〈Review〉

Investigating methods regarding diagnostic and prognostic biomarkers for malignant mesothelioma

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ABSTRACT Malignant mesothelioma (MM) is a pleural malignant tumor that results predominantly from exposure to asbestos and has a poor prognosis. After a brief review of the epidemiology, etiology, and clinical status of MM, we detail methods being used to search for diagnostic and prognostic biomarkers for MM, particularly approaches involving the use of blood samples. The soluble mesothelin-related protein (SMRP), mesothelin/ERC and osteopontin are typical biomarkers for MM. In addition to these biomarkers, fibulin-3 has recently been introduced as a biomarker for MM. Furthermore, several molecules have been reported as useful biomarkers. In addition to an introduction outlining newer approaches such as those of proteomics, we hope to summarize the recent status of biomarkers for MM in this review.

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Introduction, epidemiology and etiology of MM

Malignant mesothelioma (MM) is cancer occurring in the pleura, peritoneum, pericardium, as well as the tunica vaginalis testis[1-3]. MM predominantly occurs in the pleura, which represents more than 75% of MM cases. The frequency of deaths due to MM has been increasing in Japan. The number of MM deaths annually up to 1999 was approximately 500, whereas the number of deaths since 2000 has been gradually increasing. According to the open-data from the Ministry of Health, Labour and Welfare, Japan, the number of deaths reached 1,410 in 2013 and comprised 1,121 males and 289 females. The increase in the number of males was greater than that of females (Fig. 1)[4,5]. The import and use of asbestos peaked in 1974 and relatively high amounts were imported up to the early 1990s. The curves of MM deaths show a late phase of 40 years relative to the asbestos use curve, and the number of deaths due to MM in Japan is expected to be approximately 100,000 between 2025 and 2030[6,7].

The cause of MM is usually thought to be asbestos exposure[8-10], particularly in Japan. Although other minerals such as fibrous zeolite erionite cause MM, especially in the Cappadocia area of Turkey, these

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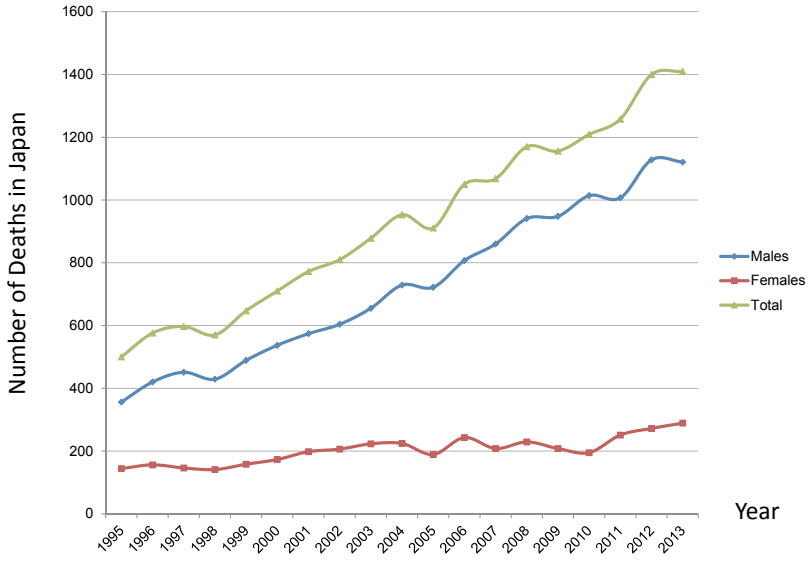

Fig. 1. Number of deaths from malignant mesothelioma in Japan (1995–2013). Reproduced using open-data from the web-site of the Ministry of Health, Labour and Welfare, Japan. The URL is as follows: http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/tokusyu/chuuhisyu13/index.html

areas, which include the Göreme National Park and Rock Site of Cappadocia, are registered as world heritage sites[11]. In addition, ionizing radiation which causes DNA damage is also considered a partial cause of MM[12,13]. Although the role of simian virus 40 (SV40) in the pathogenesis of MM has been discussed for considerable time, no definitive conclusion has been reached and researchers are still wondering whether there is a causal association between SV40 infection and MM[12,14,15]. At the experimental level, there are some reports showing that nano-tubes cause MM in animal models[16-18]. A study investigating differences between asbestos fibers and nano-tubes regarding their inclusion into cells[18] basically revealed that firm and rigid fibrous substances with an aspect ratio of more than 3 may play a significant role in one of the carcinogenic mechanisms because the phagocytosis of these fibers physically interferes with cellular function such as spindle formation at mitosis. In addition, the chemical composition of these substances, particularly that of asbestos fibers, promotes cation exchange and induced continuous reactive oxygen species (RPS), which cause direct DNA damage[12,19-21].

Diagnosis and treatments for MM

The clinical features of MM include a variety of respiratory symptoms such as chest pain, breathlessness and coughing, but there is no particular symptom that can be used to detect MM[3,22-24]. Sometimes MM cases are suspected due to the chance discovery of pleural effusion without any symptoms, or a chance radiological examination that shows an abnormal shadow in the pleura. Various radiological examinations such as a computed tomography (CT) scan, magnetic resonance imaging (MRI) and positron emission tomogrphy (PET)/CT should be performed once a MM case is suspected[3,25-29]. A histological or cytological diagnosis should then be established. If there is pleural effusion, usually unilateral, an aspiration biopsy may be performed and recent immunohistochemical staining can be implemented

using various antibodies such as calretinin, WT-1 and vimentin to detect MM with a high possibility [30-33]. One of the effective biopsy methods such as a CT-guided closed technique, biopsy under video-assisted thoracic surgery (VATS) or an open biopsy should then be performed[34-36].

The common treatments of MM are surgery, systemic chemotherapy, radiotherapy, and combined poly-modal therapies[38,39]. However, the prognosis of MM is still poor because of the difficulties of early diagnosis[40-42]. The standard cytotoxic therapy such as cisplatin and pemetrexed yields a 20 to 40% response rate with approximately 12 months overall survival[43-48]. Various molecular targeting therapies for MM have been developed recently, such as anti-angiogenic therapies using monoclonal antibodies[46,47], targeting for mutated genes such as BRCA1 (breast cancer susceptibility gene I)-associated protein 1 (BAP1)[48,49] and neurofibromatosis type 2 (NF2)[50,51] genes by histone deacetylase (HDAC) inhibitors or signal-transduction inhibitors. Moreover, immunotherapies targeting program death -1 (PD-1) or cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) have been developed[45]. Of course, various gene therapies[52,53] including reduced expression in immortalized cells (REIC)/Dickkopf-related protein 3 (Dkk3) gene have been developed[54,55] and will form the basis of future trials.

Biomarkers for MM

As mentioned above, since the prognosis of MM is still poor, diagnosis in the early stage is necessary in order for the early commencement of the various treatments. In this review, we introduce various candidates for diagnostic biomarkers of MM in serum and pleural effusion, although there are many prognostic biomarkers which have been investigated. Basically various molecules expressed in pathological specimens and/or genes including micro RNAs expressed by mesothelioma cells are studying as the prognostic markers.

1. Serum biomarkers for MM

1) Soluble mesothelin-related protein: SMRP[56-59]

Mesothelin is a glycoprotein attached to the cell surface of mesothelial cells and is involved in cell adhesion and cell-to-cell signaling. SMPR is an alternatively spliced form of mesothelin, and this variant is secreted from mesothelioma cells into extracellular spaces. The elevation of SMRP can therefore be detected in serum. Robinson *et al.* reported that SMPR can be used for the early detection and monitoring of disease progression of MM in Australian patients. The reported sensitivity and specificity are approximately 80 and 90%, respectively. There are also several reports investigating the use of SMRP to detect and monitor MM in Japan[60,61].

2) Mesothelin/ERC[62-65]

The ERC (expressed in renal carcinoma) gene was identified following investigation of Eker rat renal carcinoma, and is identified as a homolog of the human mesothelin gene. The product of the human mesothelin gene is cleaved by protease and the N-terminal fragment (N-ERC) is secreted into the blood. Hino *et al.* reported the development of an enzyme-linked immunosorbent assay (ELISA) system for the detection of N-ERC/mesothelin and demonstrated its usefulness for early diagnosis. It should be added that SMRP and N-ERC/mesothelin are also detected in cases of ovarian cancer.

3) Osteopontin[66-68]

Osteopontin (OPN), also known as bone sialoprotein I (BSP-1 or BNSP), early T-lymphocyte activation (ETA-1), secreted phosphoprotein 1 (SPP1), 2ar and Rickettsia resistance (Ric), is a glycoprotein and functions in biomineralization, bone remodeling, immune reactions such as chemotaxis, and cell adhesion. Although an elevated serum level of OPN has been reported in various tumors including lung, breast, gastro-intestinal and ovarian cancers, higher serum levels of OPN in MM

have also been reported with high sensitivity and specificity. However, its effectiveness for the early diagnosis of MM remains unconfirmed and requires further study.

4) Fibulin-3[69-71]

Recent methods to identify biomarkers of some diseases have made use of specialized and comprehensive searching methods such as those involving cDNA microarrays and proteomics. It was mentioned previously that OPN was identified using the cDNA microarray method, and the same group also demonstrated in a similar way that fibulin-3 is a biomarker for MM. According to their report, the serum fibulin-3 level can be used to distinguish asbestos-exposed patients without MM and the early (stage I and II) stages of MM.

5) Soluble syndecan-1[72]

Syndecan-1 (CD138) is a cell surface proteoglycan that plays a role in cell proliferation, differentiation, invasion migration and angiogenesis. The cellular expression as assayed by an immunohistochemical method is reported to distinguish adenocarcinoma from MM. The soluble form of syndecan-1 in serum was then proposed as a diagnostic and prognostic factor for MM.

6) Circulating fibrinogen[73]

Fibrinogen is an acute phase response protein for inflammation and is produced by the liver in response to pro-inflammatory cytokines similar to the C-reactive protein (CRP). Ghanim *et al.* reported that plasma circulating fibrinogen is a prognostic and predictive biomarker for MM. Although its sensitivity and specificity for the detection of MM and its value as a prognostic factor for overall survival in MM are not very high, it may be useful since plasma fibrinogen levels are usually measured during common medical screening for various diseases.

7) RANTES[74]

RANTES/CCL5 (chemokine (C-C motif) ligand 5) is a C-C chemokine associated with allergic immune reactions and immunomodulation in cancer. Comar *et al.* reported elevated levels of RANTES and CTACK (CCL27; chemokine (C-C motif) ligand 27) in asbestos-handling workers and MM patients using a magnetic bead multiplex immunoassay for 47 analytes including cytokines and growth factors. Although they reported significant differences in serum RANTES levels between asbestos-handling workers and MM patients, both groups showed overlapping increased levels of RANTES. However, this type of immunological approach may be important because tumor markers produced by malignant cancer cells have some limitation and depend on tumor volume, although early detection is therefore sometimes difficult using these products.

8) Hyaluronan[75]

Hyaluronan/hyaluronic acid is a linear polysaccharide associated with mesothelioma. Although hyaluronic acid is the classical marker for the detection of MM in pleural effusion[76,77], the serum level of hyaluronan shows less sensitivity for the detection of MM. Mundt *et al.* presented specific two-step prediction methods using hyaluronan and N-ERC/mesothelin. Using several markers to detect MM will be a valuable approach to increase the specificity and sensitivity of the detection process.

9) YKL-40/chitinase-3-like-1[78]

Corradi *et al.* reported that MM patients showed significantly higher serum levels of mesothelin, YKL-40, interleukin (IL)-8 and vascular endothelial growth factor (VEGF) compared to healthy controls. Higher levels of significance were obtained for mesothelin and YKL-40. However, their analysis did not distinguish MM from non-small cell lung cancer. Further analysis may be needed to clarify the effectiveness of this approach.

10) Newer approaches to identify biomarkers

i) Proteomics-based surveillance tool[79]

Ostroff *et al.* used a proteomics-based method and identified 13 genes/proteins with statistical significance between a control population and

pathogenic stages (I to IV) in MM using serum. They found that four proteins are significantly decreased in MM compared with healthy controls, namely, apolipoprotein A-I, fibronectin, KIT/stem cell factor receptor and kallistatin. Proteins showing significantly increased levels include C9 (complement component 9), C23 (chemokine (C-C motif) ligand 23), CDK5/CDK5R1 complex (cyclin-dependent kinase 5-/cyclin-dependent kinase 5, regulatory subunit 1 (p35) complex), CXCL13 (chemokine (C-X-C motif) ligand 13), F9 (coagulation factor IX), FCN2 (ficolin (collagen/fibrinogen domain containing lectin) 2), ICAM2 (intercellular adhesion molecule 2), MDK (midkine) and TNFRSF8 (CD30). Although most of the proteins have to be validated by other investigations, this type of newer approach may be useful in identifying unexpected molecules associated with mesothelioma and asbestos pathophysiology.

ii) Selected reaction monitoring (SRM) assay[80)]

Cerciello *et al.* claim to have identified a seven glycoprotein signature for MM in serum. They used selected reaction monitoring (SRM) assay technology, which relies on the ability of a triple quadrupole mass spectrometer (QQQ) to selectively isolate predefined peptides of interest in a complex protein mixture after enzymatic digestion. In addition to the seven glycoprotein signature, they identified candidate biomarkers of MM extracted by the SRM assay. The candidates with a concentration above 10^2 ng/ml include hemopexin, paraoxonase/anylesterase 1, attractin, thrombospondin-1, galectin-3-binding protein and basement membrane-specific heparin sulfate proteoglycan core protein. Candidate proteins with a concentration near 10^2 ng/ml include vasorin, ICAM-1 (intracellular adhesion molecule), phospholipid transfer protein, laminin subunit gamma-1 and CD44 antigen. The concentrations of candidates in this group are similar to those of hyaluronic acid, fibulin-3 and osteopontin. Although these candidates were not matched by the above-mentioned Ostroff report, this kind of approach will yield new insights regarding the search for biomarkers.

iii) Surface imprinting for detection of biomarkers[81)]

Mathur *et al.* reported development of a biosensor for the detection of a MM biomarker using surface imprinting and utilized hyaluronan-linked protein 1 (HAPLN1), which has been shown to be highly expressed in mesothelioma cells. They employed an amplifying detection method using surface imprinting. This approach is also important for the modification of detection methods for known molecules.

2. Biomarkers for MM in pleural effusion[82-85)]

Among the above-mentioned biomarkers, SMRP, N-ERC/mesothelin, OPN, fibulin-3 and hyaluronic acid are candidates for early diagnostic biomarkers of MM.

3. Immunological alterations caused by asbestos exposure and their use as biomarkers for MM

Our group has been investigating the effects of asbestos exposure on human immune competent cells. We found that Cd4+ T cells showed reduction of surface expression of CXCR3 (Chemokine (C-X-C motif) receptor 3) and suppressed capacity for interferon (IFN)-γ production[86,87)]. Natural killer (NK) cells represent one of the activation receptors for NK cells, and our research revealed reduced expression of NKp46 on the cell surface of freshly isolated NK cells from MM patients[88,89)]. Our group has recently been utilizing a magnetic bead multiplex immunoassay using plasma derived from asbestos-exposed patients with either pleural plaque or MM. Taken together with findings concerning alteration of cell surface molecules and RNA expression, these approaches will form the basis of a multi-factor detection assay for asbestos exposure and individuals with MM[90,91)].

Conclusion

This review details approaches concerning the

detection of diagnostic and prognostic biomarkers for MM. In Japan, the high-risk population for asbestos exposure includes people who have lived near asbestos-handling manufacturers, workers involved in building demolition, and individuals handling rubble due to earthquake and other disasters, and the screening methods used to detect asbestos exposure and MM still depend on radiological methods. However, issues regarding radiological exposure and the acceptable frequency of these examinations indicate this approach may not be suitable for the detection of MM. Screening methods that utilize peripheral blood and other body materials such as urine, saliva, hair and exhaled breath should therefore be developed and validated in the near future.

We have not mentioned newer findings regarding recently developed molecules for the pathological diagnosis of MM or prognostic marker genes including microRNAs (miRNA) specifically expressed or showing altered epigenetic status in MM tumor cells. However, a consideration of these aspects with the exploration of serum/plasma, effusion biomarkers, and the development of gene and molecular expression profiles of mesothelioma cells will result in the rapid development of detection methods that will help MM patients obtain a better prognosis.

Conflict of interest

All authors have no COI to declare regarding the content of this review.

References

1）Terracciano LM, Feroce F, Tornillo L. Pathology of malignant mesothelioma. In: Mesothelioma from bench side to clinic (Baldi A, ed.). New York, U.S.A., Nova Science Publisher, Inc., 2008, pp265-278.

2）Gibbs AR, Craighead JE. Malignant diseases of the pleura, peritoneum, and other serosal surfaces. In: Asbestos and its diseases (Craighead JE, Gibbs AR, eds.). New York, U.S.A., Oxford University Press, 2008, pp190-229.

3）Peak MD, Entwisle J, Gray S. Malignant pleural mesothelioma: Clinical presentation, radiological evaluation and diagnosis. In: Malignant pleural mesothelioma (O'Byrne K, Rusch V, eds.). New York, U.S.A., Oxford University Press, 2006, pp35-60.

4）Myojin T1, Azuma K, Okumura J, Uchiyama I. Future trends of mesothelioma mortality in Japan based on a risk function. Ind Health 50: 197-204, 2012.

5）Gemba K, Fujimoto N, Kato K, Aoe K, Takeshima Y, Inai K, Kishimoto T. National survey of malignant mesothelioma and asbestos exposure in Japan. Cancer Sci 103: 483-490, 2012. doi: 10.1111/j.1349-7006.2011.02165.x.

6）Azuma K, Uchiyama I, Chiba Y, Okumura J. Mesothelioma risk and environmental exposure to asbestos: past and future trends in Japan. Int J Occup Environ Health. 15: 166-172, 2009.

7）Murayama T, Takahashi K, Natori Y, Kurumatani N. Estimation of future mortality from pleural malignant mesothelioma in Japan based on an age-cohort model. Am J Ind Med 49: 1-7, 2006.

8）Menegozzo M, Pasetto R, Menegozzo S, Comba P. Epidemiology of mesothelioma: The role of asbestos. In: Mesothelioma from bench side to clinic (Baldi A, ed.). New York, U.S.A., Nova Science Publisher, Inc., 2008, pp25-66.

9）Hammar SP. Asbestos and mesothelioma. In: Asbestos: Risk assessment, epidemiology, and health effects, Dodson RF, Hammar SO, eds., 2nd ed.). New York, U.S.A. CRC Press, 2011, pp307-418.

10）Pavlisko EN, Sporn TA. Mesothelioma. In: Pathology of asbestos-associated diseases (Oury TD, Sporn TA, Roggli VL, eds., 3rd ed.). New York, U.S.A., Springer Science+Business Media, 2014, pp81-140.

11）Emri S, Tuncer M, Baris YI. Malignant pleural mesothelioma in Turkey. In: Malignant pleural mesothelioma (O'Byrne K, Rusch V, eds.). New York, U.S.A., Oxford University Press, 2006, pp27-34.

12）King JE, Hasleton PS. The epidemiology and aetiology of malignant mesothelioma. In: Malignant pleural mesothelioma (O'Byrne K, Rusch V, eds.). New York, U.S.A., Oxford University Press, 2006, pp1-18.

13）Lange JH, Hoskins JA, Mastrangelo G. Non-asbestos causes of mesothelioma. In: Mesothelioma from bench

side to clinic (Baldi A, ed.). New York, U.S.A., Nova Science Publisher, Inc., 2008, pp267-104.

14) Powers A, Bocchetta M, Carbone M. Viral factors in the pathogenesis of malignant mesothelioma. In: Malignant pleural mesothelioma (O'Byrne K, Rusch V, eds.). New York, U.S.A., Oxford University Press, 2006, pp147-162.

15) Manfredi JJ. The SV40 hypothesis: Evidence against a role for SV40 in the pathogenesis of mesothelioma. In: Mesothelioma from bench side to clinic (Baldi A, ed.). New York, U.S.A., Nova Science Publisher, Inc., 2008, pp243-264.

16) Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, Kitajima S, Kanno J. Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. J Toxicol Sci 33: 105-116, 2008.

17) Takagi A, Hirose A, Futakuchi M, Tsuda H, Kanno J. Dose-dependent mesothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice. Cancer Sci. 103: 1440-1444, 2012, doi: 10.1111/j.1349-7006.2012.02318.x.

18) Nagai H, Okazaki Y, Chew SH, *et al.* Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis. Proc Natl Acad Sci USA. 108: E1330-1338, 2011. doi: 10.1073/pnas.1110013108.

19) Yang H, Pass HI, Carbone M. Pathogenesis of mesothelioma. In: Mesothelioma from bench side to clinic (Baldi A, ed.). New York, U.S.A., Nova Science Publisher, Inc., 2008, pp105-116.

20) Nagai H, Ishihara T, Lee WH, Ohara H, Okazaki Y, Okawa K, Toyokuni S. Asbestos surface provides a niche for oxidative modification. Cancer Sci. 102: 2118-2125, 2011. doi: 10.1111/j.1349-7006.2011.02087.x.

21) Klebe S, Henderson DW. The molecular pathogenesis of asbestos-related disorders. In: Asbestos: Risk assessment, epidemiology, and health effects, Dodson RF, Hammar SO, eds., 2nd ed.). New York, U.S.A. CRC Press, 2011, pp109-130.

22) Cassandro R, Montrasio S, Esposito V. Natural history of human pleural mesothelioma. In: Mesothelioma from bench side to clinic (Baldi A, ed.). New York, U.S.A., Nova Science Publisher, Inc., 2008, pp337-346.

23) Cognetti, F, Ceribelli A, Cecere FL. Clinical oncology of mesothelioma. In: Mesothelioma from bench side to clinic (Baldi A, ed.). New York, U.S.A., Nova Science Publisher, Inc., 2008, pp347-356.

24) Weill D. Diagnostic features and clinical evaluation of the asbestos-associated diseases. In: Asbestos and its diseases (Craighead JE, Gibbs AR, eds.). New York, U.S.A., Oxford University Press, 2008, pp253-268.

25) Adams H, Crane MD. Radiological features of the asbestos-associated diseases. In: Asbestos and its diseases (Craighead JE, Gibbs AR, eds.). New York, U.S.A., Oxford University Press, 2008, pp269-298.

26) Au VW, Thomas M. Radiological manifestations of malignant pleural mesothelioma. Australas Radiol 47: 111-116, 2003.

27) Evans AL, Gleeson FV. Radiology in pleural disease: state of the art. Respirology 9: 300-312, 2004.

28) Wang ZJ, Reddy GP, Gotway MB, Higgins CB, Jablons DM, Ramaswamy M, Hawkins RA, Webb WR. Malignant pleural mesothelioma: evaluation with CT, MR imaging, and PET. Radiographics. 24: 105-119, 2004.

29) Marom EM, Erasmus JJ, Pass HI, Patz EF Jr. The role of imaging in malignant pleural mesothelioma. Semin Oncol 29: 26-35, 2002.

30) Inai K. Pathology of mesothelioma. Environ Health Prev Me. 13: 60-64, 2008, doi: 10.1007/s12199-007-0017-6.

31) Baldi F, Feroce F, Mellone P, Di Marino MP, Baldi A. The immunohistochemical picture of mesothelioma. In: Mesothelioma from bench side to clinic (Baldi A, ed.). New York, U.S.A., Nova Science Publisher, Inc., 2008, pp279-294.

32) King JE, Calateau-Sallé F, Hasleton PS. Histopathology of malignant pleural mesothelioma. In: Malignant pleural mesothelioma (O'Byrne K, Rusch V, eds.). New York, U.S.A., Oxford University Press, 2006, pp61-104.

33) Marchevsky AM. Application of immunohistochemistry to the diagnosis of malignant mesothelioma. Arch Pathol Lab Med. 132: 397-401, 2008, doi: 10.1043/1543-2165(2008)132[397:AOITTD]2.0.CO;2.

34) Walters J, Maskell NA. Biopsy techniques for the diagnosis of mesothelioma. Recent Results Cancer Res 189: 45-55, 2011,. doi: 10.1007/978-3-642-10862-4_4.

35) Nagendran M, Pallis A, Patel K, Scarci M. Should all patients who have mesothelioma diagnosed by video-assisted thoracoscopic surgery have their intervention sites irradiated? Interact Cardiovasc Thorac Surg 13: 66-

69, 2011,. doi: 10.1510/icvts.2011.267252.

36) Srivastava V, Dunning J, Au J. Does video-assisted thoracoscopic decortication in advanced malignant mesothelioma improve prognosis? Interact Cardiovasc Thorac Surg 8: 454-456, 2009, doi: 10.1510/icvts.2008.197160.

37) Boffa DJ, Rusch VW. Surgical and multimodal approaches to the management of operable malignant mesothelioma. In: Malignant pleural mesothelioma (O'Byrne K, Rusch V, eds.). New York, U.S.A., Oxford University Press, 2006, pp267-296.

38) Steele JPC, Rudd RM. Systemic chemotherapy for malignant pleural mesothelioma. In: Malignant pleural mesothelioma (O'Byrne K, Rusch V, eds.). New York, U.S.A., Oxford University Press, 2006, pp297-314.

39) Stevens CW, Forster KM, Smythe WR. Radiotherapy for mesothelioma. In: Malignant pleural mesothelioma (O'Byrne K, Rusch V, eds.). New York, U.S.A., Oxford University Press, 2006, pp315-334.

40) Ray M, Kindler HL. Malignant pleural mesothelioma: an update on biomarkers and treatment. Chest 136: 888-896, 2009, doi: 10.1378/chest.08-2665.

41) Su S. Mesothelioma: path to multimodality treatment. Semin Thorac Cardiovasc Surg 2009 Summer 21: 125-131, 2009, doi: 10.1053/j.semtcvs.2009.07.006.

42) Sugarbaker DJ, Wolf AS. Surgery for malignant pleural mesothelioma. Expert Rev Respir Med 4: 363-372, 2010, doi: 10.1586/ers.10.35.

43) Manegold C, Aisner J. Pemetrexed for diffuse malignant pleural mesothelioma. Semin Oncol 29(2s5): 30-35, 2002.

44) Nakano T. Current therapies for malignant pleural mesothelioma. Environ Health Prev Med 13: 75-83, 2008. doi: 10.1007/s12199-007-0016-7.

45) Remon J, Reguart N, Corral J, Lianes P. Malignant pleural mesothelioma: new hope in the horizon with novel therapeutic strategies. Cancer Treat Rev 41: 27-34, 2015, doi: 10.1016/j.ctrv.2014.10.007.

46) Antoniu SA, Dimofte G, Ungureanu D. Immune therapies for malignant mesothelioma. Expert Rev Anticancer Ther 14: 965-973, 2014, doi: 10.1586/14737140.2014.919859.

47) Palumbo C, Bei R, Procopio A, Modesti A. Molecular targets and targeted therapies for malignant mesothelioma. Curr Med Chem. 15: 855-867, 2008.

48) Testa JR, Cheung M, Pei J, *et al.* Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 43: 1022-1025, 2011, doi: 10.1038/ng.912.

49) Bott M, Brevet M, Taylor BS, *et al.* The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet 43: 668-672, 2011, doi: 10.1038/ng.855.

50) Sekido Y. Molecular pathogenesis of malignant mesothelioma. Carcinogenesis. 34: 1413-1419, 2013, doi: 10.1093/carcin/bgt166.

51) Sekido Y. Inactivation of Merlin in malignant mesothelioma cells and the Hippo signaling cascade dysregulation. Pathol Int 61: 331-344, 2011, doi: 10.1111/j.1440-1827.2011.02666.x.

52) Vachani A, Moon E, Albelda SM. Gene therapy for mesothelioma. Curr Treat Options Oncol 12: 173-180, 2011, doi: 10.1007/s11864-011-0153-5.

53) Takigawa N, Kiura K, Kishimoto T. Medical treatment of mesothelioma: anything new? Curr Oncol Rep. 13: 265-271, 2011, doi: 10.1007/s11912-011-0172-1.

54) Watanabe M, Nasu Y, Kumon H. Adenovirus-mediated REIC/Dkk-3 gene therapy: Development of an autologous cancer vaccination therapy(Review). Oncol Lett. 7: 595-601, 2014.

55) Kashiwakura Y, Ochiai K, Watanabe M, Abarzua F, Sakaguchi M, Takaoka M, Tanimoto R, Nasu Y, Huh NH, Kumon H. Down-regulation of inhibition of differentiation-1 via activation of activating transcription factor 3 and Smad regulates REIC/Dickkopf-3-induced apoptosis. Cancer Res. 68: 8333-8341, 2008, doi: 10.1158/0008-5472.CAN-08-0080.

56) Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. Soluble mesothelin-related protein--a blood test for mesothelioma. Lung Cancer. 49 (S1): S109-111, 2005.

57) Scherpereel A, Grigoriu B, Conti M, Gey T, Grégoire M, Copin MC, Devos P, Chahine B, Porte H, Lassalle P. Soluble mesothelin-related peptides in the diagnosis of malignant pleural mesothelioma. Am J Respir Crit Care Med. 173: 1155-1160, 2006.

58) Pass HI, Wali A, Tang N, Ivanova A, Ivanov S, Harbut M, Carbone M, Allard J. Soluble mesothelin-related peptide level elevation in mesothelioma serum and pleural effusions. Ann Thorac Surg. 85: 265-272, 2008.

59) Weber DG, Taeger D, Pesch B, Kraus T, Brüning T, Johnen G. Soluble mesothelin-related peptides

(SMRP) - high stability of a potential tumor marker for mesothelioma. Cancer Biomark. 3: 287-292, 20007.

60) Fukuoka K, Kuribayashi K, Yamada S, Tamura K, Tabata C, Nakano T. Combined serum mesothelin and carcinoembryonic antigen measurement in the diagnosis of malignant mesothelioma. Mol Clin Oncol 6: 942-948, 2013.

61) Fujimoto N, Gemba K, Asano M, Wada S, Ono K, Ozaki S, Kishimoto T. Soluble mesothelin-related protein in pleural effusion from patients with malignant pleural mesothelioma. Exp Ther Med 1: 313-317, 2010.

62) Shiomi K, Miyamoto H, Segawa T, Hagiwara Y, Ota A, Maeda M, Takahashi K, Masuda K, Sakao Y, Hino O. Novel ELISA system for detection of N-ERC/mesothelin in the sera of mesothelioma patients. Cancer Sci 97: 928-932, 2006.

63) Sato T, Suzuki Y, Mori T, Maeda M, Abe M, Hino O, Takahashi K. Newly established ELISA for N-ERC/mesothelin improves diagnostic accuracy in patients with suspected pleural mesothelioma. Cancer Med 5: 1377-1384, 2014, doi: 10.1002/cam4.297.

64) Hirohashi T, Igarashi K, Abe M, Maeda M, Hino O. Retrospective analysis of large-scale research screening of construction workers for the early diagnosis of mesothelioma. Mol Clin Oncol 2: 26-30, 2014.

65) Saeki H, Hashizume A, Izumi H, Suzuki F, Ishi K, Nojima M, Maeda M, Hino O. The utility of serum N-ERC/mesothelin as a biomarker of ovarian carcinoma. Oncol Lett 4: 637-641, 2012.

66) Pass HI, Lott D, Lonardo F, Harbut M, Liu Z, Tang N, Carbone M, Webb C, Wali A. Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. N Engl J Med 353: 1564-1573, 2005.

67) Park EK, Thomas PS, Johnson AR, Yates DH. Osteopontin levels in an asbestos-exposed population. Clin Cancer Res 15: 1362-1366, 2009, doi: 10.1158/1078-0432.CCR-08-0360.

68) Cristaudo A, Foddis R, Bonotti A, *et al.* Comparison between plasma and serum osteopontin levels: usefulness in diagnosis of epithelial malignant pleural mesothelioma. Int J Biol Markers 25: 164-170, 2010.

69) Pass HI, Levin SM, Harbut MR, *et al.* Fibulin-3 as a blood and effusion biomarker for pleural mesothelioma. N Engl J Med 367: 1417-1427, 2012, doi: 10.1056/NEJMoa1115050.

70) Kaya H, Demir M, Taylan M, Sezgi C, Tanrikulu AC, Yilmaz S, Bayram M, Kaplan I, Senyigit A. Fibulin-3 as a diagnostic biomarker in patients with malignant mesothelioma. Asian Pac J Cancer Prev 16: 1403-1407, 2015.

71) Creaney J, Dick IM, Meniawy TM, *et al.* Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma. Thorax 69: 895-902, 2014, doi: 10.1136/thoraxjnl-2014-205205.

72) Mundt F, Heidari-Hamedani G, Nilsonne G, Metintas M, Hjerpe A, Dobra K. Diagnostic and prognostic value of soluble syndecan-1 in pleural malignancies. Biomed Res Int. 2014; 2014: 419853. doi: 10.1155/2014/419853.

73) Ghanim B, Hoda MA, Klikovits T, *et al.* Circulating fibrinogen is a prognostic and predictive biomarker in malignant pleural mesothelioma. Br J Cancer 110: 984-990. doi: 10.1038/bjc.2013.815.

74) Comar M, Zanotta N, Bonotti A, Tognon M, Negro C, Cristaudo A, Bovenzi M. Increased levels of C-C chemokine RANTES in asbestos exposed workers and in malignant mesothelioma patients from an hyperendemic area. PLoS One 9: e104848, 2014, doi: 10.1371/journal.pone.0104848.

75) Mundt F, Nilsonne G, Arslan S, Csürös K, Hillerdal G, Yildirim H, Metintas M, Dobra K, Hjerpe A. Hyaluronan and N-ERC/mesothelin as key biomarkers in a specific two-step model to predict pleural malignant mesothelioma. PLoS One 8: e72030, 2013. doi: 10.1371/journal.pone.0072030.

76) Harington JS, Wagner JC, Smith M. The detection of hyaluronic acid in pleural fluids of cases with diffuse pleural mesotheliomas. Br J Exp Pathol 44: 81-83, 1963.

77) Pettersson T, Fröseth B, Riska H, Klockars M. Concentration of hyaluronic acid in pleural fluid as a diagnostic aid for malignant mesothelioma. Chest 94: 1037-1039, 1988.

78) Corradi M, Goldoni M, Alinovi R, Tiseo M, Ampollini L, Bonini S, Carbognani P, Casalini A, Mutti A. YKL-40 and mesothelin in the blood of patients with malignant mesothelioma, lung cancer and asbestosis. Anticancer Res. 33: 5517-5524, 2013.

79) Ostroff RM, Mehan MR, Stewart A, Ayers D, Brody EN, Williams SA, Levin S, Black B, Harbut M, Carbone M, Goparaju C, Pass HI. Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. PLoS One. 7: e46091, 2012, doi: 10.1371/journal.

pone.0046091. Epub 2012 Oct 3.

80) Cerciello F, Choi M, Nicastri A, Bausch-Fluck D, Ziegler A, Vitek O, Felley-Bosco E, Stahel R, Aebersold R, Wollscheid B. Identification of a seven glycopeptide signature for malignant pleural mesothelioma in human serum by selected reaction monitoring. Clin Proteomics. 10:16, doi: 10.1186/1559-0275-10-16.

81) Ziegler A, Cerciello F, Bigosch C, Bausch-Fluck D, Felley-Bosco E, Ossola R, Soltermann A, Stahel RA, Wollscheid B. Proteomic surfaceome analysis of mesothelioma. Lung Cancer. 75: 189-196, 2012, doi: 10.1016/j.lungcan.2011.07.009.

82) Zucali PA, Ceresoli GL, De Vincenzo F, Simonelli M, Lorenzi E, Gianoncelli L, Santoro A. Advances in the biology of malignant pleural mesothelioma. Cancer Treat Rev 37: 553-558, 2011, doi: 10.1016/j.ctrv.2011.01.001.

83) Tsujimura T, Torii I, Sato A, Song M, Fukuoka K, Hasegawa S, Nakano T. Pathological and molecular biological approaches to early mesothelioma. Int J Clin Oncol 17:40-47, 2012, doi: 10.1007/s10147-011-0369-1.

84) Henderson DW, Reid G, Kao SC, van Zandwijk N, Klebe S. Challenges and controversies in the diagnosis of mesothelioma: Part 1. Cytology-only diagnosis, biopsies, immunohistochemistry, discrimination between mesothelioma and reactive mesothelial hyperplasia, and biomarkers. J Clin Pathol 66: 847-853, 2013, doi: 10.1136/jclinpath-2012-201303.

85) Henderson DW, Reid G, Kao SC, van Zandwijk N, Klebe S. Challenges and controversies in the diagnosis of malignant mesothelioma: Part 2. Malignant mesothelioma subtypes, pleural synovial sarcoma, molecular and prognostic aspects of mesothelioma, BAP1, aquaporin-1 and microRNA. J Clin Pathol 66: 854-861, 2013, doi: 10.1136/jclinpath-2013-201609.

86) Maeda M, Nishimura Y, Hayashi H, Kumagai N, Chen Y, Murakami S, Miura Y, Hiratsuka J, Kishimoto T, Otsuki T. Reduction of CXC chemokine receptor 3 in an in vitro model of continuous exposure to asbestos in a human T-cell line, MT-2. Am J Respir Cell Mol Biol 45: 470-479, 2011, doi: 10.1165/rcmb.2010-0213OC.

87) Maeda M, Nishimura Y, Hayashi H, Kumagai N, Chen Y, Murakami S, Miura Y, Hiratsuka J, Kishimoto T, Otsuki T. Decreased CXCR3 expression in CD4+ T cells exposed to asbestos or derived from asbestos-exposed patients. Am J Respir Cell Mol Biol 45: 795-803, 2011,. doi: 10.1165/rcmb.2010-0435OC.

88) Nishimura Y, Miura Y, Maeda M, Kumagai N, Murakami S, Hayashi H, Fukuoka K, Nakano T, Otsuki T. Impairment in cytotoxicity and expression of NK cell- activating receptors on human NK cells following exposure to asbestos fibers. Int J Immunopathol Pharmacol 22: 579-590, 2009.

89) Nishimura Y, Maeda M, Kumagai N, Hayashi H, Miura Y, Otsuki T. Decrease in phosphorylation of ERK following decreased expression of NK cell-activating receptors in human NK cell line exposed to asbestos. Int J Immunopathol Pharmacol 22: 879-888, 2009.

90) Kumagai-Takei N, Maeda M, Chen Y, Matsuzaki H, Lee S, Nishimura Y, Hiratsuka J, Otsuki T. Asbestos induces reduction of tumor immunity. Clin Dev Immunol 2011;2011:481439. doi: 10.1155/2011/481439.

91) Matsuzaki H, Maeda M, Lee S, Nishimura Y, Kumagai-Takei N, Hayashi H, Yamamoto S, Hatayama T, Kojima Y, Tabata R, Kishimoto T, Hiratsuka J, Otsuki T. Asbestos-induced cellular and molecular alteration of immunocompetent cells and their relationship with chronic inflammation and carcinogenesis. J Biomed Biotechnol 2012;2012:492608. doi: 10.1155/2012/492608.