. 2014) is the increased expression of SMRP in ovarian cancer (Wu et al. 2014). Additional confounding issues include the effects of sample storage, body mass index, glomerular filtration rate, age, and smoking status (Park et al. 2010).

12.5 Osteopontin as a Biomarker of MM

Osteopontin is a 32-kDa protein that is encoded by the *SPP1* gene (secreted phosphoprotein 1) in humans (Table 12.1). It is an integrin-binding glycoprotein involved in tumorigenesis, progression, and metastasis that is overexpressed in lung cancer, MM, and several other types of cancer (Denhardt and Chambers 1994). High levels of osteopontin are correlated with tumor progression and metastasis. In a rat model, osteopontin was upregulated in asbestos-induced tumors (Sandhu et al. 2000). There is a report that osteopontin levels in plasma or serum were able to differentiate between healthy subjects exposed to asbestos and MM patients (Grigoriu et al. 2007). A number of other human population studies have reported the serum levels of osteopontin in asbestos-exposed subjects. In one study, osteopontin levels were reported for 69 asbestos-exposed subjects, 45 healthy controls, and 76 patients with surgically staged MM (Pass et al. 2005). Serum osteopontin levels were significantly higher in the group with pleural MM than in the group with exposure to asbestos but without MM. An analysis of serum osteopontin levels comparing the receiver operating characteristic (ROC) curve in the group exposed to asbestos with that of the group with MM had a sensitivity of 77.6% and a specificity of 85.5% (Pass et al. 2005). Another study, which was conducted in Turkey, had 120 healthy controls and 123 subjects exposed to naturally occurring asbestos (Bayram et al. 2014). The difference in the levels of osteopontin was significant ($p < 0.05$) in controls versus asbestos-exposed individuals but was not useful for predicting malignant transformation.

A large study analyzed SMRP and osteopontin levels in asbestos-exposed workers ($n = 1894$) together with a smaller number of unexposed controls ($n = 102$) (Felten et al. 2014). The levels of osteopontin were not significantly different between the two groups. This study also found no correlation between osteopontin levels with the length of the asbestos exposure. A systematic review and meta-analysis of six studies was conducted in order to evaluate the diagnostic accuracy of circulating osteopontin for MM (Hu et al. 2014). The overall mean diagnostic sensitivity and specificity were 65% and 81%, respectively (Fig. 12.2). The area under the ROC curve (AUC) was 0.83, and the diagnostic accuracy of both serum and plasma osteopontin was comparable. The authors concluded that osteopontin was an effective marker for MM diagnosis but that more studies with larger sample sizes and better designs were needed in order to rigorously assess its diagnostic power.

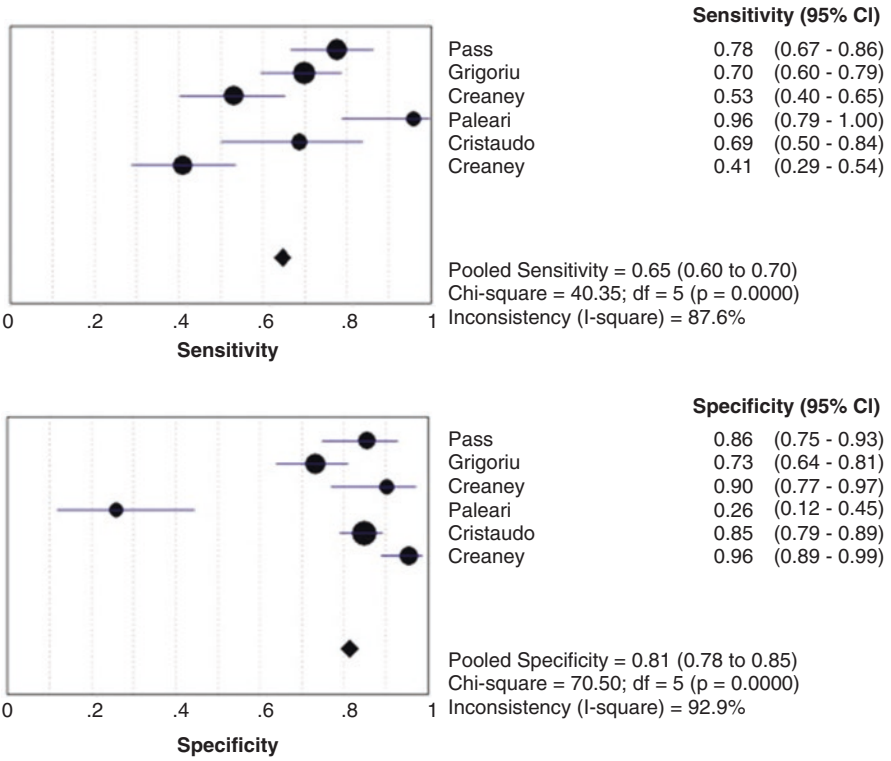

Fig. 12.2 Forest plots to estimate sensitivity and specificity of osteopontin from six studies. Each solid circle represents an eligible study. The size of the solid circle reflects the sample size of each eligible study. Error bars represent 95% CI. *Solid diamond* symbols represent mean pooled sensitivity and specificity. Reprinted with permission from Hu et al. (2014)

12.6 Fibulin-3 as a Biomarker of MM

Fibulin-3 is a 57-kDa protein, which belongs to a family of extracellular proteins expressed on the basement membranes of blood vessels. It has emerged as a potential plasma protein from the studies of Pass et al. as a biomarker of asbestos exposure with the capability of distinguishing between exposed and disease states within multiple cohorts (Table 12.1) (Pass et al. 2012). Unusually, in matched samples, fibulin-3 levels were lower in serum than in plasma probably due to the presence of thrombin cleavage sites within fibulin-3. It was also found that fibulin-3 was not able to distinguish between patients with MM and asbestosis because serum levels were elevated in both groups (Corradi et al. 2013). Plasma fibulin-3 was significantly elevated in MM patients from a Sydney patient cohort, but not a Vienna patient cohort (Fig. 12.3) (Kirschner et al. 2015).

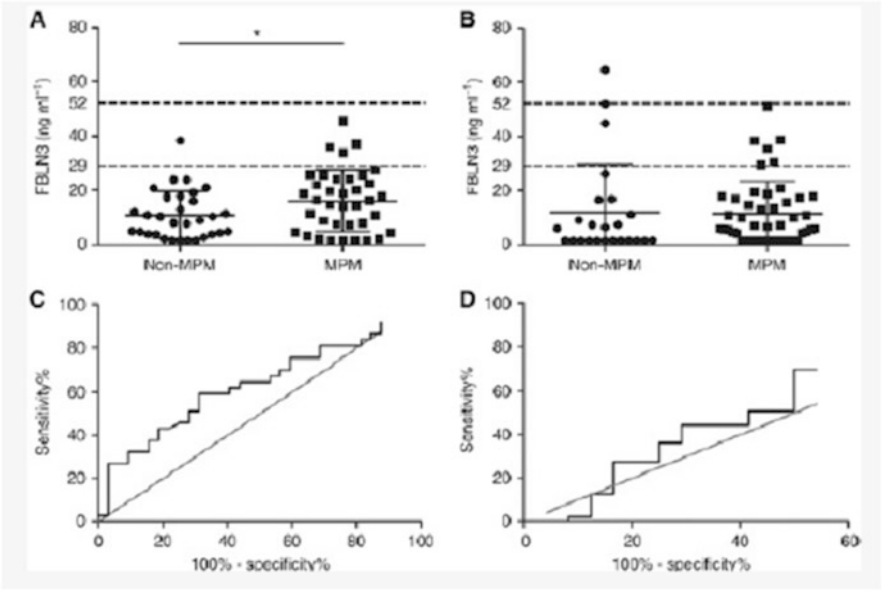

Fig. 12.3 Fibulin-3 in patient plasma. Plasma fibulin-3 protein levels in the Sydney (**a**) and Vienna cohorts (**b**). Mean levels in both cohorts were below those previously reported. Mean ± s.d. are represented by the lines in the scatter plots, and the cutoffs applied in the original study (Pass et al. 2012) are indicated by dotted lines. The diagnostic accuracy of plasma fibulin-3 was low in both investigated cohorts: (**c**) Sydney cohort AUC = 0.63 (95% CI, 0.50–0.76) and (**d**) Vienna cohort AUC = 0.56 (95% CI, 0.41–0.71). Reprinted with permission from Kirschner et al. (2015)

In addition, the diagnostic accuracy was low, which raised questions as to whether fibulin-3 actually has diagnostic value (Kirschner et al. 2015). However, in another recent study, patients with fibulin-3 levels >34.25 ng/mL before treatment had more than four times higher probability for developing progressive disease within 18 months than patients with levels ≤34.25 ng/mL (Kovac et al. 2015). In addition, patients with fibulin-3 levels >34.25 ng/mL after treatment had increased odds for progressive disease within 18 months even if they had a complete response or stable disease. The authors concluded that a combination of serum SMRP and plasma fibulin-3 levels might be helpful in detecting the progression of MM. Clearly, additional validation studies will be required to fully elucidate the utility of fibulin-3 as a dependable biomarker of asbestos exposure in human populations. It would be interesting to assess multiplexed assays with other protein biomarkers for MM (Kovac et al. 2015; Creaney et al. 2015). In fact, a recent study has demonstrated that compared to an asbestos exposure group of 48 subjects, a group of 42 MM patients had significantly higher mean epidermal growth factor (EGFR), thioredoxin-1 (TRX), SMRP, and fibulin-3 levels (Demir et al. 2016).

12.7 High Mobility Group Box (HMGB) 1 Protein as a Biomarker of MM and Asbestos Exposure

HMGB1 is a 30-kDa DNA-binding nonhistone protein that is present in nucleus (Lotze and Tracey 2005). It consists of 215 amino acid residues that are organized into three domains, which include two tandem HMG box domains (A box and B box) that are arranged in an L-shaped configuration, and a C-terminal tail of 30 amino acid residues (Fig. 12.4). The nuclear localization of HMGB1 is due to the presence of two lysine-rich nuclear localization sequences (NLSs), spanning amino acids 28–44 (NLS1) and 179–185 (NLS2) (Fig. 12.4) (Lotze and Tracey 2005). HMGB1 is released from macrophages and monocytes by endogenous pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin [IL]-1β, and interferon [IFN]-γ (Wang et al. 2004). Because its N-terminus lacks a signal sequence, HMGB1 cannot

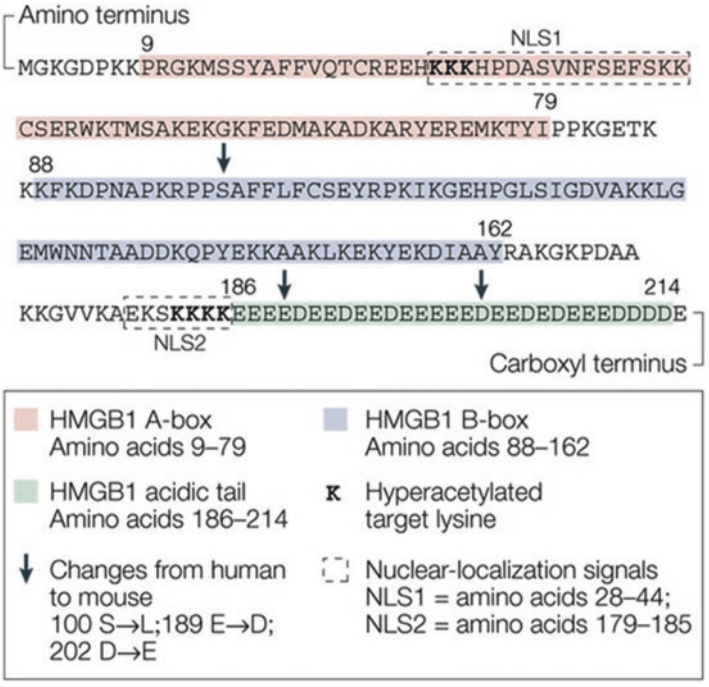

Fig. 12.4 HMGB1 structure. A linear diagram of high-mobility group box 1 protein (HMGB1) is shown, including the residues that constitute the A-box (*pink*), B-box (*purple*), and acidic tail (*green*). The proximal A-box and B-box of HMGB1 both contain putative nuclear-emigration signals, as identified by binding to the nuclear exportin chromosome-region maintenance 1. HMGB1 also contains 43 lysine residues, some of which are frequently acetylated in lipopolysaccharide-activated macrophages (shown in *bold*). These lysine residues are found within two nuclear-localization signals (indicated by *dashed boxes*): NLS1, which spans amino acids 28–44; and NLS2, which spans amino acids 179–185. Reprinted with permission from Lotze and Tracey (2005)

be released via the classical endoplasmic reticulum-Golgi secretory pathway. Instead, activated macrophages/monocytes acetylate the HMGB1 at three lysine residues in the NLS1 region and five lysine residues in the NLS2 region (Fig. 12.4) (Lotze and Tracey 2005), which leads to translocation into the cytoplasm and release into the extracellular milieu (Bonaldi et al. 2003; Chen et al. 2005).

The HMGB1 protein, which has a regulatory role in inflammatory immune responses, has received substantial attention as a potential biomarker of MM (Qi et al. 2013) (Table 12.1). Gene expression profiling of mesothelial cells exposed to asbestos has shown upregulation of many genes targeted by HMGB1 (Qi et al. 2013). Furthermore, exposing mice to asbestos resulted in increased serum levels of HMGB1 for 10 or more weeks after crocidolite exposure, but returned to background levels within 8 weeks after chrysotile exposure. Continuous administration of chrysotile was required for sustained high serum levels of HMGB1 (Qi et al. 2013). One study found elevated serum levels of HMGB1, in asbestos-exposed individuals when compared with both smoking and nonsmoking controls (Yang et al. 2010), indicating that serum HMGB1 could be exploited for assessing asbestos exposure in human populations. In agreement with this finding, elevated serum levels of HMGB1 have been found in MM patients (Jube et al. 2012; Tabata et al. 2013b; Yamada et al. 2011).

More recently, it was found that serum HMGB1 is extensively acetylated in the serum of MM patients (Napolitano et al. 2016). The quantification of HMGB1 has generally been conducted using immunoassay-based methodology such as ELISAs, which cannot readily distinguish the nonacetylated and acetylated forms (Zhou et al. 2016; Yamada et al. 2003; Zangar et al. 2006; Barnay-Verdier et al. 2011; Bergmann et al. 2016). Development of high-specificity liquid chromatography-mass spectrometry (LC-MS)-based methods by Antoine and his colleagues (Antoine et al. 2012; Ge et al. 2014; Napolitano et al. 2016) made it possible to distinguish the HMGB1 hyperacetylated forms from the unmodified form normally found in the nucleus (Lotze and Tracey 2005). The MS-based methods are based upon the use of electrospray ionization (ESI) analysis of intact HMGB1 protein followed by spectral deconvolution (Fig. 12.5) (Napolitano et al. 2016) or by the use of Glu-C protease digestion of the HMGB1 followed by LC-ESI/tandem mass spectrometry (MS/MS) analysis of the hyperacetylated NLS2-derived decapeptide $K^{180}SKKKKEEEE^{189}$, which contains five acetylated lysine residues (Fig. 12.6) (Ge et al. 2014). LC-MS analysis of synthetic acetylated peptide from the NLS2 region of HMGB1–K(Ac)SK(Ac)K(Ac)K(Ac)K(Ac)EEEE – revealed that it could be rapidly adsorbed on plastic surfaces (Antoine et al. 2012). As a result, glass vials were required throughout the assay procedure. To minimize losses during LC-MS analysis, a desalted tryptic digest of human serum albumin was used as a proteinaceous carrier for the Glu-C-derived peptides.

Hyperacetylated and nonacetylated HMGB1 (together referred to as total HMGB1) were analyzed blindly in blood collected from MM patients ($n = 22$), individuals with verified chronic asbestos exposure ($n = 20$), patients with benign pleural effusions ($n = 13$), malignant pleural effusions not due to MM ($n = 25$), and healthy control subjects ($n = 20$) (Napolitano et al. 2016). Blood levels of previously

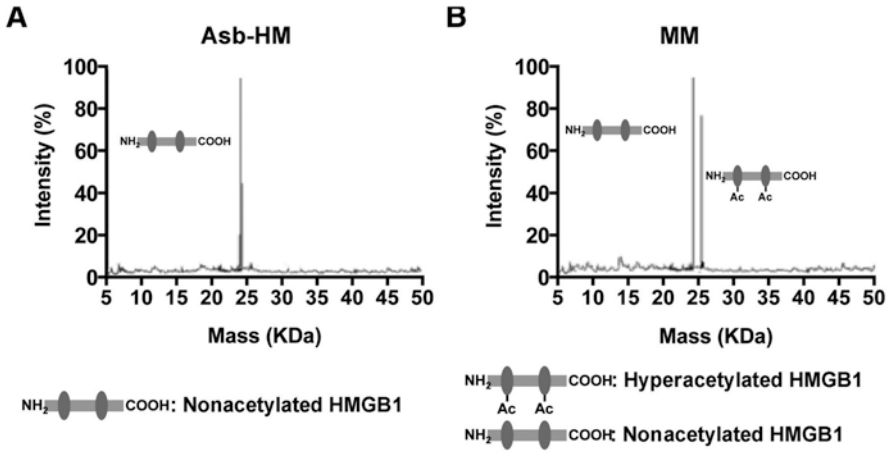

Fig. 12.5 Asbestos-exposed human mesothelial (HM) and malignant mesothelioma (MM) cells release different HMGB1 isoforms. (**a**) Representative spectrum of whole protein ESI/MS analysis of HMGB1 in crocidolite asbestos-exposed HM, where only nonacetylated HMGB1 was detected. (**b**) Representative spectrum of whole protein ESI/MS spectrum of HMGB1 in MM cells where both hyperacetylated and nonacetylated HMGB1 were detected. Reprinted with permission from Napolitano et al. (2016)

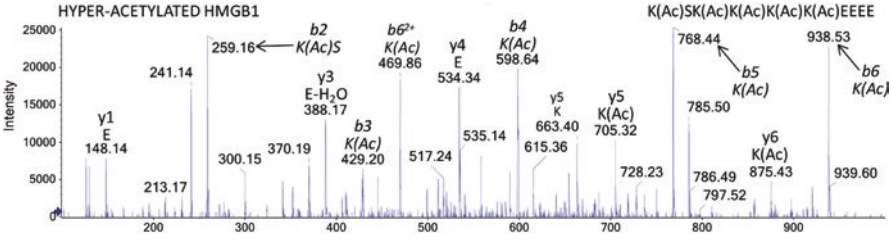

Fig. 12.6 Diagnostic LC–MS/MS spectrum of Glu-C-derived peptide confirming the identification of hyper-acetylated HMGB1 derived from inflammatory cells present in patient sera during acetaminophen hepatotoxicity. Amino acids, b and y ions and peptide sequences are indicated on each spectrum. Acetylated lysine residues within HMGB1 are represented by K(Ac). Reprinted with permission from Antoine et al. (2012)

proposed biomarkers (fibulin-3, SMRP, and osteopontin) were also analyzed in the non-healthy individuals. HMGB1 serum levels reliably distinguished MM patients, asbestos-exposed individuals, and unexposed controls (Napolitano et al. 2016). Total HMGB1 was significantly higher in MM patients and asbestos-exposed individuals compared with healthy controls. Hyperacetylated HMGB1 was significantly higher in MM patients compared with asbestos-exposed individuals and healthy controls and did not vary with tumor stage. At the cutoff value of 2.00 ng/mL, the sensitivity and specificity of serum hyperacetylated HMGB1 in differentiating MM patients from asbestos-exposed individuals and healthy controls was 100%, outperforming other previously proposed biomarkers. Furthermore, by combining HMGB1

and fibulin-3, increased sensitivity and specificity was obtained for differentiating MM patients from patients with cytologically benign or malignant non-mesothelioma pleural effusion. If confirmed by other groups, these results are clearly highly significant and clinically relevant because they provide the first biomarker of asbestos exposure and indicate that hyperacetylated HMGB1 is an accurate biomarker to differentiate MM patients from individuals occupationally exposed to asbestos and unexposed controls. More extensive studies will reveal whether these exciting new findings offer an approach to distinguish subjects exposed to asbestos from those who have progressed to MM.

12.8 Proteomic Biomarkers of MM

A targeted proteomics approach using Slow Off-rate Modified Aptamer (SOMAmer) technology (Ostroff et al. 2012) was employed for the discovery, verification, and validation of MM biomarkers. SOMAmers have slow specific off-rates for dissociation of targeted analytes, which results in highly selective protein detection. This makes it possible to simultaneously quantify over 1000 proteins in unfractionated biologic samples (Vaught et al. 2010). The biomarker study by Ostroff and colleagues used serum from 117 MM cases and 142 asbestos-exposed control individuals. An initial set of 64 candidate high abundance protein biomarkers was discovered. A training set identified a panel of 13-protein biomarkers for the validation studies (Table 12.1). In a paired sample analysis, the sensitivity (91%) and specificity (94%) of the 13-protein panel and the AUC of the ROC curve of 0.99 were far superior to those observed for serum SMRP (Fig. 12.7). The 13-protein panel consists of both inflammatory and proliferative proteins, which are involved in biological processes that are strongly associated with asbestos-induced malignancy. Further validation studies will be required to determine whether this panel will be useful for screening and diagnosis of high-risk individuals.

12.9 Conclusions and Future Perspectives

There is a compelling need to rigorously validate the serum and plasma proteins that are upregulated in MM as a panel of useful biological response biomarkers of asbestos exposure (Table 12.1). In spite of the ban on mining it in the USA, asbestos is still being mined in other parts of the world where there are poor controls on potential exposure of workers to asbestos (Linton et al. 2012). Furthermore, the 20–40-year latency period before ARDs are detected means that they will continue to be a public health problem in the USA for many years (Carbone et al. 2012). A reliable biomarker panel capable of assessing whether a particular individual is at risk for ARDs would be a useful clinical tool in screening, diagnosis, and prevention. Perhaps more importantly, such a diagnostic biomarker panel could help alleviate

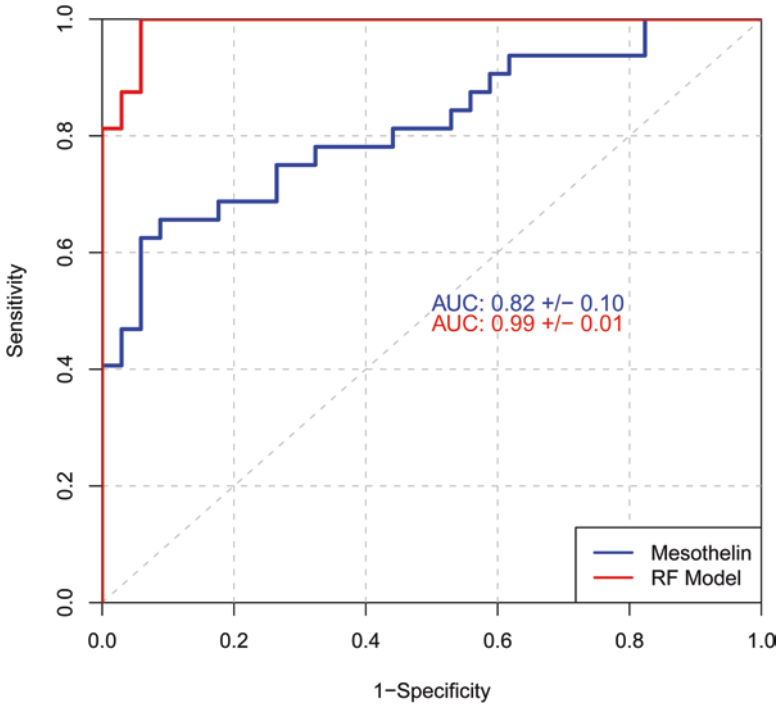

Fig. 12.7 ROC curves comparing 13-protein MM biomarker panel to mesothelin (SMRP). Performance of the 13-protein random forest (RF) classifier panel (*red*) compared to a commercial mesothelin (SMRP) assay (*blue*) on the same cohort of 32 MM cases and 34 asbestos-exposed controls. ROC curves are plotted with corresponding AUC values and 95% confidence intervals. Reprinted with permission from Ostroff et al. (2012)

concerns about possible environmental exposure as well as help to ensure that effective removal of asbestos from the environment has been implemented. In the past, response biomarker studies have focused on small molecule biomarkers of oxidative stress, signaling factors for cell-mediated and humoral immune responses, and growth factors generated in response to inhalation of asbestos fibers. These studies were able to elucidate many of the important factors involved with the pathogenesis of ARDs as well as guide efforts for developing effective asbestos exposure biomarkers. This has led to the potentially exciting observation that acetylated HMGB1 may be a highly specific biomarker of MM and that elevated nonacetylated HMGB1 might be a useful response biomarker of asbestos exposure.

The discovery of acetylated HMGB1 in MM patients has highlighted the potential utility of LC-MS-based methodology, which is more specific than ELISA-based procedures as also evidenced in our recent studies of apolipoprotein A-I (Wang et al. 2015). Furthermore, LC-MS methodology can be readily adapted to multiplexing, so that multiple candidate proteins can be quantified with high sensitivity and specificity in a single analysis (Mesaros and Blair 2016). Another approach that could be applied to the discovery of biomarkers of response resulting from asbestos

exposure involves the implementation of untargeted serum lipidomics using ultrahigh-performance LC coupled with high-resolution MS (Snyder et al. 2015). This could lead to the discovery of a panel of serum lipid biomarkers of response to asbestos exposure that could be validated in more extensive biomarker studies. A serum lipid biomarker panel when combined with serum protein biomarkers would provide an extremely rigorous approach to monitoring populations at risk for environmental asbestos exposure such as near the BoRit site in Pennsylvania and the disused mine in Libby, Montana. Finally, highly specific and sensitive LC-MS assays of rigorously validated MM biomarkers would be extremely effective in assessing the impact of novel approaches to the treatment of MM such as the immune checkpoint inhibitors (Lievense et al. 2014; Ceresoli et al. 2016).

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References

Altomare DA, Menges CW, Xu J et al (2011) Losses of both products of the Cdkn2a/Arf locus contribute to asbestos-induced mesothelioma development and cooperate to accelerate tumorigenesis. PLoS One 6:e18828

Antoine DJ, Jenkins RE, Dear JW et al (2012) Molecular forms of HMGB1 and keratin-18 as mechanistic biomarkers for mode of cell death and prognosis during clinical acetaminophen hepatotoxicity. J Hepatol 56:1070–1079

Armstrong AW, Voyles SV, Armstrong EJ et al (2011) Angiogenesis and oxidative stress: common mechanisms linking psoriasis with atherosclerosis. J Dermatol Sci 63:1–9

Bang KM, Mazurek JM, Storey E et al (2009) Malignant mesothelioma mortality-United States, 1999–2005. MMWR Morb Mortal Wkly Rep 58:393–396

Barnay-Verdier S, Gaillard C, Messmer M et al (2011) PCA-ELISA: a sensitive method to quantify free and masked forms of HMGB1. Cytokine 55:4–7

Bayram M, Dongel I, Akbas A et al (2014) Serum biomarkers in patients with mesothelioma and pleural plaques and healthy subjects exposed to naturally occurring asbestos. Lung 192:197–203

Becklake MR (1976) Asbestos-related diseases of the lung and other organs: their epidemiology and implications for clinical practice. Am Rev Respir Dis 114:187–227

Bergmann C, Strohbuecker L, Lotfi R et al (2016) High mobility group box 1 is increased in the sera of psoriatic patients with disease progression. J Eur Acad Dermatol Venereol 30:435–441

Beyer HL, Geschwindt RD, Glover CL et al (2007) MESOMARK: a potential test for malignant pleural mesothelioma. Clin Chem 53:666–672

Bianchi AB, Mitsunaga SI, Cheng JQ et al (1995) High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. Proc Natl Acad Sci U S A 92:10854–10858

Blair IA (2008) DNA adducts with lipid peroxidation products. J Biol Chem 283:15545–15549

Bonaldi T, Talamo F, Scaffidi P et al (2003) Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J 22:5551–5560

Boulanger G, Andujar P, Pairon JC et al (2014) Quantification of short and long asbestos fibers to assess asbestos exposure: a review of fiber size toxicity. Environ Health 13:59

Britton M (2002) The epidemiology of mesothelioma. Semin Oncol 29:18–25

Carbone M, Flores EG, Emi M et al (2015) Combined genetic and genealogic studies uncover a large BAP1 cancer syndrome kindred tracing back nine generations to a common ancestor from the 1700s. PLoS Genet 11:e1005633

Carbone M, Ly BH, Dodson RF et al (2012) Malignant mesothelioma: facts, myths, and hypotheses. J Cell Physiol 227:44–58

Carbone M, Yang H, Pass HI et al (2013) BAP1 and cancer. Nat Rev Cancer 13:153–159

Ceresoli GL, Bonomi M, Sauta MG (2016) Immune checkpoint inhibitors in malignant pleural mesothelioma: promises and challenges. Expert Rev Anticancer Ther 16:673–675

Chen G, Li J, Qiang X et al (2005) Suppression of HMGB1 release by stearoyl lysophosphatidylcholine: an additional mechanism for its therapeutic effects in experimental sepsis. J Lipid Res 46:623–627

Cheng JQ, Jhanwar SC, Klein WM et al (1994) p16 alterations and deletion mapping of 9p21-p22 in malignant mesothelioma. Cancer Res 54:5547–5551

Cheresh P, Morales-Nebreda L, Kim SJ et al (2015) Asbestos-induced pulmonary fibrosis is augmented in 8-oxoguanine DNA glycosylase knockout mice. Am J Respir Cell Mol Biol 52:25–36

Cheung M, Talarchek J, Schindeler K et al (2013) Further evidence for germline BAP1 mutations predisposing to melanoma and malignant mesothelioma. Cancer Genet 206:206–210

Chew SH, Toyokuni S (2015) Malignant mesothelioma as an oxidative stress-induced cancer: an update. Free Radic Biol Med 86:166–178

Corradi M, Goldoni M, Alinovi R et al (2013) YKL-40 and mesothelin in the blood of patients with malignant mesothelioma, lung cancer and asbestosis. Anticancer Res 33:5517–5524

Creaney J, Dick IM, Robinson BW (2015) Comparison of mesothelin and fibulin-3 in pleural fluid and serum as markers in malignant mesothelioma. Curr Opin Pulm Med 21:352–356

Creaney J, Musk AW, Robinson BW (2010a) Sensitivity of urinary mesothelin in patients with malignant mesothelioma. J Thorac Oncol 5:1461–1466

Creaney J, Olsen NJ, Brims F et al (2010b) Serum mesothelin for early detection of asbestos-induced cancer malignant mesothelioma. Cancer Epidemiol Biomark Prev 19:2238–2246

Cui A, Jin XG, Zhai K et al (2014) Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analysis. BMJ Open 4:e004145

de Assis LV, Locatelli J, Isoldi MC (2014) The role of key genes and pathways involved in the tumorigenesis of malignant mesothelioma. Biochim Biophys Acta 1845:232–247

Demir M, Kaya H, Taylan M et al (2016) Evaluation of new biomarkers in the prediction of malignant mesothelioma in subjects with environmental asbestos exposure. Lung 194:409–417

Denhardt DT, Chambers AF (1994) Overcoming obstacles to metastasis--defenses against host defenses: osteopontin (OPN) as a shield against attack by cytotoxic host cells. J Cell Biochem 56:48–51

Ellegaard PK, Poulsen HE (2016) Tobacco smoking and oxidative stress to DNA: a meta-analysis of studies using chromatographic and immunological methods. Scand J Clin Lab Invest 76:151–158

Felten MK, Khatab K, Knoll L et al (2014) Changes of mesothelin and osteopontin levels over time in formerly asbestos-exposed power industry workers. Int Arch Occup Environ Health 87:195–204

Frank AL, Joshi TK (2014) The global spread of asbestos. Ann Glob Health 80:257–262

Fung H, Kow YW, Van HB et al (1997) Patterns of 8-hydroxydeoxyguanosine formation in DNA and indications of oxidative stress in rat and human pleural mesothelial cells after exposure to crocidolite asbestos. Carcinogenesis 18:825–832

Ge X, Antoine DJ, Lu Y et al (2014) High Mobility Group Box-1 (HMGB1) participates in the pathogenesis of alcoholic liver disease (ALD). J Biol Chem 289:22672–22691

Grigoriu BD, Scherpereel A, Devos P et al (2007) Utility of osteopontin and serum mesothelin in malignant pleural mesothelioma diagnosis and prognosis assessment. Clin Cancer Res 13:2928–2935

Guo Y, Chirieac LR, Bueno R et al (2014) Tsc1-Tp53 loss induces mesothelioma in mice, and evidence for this mechanism in human mesothelioma. Oncogene 33:3151–3160

Hanaoka T, Tsugane S, Yamano Y et al (1993) Quantitative analysis of 8-hydroxyguanine in peripheral blood cells: an application for asbestosis patients. Int Arch Occup Environ Health 65:S215–S217

Hillegass JM, Miller JM, Macpherson MB et al (2013) Asbestos and erionite prime and activate the NLRP3 inflammasome that stimulates autocrine cytokine release in human mesothelial cells. Part Fibre Toxicol 10:39

Hillegass JM, Shukla A, Lathrop SA et al (2010) Inflammation precedes the development of human malignant mesotheliomas in a SCID mouse xenograft model. Ann N Y Acad Sci 1203:7–14

Hu ZD, Liu XF, Liu XC et al (2014) Diagnostic accuracy of osteopontin for malignant pleural mesothelioma: a systematic review and meta-analysis. Clin Chim Acta 433:44–48

Jakubec P, Pelclova D, Smolkova P et al (2015) Significance of serum mesothelin in an asbestos-exposed population in the Czech Republic. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 159:472–479

Jube S, Rivera ZS, Bianchi ME et al (2012) Cancer cell secretion of the DAMP protein HMGB1 supports progression in malignant mesothelioma. Cancer Res 72:3290–3301

Kadiiska MB, Gladen BC, Baird DD et al (2005) Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 38:698–710

Kalra N, Zhang J, Thomas A et al (2015) Mesothelioma patient derived tumor xenografts with defined BAP1 mutations that mimic the molecular characteristics of human malignant mesothelioma. BMC Cancer 15:376

Kirschner MB, Pulford E, Hoda MA et al (2015) Fibulin-3 levels in malignant pleural mesothelioma are associated with prognosis but not diagnosis. Br J Cancer 113:963–969

Kovac V, Dodic-Fikfak M, Arneric N et al (2015) Fibulin-3 as a biomarker of response to treatment in malignant mesothelioma. Radiol Oncol 49:279–285

Lang-Lazdunski L (2014) Surgery for malignant pleural mesothelioma: why, when and what? Lung Cancer 84:103–109

Lemen RA, Dement JM, Wagoner JK (1980) Epidemiology of asbestos-related diseases. Environ Health Perspect 34:1–11

Lievense LA, Hegmans JP, Aerts JG (2014) Biomarkers for immune checkpoint inhibitors. Lancet Oncol 15:e1

Linton A, Vardy J, Clarke S et al (2012) The ticking time-bomb of asbestos: its insidious role in the development of malignant mesothelioma. Crit Rev Oncol Hematol 84:200–212

Lotze MT, Tracey KJ (2005) High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat Rev Immunol 5:331–342

Marczynski B, Kraus T, Rozynek P et al (2000a) Association between 8-hydroxy-2′-deoxyguanosine levels in DNA of workers highly exposed to asbestos and their clinical data, occupational and non-occupational confounding factors, and cancer. Mutat Res 468:203–212

Marczynski B, Rozynek P, Kraus T et al (2000b) Levels of 8-hydroxy-2′-deoxyguanosine in DNA of white blood cells from workers highly exposed to asbestos in Germany. Mutat Res 468:195–202

Marini V, Michelazzi L, Cioe A et al (2011) Exposure to asbestos: correlation between blood levels of mesothelin and frequency of micronuclei in peripheral blood lymphocytes. Mutat Res 721:114–117

Mesaros C, Arora JS, Wholer A et al (2012) 8-Oxo-2′-deoxyguanosine as a biomarker of tobacco-smoking-induced oxidative stress. Free Radic Biol Med 53:610–617

Mesaros C, Blair IA (2016) Mass spectrometry-based approaches to targeted quantitative proteomics in cardiovascular disease. Clin Proteomics 13:20

Mesaros C, Worth AJ, Snyder NW et al (2015) Bioanalytical techniques for detecting biomarkers of response to human asbestos exposure. Bioanalysis 7:1157–1173

Milne GL, Musiek ES, Morrow JD (2005) F2-Isoprostanes as markers of oxidative stress in vivo: an overview. Biomarkers 10(Suppl 1):S10–S23

Montjoy C, Parker J, Petsonk L et al (2009) Mesothelioma review. W V Med J 105:13–16

Mossman BT, Shukla A, Heintz NH et al (2013) New insights into understanding the mechanisms, pathogenesis, and management of malignant mesotheliomas. Am J Pathol 182:1065–1077

Napolitano A, Antoine DJ, Pellegrini L et al (2016) HMGB1 and its hyperacetylated isoform are sensitive and specific serum biomarkers to detect asbestos exposure and to identify mesothelioma patients. Clin Cancer Res 22(12):3087–3096

Navarro-Compan V, Melguizo-Madrid E, Hernandez-Cruz B et al (2013) Interaction between oxidative stress and smoking is associated with an increased risk of rheumatoid arthritis: a case-control study. Rheumatology (Oxford) 52:487–493

Ohar JA, Cheung M, Talarchek J et al (2016) Germline BAP1 mutational landscape of asbestos-exposed malignant mesothelioma patients with family history of cancer. Cancer Res 76:206–215

Ostroff RM, Mehan MR, Stewart A et al (2012) Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. PLoS One 7:e46091

Park EK, Thomas PS, Creaney J et al (2010) Factors affecting soluble mesothelin related protein levels in an asbestos-exposed population. Clin Chem Lab Med 48:869–874

Park EK, Yates DH, Creaney J et al (2012) Association of biomarker levels with severity of asbestos-related diseases. Saf Health Work 3:17–21

Pass HI, Levin SM, Harbut MR et al (2012) Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. N Engl J Med 367:1417–1427

Pass HI, Lott D, Lonardo F et al (2005) Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. N Engl J Med 353:1564–1573

Pass HI, Wali A, Tang N et al (2008) Soluble mesothelin-related peptide level elevation in mesothelioma serum and pleural effusions. Ann Thorac Surg 85:265–272

Peipins LA, Lewin M, Campolucci S et al (2003) Radiographic abnormalities and exposure to asbestos-contaminated vermiculite in the community of Libby, Montana, USA. Environ Health Perspect 111:1753–1759

Pelclova D, Fenclova Z, Kacer P et al (2008) Increased 8-isoprostane, a marker of oxidative stress in exhaled breath condensate in subjects with asbestos exposure. Ind Health 46:484–489

Peluso I, Morabito G, Urban L et al (2012) Oxidative stress in atherosclerosis development: the central role of LDL and oxidative burst. Endocr Metab Immune Disord Drug Targets 12:351–360

Pilger A, Rudiger HW (2006) 8-Hydroxy-2′-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. Int Arch Occup Environ Health 80:1–15

Pooley FD (1972) Electron microscope characteristics of inhaled chrysotile asbestos fibre. Br J Ind Med 29:146–153

Prazakova S, Thomas PS, Sandrini A et al (2014) Asbestos and the lung in the 21st Century: an update. Clin Respir J 8:1–10

Qi F, Okimoto G, Jube S et al (2013) Continuous exposure to chrysotile asbestos can cause transformation of human mesothelial cells via HMGB1 and TNF-alpha signaling. Am J Pathol 183:1654–1666

Ramos-Nino ME, Testa JR, Altomare DA et al (2006) Cellular and molecular parameters of mesothelioma. J Cell Biochem 98:723–734

Reid A, de Klerk NH, Magnani C et al (2014) Mesothelioma risk after 40 years since first exposure to asbestos: a pooled analysis. Thorax 69:843–850

Robinson BW, Creaney J, Lake R et al (2003) Mesothelin-family proteins and diagnosis of mesothelioma. Lancet 362:1612–1616

Rodriguez Portal JA, Rodriguez BE, Rodriguez RD et al (2009) Serum levels of soluble mesothelin-related peptides in malignant and nonmalignant asbestos-related pleural disease: relation with past asbestos exposure. Cancer Epidemiol Biomark Prev 18:646–650

Sandhu H, Dehnen W, Roller M et al (2000) MRNA Expression patterns in different stages of asbestos-induced carcinogenesis in rats. Carcinogenesis 21:1023–1029

Sartorelli P, Romeo R, Scancarello G et al (2007) Measurement of asbestos fibre concentrations in fluid of repeated bronchoalveolar lavages of exposed workers. Ann Occup Hyg 51:495–500

Schulze PC, Lee RT (2005) Oxidative stress and atherosclerosis. Curr Atheroscler Rep 7:242–248

Schurkes C, Brock W, Abel J et al (2004) Induction of 8-hydroxydeoxyguanosine by man made vitreous fibres and crocidolite asbestos administered intraperitoneally in rats. Mutat Res 553:59–65

Sekido Y (2013) Molecular pathogenesis of malignant mesothelioma. Carcinogenesis 34:1413–1419

Selikoff IJ, Hammond EC, Seidman H (1980) Latency of asbestos disease among insulation workers in the United States and Canada. Cancer 46:2736–2740

Serban C, Dragan S (2014) The relationship between inflammatory and oxidative stress biomarkers, atherosclerosis and rheumatic diseases. Curr Pharm Des 20:585–600

Snyder NW, Mesaros C, Blair IA (2015) Translational metabolomics in cancer research. Biomark Med 9:821–834

Stayner L, Welch LS, Lemen R (2013) The worldwide pandemic of asbestos-related diseases. Annu Rev Public Health 34:205–216

Sterman DH, Albelda SM (2005) Advances in the diagnosis, evaluation, and management of malignant pleural mesothelioma. Respirology 10:266–283

Swain WA, O'Byrne KJ, Faux SP (2004) Activation of p38 MAP kinase by asbestos in rat mesothelial cells is mediated by oxidative stress. Am J Phys Lung Cell Mol Phys 286:L859–L865

Tabata C, Kanemura S, Tabata R et al (2013a) Serum HMGB1 as a diagnostic marker for malignant peritoneal mesothelioma. J Clin Gastroenterol 47:684–688

Tabata C, Shibata E, Tabata R et al (2013b) Serum HMGB1 as a prognostic marker for malignant pleural mesothelioma. BMC Cancer 13:205–211

Testa JR, Cheung M, Pei J et al (2011) Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 43:1022–1025

Valavanidis A, Vlachogianni T, Fiotakis C (2009) 8-Hydroxy-2′-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27:120–139

Vaught JD, Bock C, Carter J et al (2010) Expanding the chemistry of DNA for in vitro selection. J Am Chem Soc 132:4141–4151

Victor VM, Rocha M, Sola E et al (2009) Oxidative stress, endothelial dysfunction and atherosclerosis. Curr Pharm Des 15:2988–3002

Wang H, Yang H, Tracey KJ (2004) Extracellular role of HMGB1 in inflammation and sepsis. J Intern Med 255:320–331

Wang Q, Zhang S, Guo L et al (2015) Serum apolipoprotein A-1 quantification by LC-MS with a SILAC internal standard reveals reduced levels in smokers. Bioanalysis 7:2895–2911

Wu L, Ortiz C, Xu Y et al (2015) In situ liquid cell observations of asbestos fiber diffusion in water. Environ Sci Technol 49:13340–13349

Wu X, Li D, Liu L et al (2014) Serum soluble mesothelin-related peptide (SMRP): a potential diagnostic and monitoring marker for epithelial ovarian cancer. Arch Gynecol Obstet 289:1309–1314

Xu J, Kadariya Y, Cheung M et al (2014) Germline mutation of Bap1 accelerates development of asbestos-induced malignant mesothelioma. Cancer Res 74:4388–4397

Yamada S, Inoue K, Yakabe K et al (2003) High mobility group protein 1 (HMGB1) quantified by ELISA with a monoclonal antibody that does not cross-react with HMGB2. Clin Chem 49:1535–1537

Yamada S, Tabata C, Tabata R et al (2011) Clinical significance of pleural effusion mesothelin in malignant pleural mesothelioma. Clin Chem Lab Med 49:1721–1726

Yang H, Rivera Z, Jube S et al (2010) Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release and resultant inflammation. Proc Natl Acad Sci U S A 107:12611–12616

Yoshida R, Ogawa Y, Shioji I et al (2001) Urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine and biopyrrins levels among construction workers with asbestos exposure history. Ind Health 39:186–188

Zangar RC, Daly DS, White AM (2006) ELISA microarray technology as a high-throughput system for cancer biomarker validation. Expert Rev Proteomics 3:37–44

Zhou Q, Zhu Z, Hu X et al (2016) HMGB1: A critical mediator for oxidized-low density lipoproteins induced atherosclerosis. Int J Cardiol 202:956–957