GESDAV

Journal of Environmental and Occupational Science

available at www.scopemed.org

Review Article

A key role of microRNAs as early biomarkers in mesothelioma

Barbara Ficociello[1], Claudio Minoia[2], Elena Sturchio[1]

[1]INAIL Research, Department for Production Plants and Anthropic Settlements, Via Alessandria, Rome, Italy
[2]Laboratory for Environmental and Toxicological Testing, IRCCS Pavia, S. Maugeri Foundation, Via S. Maugeri 10, Pavia, Italy.

Received: August 09, 2013

Accepted: August 09, 2013

Published: August 22, 2013

DOI : 10.5455/jeos.20130809110116

Corresponding Author:
Elena Sturchio,
INAIL Research, Department for Production Plants and Anthropic Settlements, Via Alessandria, 220/E - 00198 Rome, Italy
e.sturchio@inail.it

Key words: Biomarkers, asbestos exposure; mesothelioma, microRNAs

Abstract
Mesothelioma is a rare cancer that is caused almost exclusively by exposure to asbestos. Although the use of asbestos has been reduced dramatically in recent decades, the incidence of mesothelioma has remained steady. Scientific literature has consistently shown that this disease has a latency period ranging between 20 and 60 years, then it will continue to rank among major social and healthcare issues for decades to come. There is no single established path to a mesothelioma diagnosis. However, the process often involves multiple procedures. The lack of biomarkers capable of providing predictive estimates for malignant pleural mesothelioma in relation to asbestos exposure in work and environment settings is a significant shortfall. The latter shortfall is compounded by a present-day lack of clinical or therapeutic options capable of stalling the development of pathology; hence the mean survival rate (from time of diagnosis) of approximately 10 months.
This study provides a review of current knowledge on etiopathogenetic mechanisms in mesothelioma, and on diagnostic/prognostic biomarkers. As reported by recent literature, studies on microRNAs have proved to be of special interest. Main focus is addressed in particular at current knowledge progresses concerning the role of microRNAs in malignant pleural mesothelioma, showing the significance and uses of such biomarkers.
A comparative analysis of different data from various reported papers reveals the consistency or the divergence of these results providing useful clues to suggest new directions in future research studies.

© 2013 GESDAV

INTRODUCTION

The term “asbestos” is used to identify a group of naturally occurring fibrous silicates, and it is present in nature in two main forms: amphibole (crocidolite, amosite, tremolite, anthophyllite and actinolite) and serpentine (chrysotile) fibers. Asbestos, classified as a Group 1 “carcinogenic to humans" by the International Agency for Research on Cancer [1], has been in the past and will continue to be for the next decades a major social and public health issue. Environmental and occupational exposure to asbestos fibers induces typical diseases, in particular asbestosis, pleural plaques, lung cancer and mesothelioma. Although asbestos production and use has been banned in Italy since 1992, the mineral is still present in a large number of materials and manufacts and continues to be a significant source of occupational and environmental exposure of a possible risk that is particularly high in areas where the presence of asbestos is well documented. Cases show the highest incidence in areas, where asbestos industries laid, such as asbestos cement production plants, the shipbuilding and repair. Malignant pleural mesothelioma (MPM) may affect not only workers but also their families, which are exposed through contaminated workers’ clothes and hairs. Therefore, the number of mesothelioma cases in worker's family members significantly contributes to the global burden of this asbestos-related disease [2]. Scientific literature has consistently shown that MPM

has a latency period ranging between 20 and 60 years, hence it is anticipated that the incidence of this disease will peak in Italy in the time-period 2017-2020, with a number of cases per year in the order of 1,000. It is still necessary to develop efforts aimed at refining tools adequate for early diagnosis and therapy of the disease [3]. Accordingly with the above mentioned needs, the present review addressed at collecting the existing evidence regarding the relationship between MPM and microRNAs (miRNAs). In particular, we will evaluate the possibility of using miRNAs as biomarkers of the presence of MPM at its early stage, as well as collecting information regarding the prognosis and even the aetiology of the disease. A comparison between the results of several different studies should reveal data consistency or divergence, providing useful clues as to which direction to take in future research.

EXPOSURE TO ASBESTOS AND ETIOPATHOGENETIC MECHANISMS

Malignant mesothelioma is a rare form of cancer arising from the thin serous membrane of the body cavities: pleura, peritoneum, tunica vaginalis testis, and pericardium. MPM is the prevalent form of malignant mesothelioma originating in the thoracic cavity. The disease tends to progress slowly and often does not cause symptoms in its early stages. Peritoneal mesothelioma is the second form of the disease, accounting for less than 30% of all mesothelioma cases, it has a high infiltration capacity, and the severity of the prognosis exceeds even that of MPM. Pericardium as well as male and female reproductive organs mesotheliomas are very rare [4]. Very rare forms of mesothelioma occur in the pericardium, as well as the mesothelium of the male and female reproductive organs.

All forms of asbestos can cause MPM, but it is well known that about 80% of cases is correlated to asbestos amphibole forms (crocidolite and amosite) while chrysotile asbestos appears less potent for the induction of these diseases [5]. The damage induced by asbestos relates to route and duration of exposure, dose and geometry, size, physical and chemical properties of the fibers. In particular, size and chemical composition have a strong impact on fiber cytotoxicity, biopersistence and biodegradability, determining biological differences in pathogenicity [6].

If asbestos fibers are sufficiently small, they can pass through the alveolar wall. Penetration might be passive, but fibers may be transferred by macrophages via the lymphatic or haematic systems, reaching iliac lymph nodes, the large intestine and pleura. Generally, while larger and longer fibers penetrate no further than the alveoli, they undergo various forms of transformation and decay which can lead to their downsizing and, hence, a subsequent increase in their penetration capacity. Fibers longer than 5μm are not subject to phagocytosis by macrophages, or are only partially phagocytised, triggering intense oxidative and inflammatory reactions which are at the basis of the developing of asbestosis [7]. The mechanisms by which asbestos induces mesothelioma are not yet clearly defined (Figure 1), but the concurrence of several cellular alterations (creation of ROS, RNS and apoptosis) and molecular mechanisms (genetic and epigenetic alterations, chromosomal damage, altered gene expression) in mesothelial cells is very likely. In fact, asbestos activates cellular signalling pathways that regulate gene expression and cell fate, either through direct interactions with receptors or via genesis of ROS [8]. In this view, it is particularly important collecting a better understanding of the capacity and the mechanism by which asbestos fibers affect signalling pathways, in order to point out strategies for prevention and therapy of asbestos-related diseases.

Fibers directly bind to Epidermal Growth Factor Receptors (EGFR) on the surface of the mesothelial cells, activating, *via* phosphorylation, mitogen-activated protein kinase/extracellular signal-regulated kinases-mediated pathways (MAPK/ERK). The MAPK pathways, involving a series of protein kinase cascades, play a critical role in cell proliferation. Especially, the *c-fos* and *c-jun* proto-oncogenes are positively regulated by phosphorylation of its N-terminal activation domain by MAPK, with a significant increased of activator protein 1 (AP-1) [9].

Furthermore, the pathogenetic mechanism of asbestos fibers is also responsible for increased tumour necrosis factor-alpha (TNF-α) expression also resulting from macrophage build-up due to activation within pulmonary and pleural tissues [10]. According to Yang et al. (2006) [11], asbestos fibers induce alveolar macrophages to release TNF-α, which, in turn, stimulates mesothelial cells to enhance the production of TNF-α, promotes higher levels of tumour necrosis factor receptor-1 (TNF-R1) expression and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signalling pathways activation in mesothelial cells. Then, NF-kB increases the cell survival and thereby the amount of cells asbestos-damaged susceptible to malignant transformation [12]. Data suggest that asbestos-induced ROS and RNS have a critical role in triggering cell injury directly by alveolar macrophages, polymorphonuclear neutrophils and indirectly by target cell damaged because of unsuccessful phagocytosis [13]. Then TNF-α, ROS and RNS are key proximal mediators of asbestos pulmonary toxicity to activate redox-sensitive transcription factors such as AP-1 and NF-kB, functioning by activating apoptotic death

pathways and inducing a local chronic inflammation. Chronic inflammation is compounded by neoplastic invasion supported by autocrine and paracrine induction of multiple growth factors [14].

Furthemore, asbestos-induced DNA damage and mutagenesis are dependent on the intracellular levels of redox ROS and RNS, that may cause DNA adducts, mutations, transversions or insertions, chromosomal DNA damages [15,16,17]. The genetic alterations, such as, chromosomal mutations, micronuclei formation, lagging chromosomes are caused by direct interaction between the asbestos fibers and mitotic spindle. The chromosomal mutations may affect oncogenes and tumour suppressor genes encoding critical growth regulators involved in the transformation of the cells [8,9,12]. The hypothesis that an increased damage to DNA could promote asbestos carcinogenicity is supported by several studies. In fact DNA damage caused by continuous production of ROS and RNS may determine a genetic instability that alters normal expression of many DNA repair genes (GADD153, Cip1, p53, Ku70) [8,18]. The asbestos effects are directly or indirectly induced by various factors that can lead to a wide range of genetic and molecular alterations, including epigenetic changes. The correlation between the epigenetic changes with the development of mesothelioma has not been fully clarified yet. Epigenetic modulation (e.g. promoter methylation, miRNA and histone acetylation) plays a crucial role in the control of transcription and in the maintenance of normal homeostasis of cells.

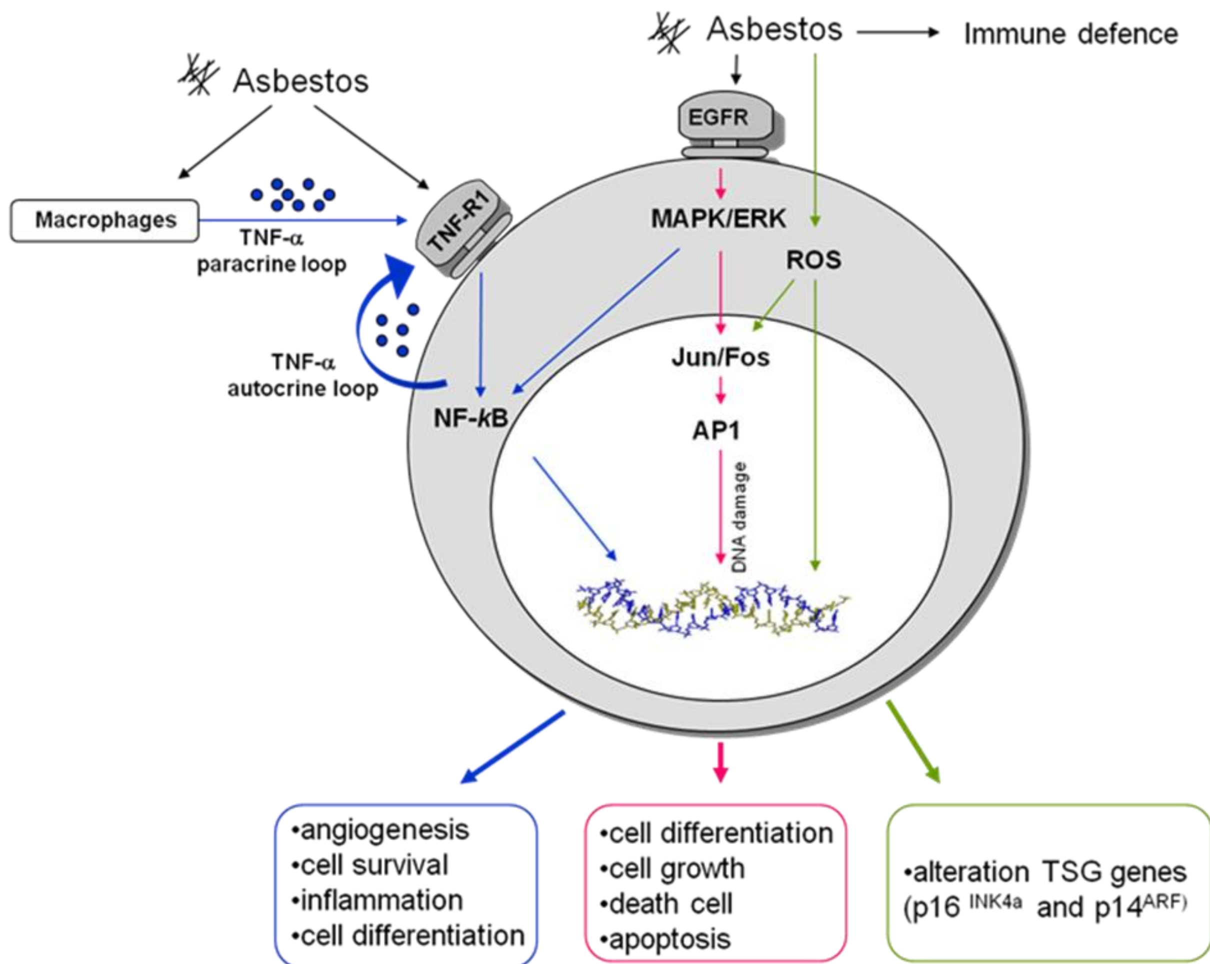

Figure 1. Asbestos's pathogenicity in mesothelial cell

Epidermal growth factor receptors (EGFR), mitogen-activated protein kinase/extracellular signal-regulated kinases-mediated pathways (MAPK/ERK), activator protein 1 (AP-1), tumour necrosis factor-alpha (TNF-α), tumour necrosis factor receptor-1 (TNF-R1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), reactive oxygen species (ROS), Tumor Suppressor Gene (TSG).

BIOMARKERS

The principal aim in cancer surveillance and screening programs is the diagnosis of the disease in the early stages of the disease. In recent years research has, therefore, also concentrated on identifying and developing diagnostic and prognostic tests in ex-exposed subjects [19,20]. To date, imaging techniques (chest radiography, chest computed and positron emission tomography), that are not always effective and appropriate for screening purposes, represent the main tools for the screening, diagnosis and prognosis of MPM, and to monitor the response to treatments.

With reference to MPM, research has focused on identifying significant tumour markers, but, none of the classical tumour biomarkers measured in biological fluids (serum, or pleural effusions), either alone or in combination, is sensitive or specific enough for the early diagnosis of MPM (e.g. Hyaluronic Acid, Carbohydrate Antigen (CA) 15-3, CA15-5, CA125, CA19-9, Carcinoembryonic Antigen, Cytokeratin Fragments, Tissue Polypeptide Antigen) [21-26].

Soluble mesothelin (SMRP) and osteopontin (OPN) are currently considered as promising sensitive and specific tumour markers for MPM but are still subject to some limitations. While several studies evidenced that the use of SMPR is useful in screening for early signs of disease [23,27], other evidence, in a large-scale prospective study, on the contrary considered that the use of this marker in detection of asbestos cancer not to be useful in MPM screening due to the high false-positive rate [28,29]. Otherwise, authors, do not deem OPN serum concentration levels an adequate diagnostic marker due to its lack of specificity to differentiate between subjects with pleural mesothelioma and subjects with lung carcinoma metastasis or subjects with benign pleural lesions associated with asbestos exposure [30,31].

Recent research has suggested the possibility of pointing out novel approaches for differential diagnosis of malignant mesothelioma, alternative or complementary to classic approaches such as cytologic, histochemical and immunohistochemical methods. In particular, studies of epigenetic modulation, transcriptomic and gene expression analysis by microarray technology could lead to identifying new diagnostic biomarkers for mesothelioma, potentially adequate to achieve early diagnosis and selective therapy. Altered epigenetic transcriptional regulation is typical of neoplasia. Comparative studies of MPM subsets evidenced interindividual variations of molecular alterations due to both genetic and epigenetic alterations. As known, changes of hypermethylation of CpG islands in the promoter region and/or hypoacetylation and hypermethylation of histones H3 and H4 may contribute to the development of malignant phenotype mediated by a downregulation of tumour suppressor genes. Recent researches have shown that CpG island hypermethylation can be used as an epigenetic biomarker in cancer, using pattern variability for diagnostic and classification purposes [32,33]. The asbestos-induced epigenetic changes, as promoter hypermethylation may determine changes in transcriptional silencing critical for neoplastic development. Data analysis confirmed distinct methylation profiles between MPM from asbestos exposed and from non-exposed patients and a significant positive association between asbestos fibers burden and methylation status of cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B), *Ras* association (RalGDS/AF-6) domain family member 1 (RASSF1), metallothionein 1A and 2A (MT1A, MT2A) [34]. Studies on the correlations between the degree of gene methylation and asbestos exposure have focused on two promoters of the CDKN2A gene which encodes p16INK4a and p14ARF transcription factors. The p16INK4a protein is a negative regulator of the CCND1/CDK4/P16INK4a/RB1/E2F pathway that play a role in the G1-S transition of the cell cycle, blocking cell cycle at the G1 phase [35].

On the contrary, p14ARF protein interacts with MDM2 protein and is able to block p53 decay. Results show that in 31% of mesothelioma tissue samples are detectable p16 alterations, in 8.8% promoter sequence methylation and in 22% p16 gene deletion, with only 2% showing signs of point mutations of the gene itself.

In two separate studies, Christensen et al. (2008) (2009) confirm the presence of significant relationship between asbestos exposure and altered DNA methylation levels in mesothelioma patients' tissues [34,36]. The latter epigenetic alterations correlate with the disease's progression; long latency periods may cause alterations to the methylation of specific gatekeeper genes and engender a cell turnover within specific altered cells capable of promoting the carcinogenic process. Furthermore, differential DNA methylation asbestos-related, investigated on several cancer-related genes defined the distinct profiles of DNA methylation in mesothelioma compared to non tumorigenic pleural tissues. Results of the study have underlined specific pathways (Fc epsilon RI Signaling and Calcium Signaling) with gene-loci enriched for methylation in mesothelioma *versus* lung adenocarcinoma. Christensen et al. propose that methylation profiles could be considered powerful markers for differential diagnosis of lung adenocarcinoma, mesothelioma and non malignant lung disease.

Goto at al. showed that MPM and adenocarcinoma

have characteristic methylation patterns likely resulting by different pathologic processes [37]. Their findings evidenced that three DNA hypermethylated genes (TMEM30B, KAZALD1, and MAPK13) were specifically methylated only in MPM and could serve as potential diagnostic markers. Interestingly, MPM cases that had very low levels of DNA methylation were related to a longer survival. These results suggested that DNA hypermethylation in MPM could lead to the silencing of tumour suppressor genes and consequently influence mechanisms affecting progression of this disease. However, DNA methylation as diagnostic biomarkers presents some critical issues, because in pleural mesothelioma DNA methylation is dependent on age, ethnicity, histologic subtype, and asbestos exposure.

THE ROLE OF microRNAs

MiRNAs are endogenous, single-stranded, non-coding RNA molecules ~21 nucleotides long which act as negative regulators of gene expression at the post-transcriptional level [38]. MiRNAs have gained great attention in recent years due to their important role in crucial cellular processes, such as apoptosis, differentiation, proliferation mechanisms, angiogenesis, transformation, resistance to antiblastic drugs and development [39]. To date, over 1500 different miRNAs have been identified in human and each of them is predicted to regulate hundreds of target genes [40,41].

It is now recognized that miRNAs are deregulated in a variety of human tumours and that they can function as both oncogenes and tumour suppressors [42-45]. The factors that affect alterations to miRNA expression profiles and that may determine the loss of function of a miRNA, are not entirely understood. One hypothesis is that alterations to cellular mechanisms, such as transcriptional regulation and epigenetic silencing, as well as miRNA biosynthesis mechanisms, are directly involved [46]. Another hypothesis involves anomalies in chromosomal regions, such as genomic deletion, mutation, where miRNA-coding genes are located [42]. A significant variety of miRNAs are mapped to cancer-associated genomic regions or to fragile sites (e.g. let-7 as a putative tumour suppressor) [47,48,49].

It is evident that miRNA expression profiling may be useful to detect the presence in the organism of pathological tissues and organs, and even to identify the histologic origin of poorly differentiated cancers. The first study on 2002 was focused on chronic lymphocytic leukemia, then other researches on colon, breast, lung, ovarian and pancreatic carcinomas were followed [50-56]. Lawrie et al. (2008) published the first study leading to the identification of miRNAs in serum; their work revealed that miR-155, miR-21 and miR-210 miRNAs are over-expressed in patients affected by diffuse large B-cell lymphoma when compared to healthy subjects [57].

Furthermore, high miR-21 levels have been linked to longer survival times in the absence of recidivism. Additional researches suggest that miRNA signatures may be useful in detecting and predicting the course of several human cancers [58,59,60].

Chen et al., (2008) performed their research on patients with rectal cancer and lung carcinoma [61]. The study led to the identification of 8 serum miRNAs (including miR-25 and miR-223, which are known to be cancer-specific) in lung cancer patients, and 14 serum miRNAs (including miR-485-5p, miR-361-3p, miR-326 and miR-487b) in colorectal cancer patients, providing evidence that circulating miRNAs could be considered as signatures for different pathologies.

The MPM is a tumour difficult to diagnose, clinically similar to other types of lung neoplasia, in particular lung adenocarcinoma [45]. The first ever commercial release of a miRNA-based diagnostic test (ProOnc Mesothelioma) took place in the United States in 2009; its reliability is disputed [62]. Even if potential miRNAs expression profiles involved in lung adenocarcinoma etiopathogenesis have been defined, they neither allow for differential diagnosis nor do they clarify the biological pathways involved in disease progression. Studies by Gee et al., (2010) have identified a cluster of 7 miRNAs considered to be mesothelioma-specific through a study based on 250 miRNAs in lung adenocarcinoma and MPM histological samples [63]. Their data, however, does not allow for differentiation between mesothelioma histotypes. They demonstrated that miRNAs belonging to the miR-200 gene family, miR-203 and miR-205 are down-regulated in mesothelioma but not in lung carcinoma patients. Significantly, four of the miR-200 family members (miR-200b, miR-200c, miR-141 and miR-429) could be used for discriminating between adenocarcinoma and mesothelioma. Regarding these selected miRNAs, ROC curve analysis, with AUC (95% CI), confirms their high predictivity, specificity and sensitivity values as mesothelioma markers. These values are consistent with International Mesothelioma Panel recommendations set, in fact they require an 80% specificity and sensitivity to identify the effectiveness of biomarkers.

These panel of miRNAs play a role in modulation of proteins associated with *Wnt* signaling pathway. In fact, the study has revealed that *c-jun, c-myc, EGR* and *Wnt5B* genes involved in *wnt*-mediated pathways are up-regulated in MPM. MiR-200 family and miR-205 are silenced in advanced cancer and may inhibit

tumour-cell invasion and metastasis [64,65].

Guled et al. have identified 12 highly expressed miRNAs (e.g. let-7b*, miR-1228*, miR-195*, miR-30b*, miR-32*, miR-345, miR-483-3p, miR-584, miR-595, miR-615-3p, and miR-885-3p) exclusively within affected tissues and 9 miRNAs (e.g. let-7e*, miR-144*, miR-203, miR-340*, miR-34a*, miR-423, miR-582, miR-7-1*, and miR-9) whose expression showed no differentiation compared to the control sample [66]. The study has also identified target genes predicted by computational algorithms (Sanger miRBase and miRanda). Among predicted gene targets related to miRNA highly expressed (miR-885-3p) there are oncosuppressor genes (CDK2A and NF2). CDK2A gene is inactivated in more than 80% of MPM and NF2 is linked to cellular invasiveness. The target genes of unexpressed miRNA included oncogenes (miR-9 and miR-203) that are related to proto-oncogene *Jun* involved in cell transformation, division and proliferation. Moreover, miR-203 is associated to Hepatocyte Growth Factor regulation which is involved in differential cell growth. Recent data support a possible link between mesothelioma and dysregulation of proto-oncogene expression. Results have shown that activation of mesothelioma malignant cell with ephrinA1 could lead to an induction of miRNA let-7 expression, with resulting repression of proto-oncogene *Ras* and the reduction of tumour growth [67]. Guled et al. did not reveal differential miRNA expressions in asbestos-exposed and non-exposed mesothelioma patients, the data allow to distinguish different mesothelioma using different miRNA expression profiles. However, the different and specific miRNA expression profiles are related to histological subtype of mesothelioma. Correlation-based analysis has shown that epithelioid and biphasic subtype miRNA expression profiles are similar, while sarcomatoid subtype shows a different miRNA expression profile.

A research by Busacca et al. identified 7 miRNAs (miR-17-5p, miR-21, miR-29a, miR-30c, miR-30e-5p, miR-106a, and miR-143) in epithelial, sarcomatoid and biphasic malignant pleural mesothelioma tissue samples [68]. Differential expression of these miRNAs was associated with the histopathological subtypes. Reduced miR-17-5p and miR-30c expression was seen to correlate with a favourable prognosis in patients affected by sarcomatoid mesothelioma. While another study has shown that miR-205 down-regulation correlated significantly with both a mesenchymal phenotype and a more aggressive growth [69].

MiRNA expression pattern comparative analysis between normal pleural mesothelial and malignant pleural mesothelioma cell cultures, by Balatti et al. (2011) [70]. A different miRNA expression profiling, miR-7, miR-182, miR-214, and miR-497 were dysregulated in malignant pleural mesothelioma cell cultures, where miR 17-92 cluster and its paralogous (miR-17-5p, 18a, 19b, 20a, 20b, 25, 92, 106a, 106b) were over-expressed. Benjamin et al. have proposed a standardized test to identify miRNAs as possible molecular markers for the differential diagnosis of MPM from peripheral lung adenocarcinoma [71]. The study has revealed differential miRNA expression profiles in mesothelioma and carcinoma tissues, specifically, an overexpression of the miR-200 family (miR-200a/b/c), miR-141, miR-429, and miR-192/194, in carcinoma *versus* mesothelioma samples. Furthermore, mesothelioma samples showed higher expression of miR-193 family (miR-193a-3p/5p and miR-193b) and miR-152. The authors have proposed that over-expression of miR-193–3p in mesothelioma and miR-200c and miR-192 in peripheral lung adenocarcinoma and pleural carcinoma can represent validated set markers (100% sensitivity, 94% specificity) for the differential diagnosis of malignant pleural mesothelioma.

According to Pass et al. miRNA expression profiles could be also used as prognostic biomarkers [72]. Authors rank miR-29c* as a reliable marker due to the good correlation between high levels of expression in epithelial mesothelioma patients and a favourable prognosis. A limitation to the marker's usefulness is that it does not allow for a differentiation between asbestos-exposed and non-exposed patients. Further, *in vitro* studies have shown that miR-29c* up-regulation reduces cell proliferation, clone formation and migration activity. Furthermore, miR-29c* is able to control several pathways, such as the phosphoinositide 3-kinase and NF-κB pathways and those involved in apoptosis and immune system regulation. Further researches suggest that miR-29c* plays a role in mesothelioma by down-regulation of DNA methyltransferase and up-regulation of demethylating genes. The latter mechanisms allow further assessments on altered promoter methylation levels arising with asbestos exposure, and on altered expression in genes with various degrees of methylation as with mesothelioma [36]. Considering the same prognostic purposes, Ivanov et al., (2010) have studied the role of miR-31 both in immortalized mesothelioma cells and in mesothelioma cell lines produced from surgical specimens derived from patients with resected MPM; their work have shown a strong relationship between the absence of miR-31 and the degree of tumour aggressiveness [73]. Functional activity of miR-31 revealed its ability to inhibit proliferation, migration, invasion of malignant mesothelioma cells. Loss of miR-31 in patients with an unfavourable prognosis, frequently observed in aggressive forms of mesothelioma, is connected to homozygous deletion of the CDKN2A and CDKN2B, tumour suppressor genes.

MiR-31, CDKN2A and CDKN2B genes are mapped on fragile sites of chromosome region 9p21.3 with high rate of loss of heterozygosity. The authors revealed that reintroduction of miR-31 into malignant mesothelioma cell line inhibits cell cycle progression and suppresses invasion, migration, and clonogenicity, supporting the hypothesis of a possible role of this miRNA in the progression of the disease. This thesis is supported by genome-wide expression profiling of mir-31 affected genes that showed as this miRNA may regulate cell division, DNA replication and repair.

Most of the studies related to miRNAs are performed in tissue specimens, while predictive and diagnostic biomarkers should be detectable in easily accessible samples such as body fluids. Scientific evidences show that neoplasia may generate miRNA fingerprints in the cellular fraction of human peripheral blood and other studies have investigated the possibility that cell-free miRNAs in plasma, serum or in cellular fraction could be useful biomarkers for diagnosis of malignant mesothelioma [74].

A study has identified circulating miR-126 in the serum that may differentiate asbestos exposed subjects from MPM patients and healthy controls. MiR-126 was correlated with the serum levels of the angiogenic factor Vascular Endothelial Growth Factor and the SMRPs. Results showed that the combination of miR-126 and SMRP can be used as a diagnostic marker, providing useful tools available for exposed subject monitoring purposes [75].

Weber et al. (2012) have studied miRNAs deregulation in cellular fraction of human peripheral blood of patients with MPM. Results described a global downregulation of miRNA expression; in particular, the authors believe that miR-103 may be a significant biomarker for the diagnosis of mesothelioma and it may be useful in combination with other biomarkers like SMRP to improve sensitivity and specificity [76].

Kirschner et al. (2012) have investigated circulating miRNAs in serum and plasma of MPM patients respect to healthy controls, performing microarray analysis of 90 miRNAs previously associated with MPM. They proposed miR-625-3p as potential diagnostic biomarker for MPM and confirmed the role of miR-29c*, miR-92a as candidate tumour markers [77] (Table 1).

Table 1. Summary of reported microRNAs related to malignant mesothelioma (Legend: MPM: Malignant Pleural Mesothelioma, MiRNA: MicroRNA, VEGF: Vascular endothelial growth factor, SMRP: Soluble mesothelin-related peptides)

Samples: tumour tissues	Relevant altered miRNAs	Biological pathways/gene targets predicted to be affected by selected miRNAs	Conclusions	Ref
Mesothelioma: 39 epithelioid 19 biphasic 10 sarcomatoid 32 uncharacterized 32 lung adenocarcinoma 4 non diseased lung	Downregulation in MPM *vs* lung adenocarcinoma miR-200 family (miR-200c, miR-141, miR-200b and miR-429) miR-200a* miR-203 miR-205	MiR-200 family play a role in modulation of proteins associated with *wnt* signaling pathway. Upregulated genes in MPM: Jun, Myc, EGR1 and Wnt5B.	Downregulation of miR-200 family can be used in the differential diagnosis between MPM and lung adenocarcinoma.	[63]
Mesothelioma: 29 Epithelioid 6 Biphasic 6 Sarcomatoid 6 Unspecified	Highly expressed in MPM: miR-193 family (miR-193a-3p/5p, miR-193b) miR-152		Standardized set markers (100% sensitivity, 94% specificity) for the differential diagnosis of MPM vs lung adenocarcinoma.	[71]
Carcinoma: 15 Bladder 26 Breast 36 Colon 12 Endometrium 11 Esophagus 33 Kidney 1 Kidney 10 Liver 76 Lung 19 Ovary 11 Pancreas 3 Prostate 6 Stomach	Highly expressed in carcinoma: miR-200 family (miR-200a/b/c, miR-141, miR-429) miR-192/194			

Table 1. Continued

Samples: tumour tissues	Relevant altered miRNAs	Biological pathways/gene targets predicted to be affected by selected miRNAs	Conclusions	Ref
Mesothelioma: 11 epithelioid 5 biphasic 1 sarcomatoid 1deciduoid variant	Highly expressed let-7b* miR-1228* miR-195* miR-30b* miR-32* miR-345, miR-483-3p miR-584 miR-595 miR-615-3p miR-885-3p	RB 1 CDKN2A NF2	MiRNAs differentially expressed allow to discriminate different histotypes: epithelioid miR-135b miR-181a-2* miR-499-5p miR-517b miR-519d miR-615-5p miR-624 biphasic miR-218-2* miR-346 miR-377* miR-485-5p miR-525-3p sarcomatoid miR-301b miR-433 miR-543	[66]
	Not expressed let-7e* miR-144* miR-203 miR-340* miR-34a* miR-423 miR-582 miR-7-1* miR-9	HGF EGF PDGFA JUN		
Mesothelioma 142 tumour tissues 9 human mesothelioma cell lines 3 human normal mesothelial cell lines	Highly expressed in epithelial MPM: miR-29c*	MiR-29c* downregulates DNA methyltransferases and upregulates demethylating genes. MiR-29c* controls different pathways: PI3 Kinase pathway, NF-kB, and those involved in apoptosis, and immunity system regulation.	Increased expression of miR-29c* could be used as predictive biomarker of favourable prognosis. MiR-29c* overexpression in mesothelioma cell lines is related to significantly decreased proliferation, migration, invasion, and colony formation.	[72]

Table 1. Continued

Samples: tumour tissues	Relevant altered miRNAs	Biological pathways/gene targets predicted to be affected by selected miRNAs	Conclusions	Ref
Human mesothelioma cells Immortalizzated human mesothelioma cells	mesothelioma cells: up-regulated miR-17–92 cluster (miR-17–3p, miR-17–5p, miR-18a, miR-20a) miR-30c let 7 family significantly dysregulated miR-21 miR-29a miR-30b, miR-106a strongly overexpressed miR-143 down-regulated miR-221 miR-222	Pathways controlling programmed cell death, chronic inflammatory response, NO-mediated signaling, cell growth and motility, and protein kinase activity. Up-regulation of miR-17–92 cluster involved in the activation of oncogenic mechanisms, can promote proliferation, inhibit apoptosis, induce tumour angiogenesis, and it is transactivated by *c-myc* [78].	Specific 7 miRNAs (miR-17–5p, miR-21, miR-29a, miR-30c, miR-30e–5p, miR-106a and miR-143) differentially expressed allow to discriminate different histotypes: epithelioid highest expression biphasic intermediate expression sarcomatoid lowest expression, Reduced expression of miR-17–5p and miR-30c is correlated with better survival of patients.	[68]
Mesothelioma: 8 epithelioid 8 biphasic 8 sarcomatoid	mesothelioma: differentially expressed miR-143 miR-106a miR-30e-5p miR-30c miR-29a miR-21 miR-17–5p			
5 Mesothelioma cell lines 5 human normal pleural mesothelial cells obtained from biopies	Highly upregulated miR-17-92 cluster (miR-17-5p, 18a, 19b, 20a, 20b, 25, 92, 106a, 106b) Highly dysregulated miR-7, miR-182, miR-214, miR-497	CDKN1A/p21 shown to be *miR 17-92* cluster target. It is a potent negative regulator of the G1-S checkpoint. Absence of p21 expression in MPM samples could be due to the overexpression of the miR 17-92 cluster.	These miRNAs may represent MPM markers	[70]
22 tissues with suspected MPM 10 tissues with clear signs of the pathology 5 non-malignant tissue Serum of MPM patients: 23 epithelioid 3 biphasic 1 sarcomatoid	Highly downregulated: miR-126 in the malignant tissue.	High VEGF levels (regulated by miR-126) and SMRP were found in the serum of MPM patients compared with asbestos-exposed subjects and healthy controls.	Expression of miR-126 can be evaluated in the serum and in combination with SMRP may be used as a diagnostic marker, providing tools available for ex-exposed subject monitoring purposes	[75]

Table 1. Continued

Samples: tumor tissues	Relevant altered miRNAs	Biological pathways/gene targets predicted to be affected by selected miRNAs	Conclusions	Ref
Plasma of MPM patients: 9 epithelioid 3 biphasic 2 sarcomatoid 1 Unspecified Plasma of 14 healthy subjects Plasma: 10 patients with asbestosis 30 with MPM Tumour tissues: 15 epithelioid 3 biphasic 0 sarcomatoid 7 non-malignant tissues	Highly upregulated miR-29c* miR-92a miR-625-3p		MiR-29c*, miR-92a and miR-625-3p are a promising novel diagnostic marker for MPM in plasma.	[77]
Subjects with MPM 12 epithelioid 7 biphasic 1 sarcomatoid 17 subjects exposed to asbestos 25 healty subjects	Highly downregulated: miR-103 in epithelioid and biphasic mesothelioma	MiR-103 is part of the miR-15/107 group involved in the following biological functions: cell division, cellular metabolism, stress response, and angiogenesis.	MiR-103 as a new potential biomarker for the diagnosis of mesothelioma, showing a promising sensitivity and specificity.	[76]

FUTURE PERSPECTIVE

The MPM is a tumour difficult to diagnose, chemoresistant, and with rising incidence. Histopathological research (e.g. immunohistochemistry and histology) and clinical studies currently provides little in the way of diagnostic and prognostic information. Traditional techniques are being flanked by new molecular techniques, capable of correlate cancer genotype and phenotype profiles with clinical and pathological parameters. Another major issue in clinics is represented by the need of biomarkers for early diagnosis. MiRNAs have revealed a great potential as new early diagnosis biomarkers. Deregulated miRNA expression pattern is specific for different cancers, including MPM. MiRNA expression analysis is a promising tool for diagnosis, typing of MPM than normal tissue and other lung tumours and monitoring of new therapies.

REFERENCES

1. International Agency for Research on Cancer (IARC) 1977. Asbestos. IARC Monogr. Eval. Carcinogen. Risk Chem. Man vol.14, pp.1–106.

2. Marinaccio A, Binazzi A, Marzio DD, Scarselli A, Verardo M, Mirabelli D, et al. Pleural malignant mesothelioma epidemic: incidence, modalities of asbestos exposure and occupations involved from the Italian National Register. Int. J. Cancer. 2012; 130:2146-54.

3. Carbone M., Kratzke R A, Testa JR. The pathogenesis of mesothelioma. Semin. Oncol. 2002; 29(1):2-17.

4. Bunderson-Schelvan M, Pfau JC, Crouch R, Holian A. Nonpulmonary outcomes of asbestos exposure. J. of Toxicol. Environ. Health. 2011; 14(1-4):122-152.

5. Robinson BW and Lake RA. Advances in malignant mesothelioma. N. Engl. J. Med. 2005; 353:1591–1603.

6. Browne K. The quantitative risks of mesothelioma

and lung cancer in relation to asbestos exposure. Ann. Occup. Hyg. 2001; 45(4): 327–329; author reply 336–338.

7. Tomatis L, Cantoni S, Carnevale F, Merler E, Mollo F, Ricci P, et al. The role of asbestos fiber dimensions in the prevention of mesothelioma. Int. J. Occup. Environ. Health 2007; 13(1):64-69.
8. Nymark P, Wikman H, Hienonen-Kempas T, Anttila S. Molecular and genetic changes in asbestos-related lung cancer. Cancer Lett. 2008; 265 (1):1–15.
9. Heintz NH, Janssen YM, Mossman BT. Persistent induction of c-fos and c-jun expression by asbestos. Proc. Natl. Acad. Sci. 1993; 90(8):3299–3300.
10. Ramos-Nino ME, Timblin CR, Mossman BT. Mesothelial cell transformation requires increased AP-1 binding activity and ERK-dependent Fra-1 expression. Cancer Res. 2002; 62(21):6065-6069.
11. Yang H, Bocchetta M, Kroczynska B, Elmishad AG, Chen Y, Liu Z, et al. TNF-α inhibits asbestos induced cytotoxicity via a NF-KB-dependent pathway, a possible mechanism for asbestos-induced oncogenesis. Proc. Natl. Acad. Sci. 2006; 103(27):10397-10402.
12. Huang SXL, Jaurand MC, Kamp DW, Whysner J, Hei TK. Role of mutagenicity in asbestos fiber-induced carcinogenicity and other diseases. J. Toxicol. and Environ. Health. 2011; 14(1-4):179-245.
13. Kamp DW, Weitzman SA. The molecular basis of asbestos induced lung injury. Thorax 1999; 54(7):638-652.
14. Zucali PA, Ceresoli GL, De Vincenzo F, Simonelli M, Lorenzi, E, Giannoncelli L, et al. Advances in the biology of malignant pleural mesothelioma. Cancer Treat Rev. 2011; 37(7):543–558.
15. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat. Rev.Cancer. 2003; 3(4):276-285.
16. Unfried K, Schurkes C, Abel J. Distinct spectrum of mutations induced by crocidolite asbestos: clue for 8-hydroxydeoxyguanosine-dependent mutagenesis in vivo. Cancer Res. 2002; 62(1):99–104.
17. Heintz NH, Janssen-Heininger YMW, Mossman BT. Asbestos, lung cancers, and mesotheliomas from molecular approaches to targeting tumour survival pathways. Am. J. Respir. Cell Mol. Biol. 2010; 42(2):133-139.
18. Johnson N, Jaramillo R. p53, Cip1, and Gadd153 expression following treatment of A549 cells with natural and man-made vitreous fibers. Environ. Health Perspect. 1997; 105(5):1143–1145.
19. Tomasetti M, Santarelli L. Biomarkers for Early Detection of Malignant Mesothelioma: Diagnostic and Therapeutic Application. Cancers. 2010; 2(2):523-548.
20. Grigoriu B, Grigoriu C, Chahine B, Gey T, Scherpereel A. Clinical utility of diagnostic markers for malignant pleural mesothelioma. Monaldi Arch Chest Dis 2009; 71(1):31-38.
21. Van der Bij S, Schaake E, Koffijberg H, Burgers JA, de Mol BA, Moons KG. Markers for the non-invasive diagnosis of mesothelioma: a systematic review. Br. J. Cancer 2011; 104(8):1325–1333.
22. Gube M, Taeger D, Weber DG, Pesch B, Brand P, Johnen G, et al. Performance of biomarkers SMRP, CA125, and CYFRA 21-1 as potential tumour markers for malignant mesothelioma and lung cancer in a cohort of workers formerly exposed to asbestos. Arch. Toxicol. 2011; 85(3):185–192.
23. Hollevoet K, Nackaerts K, Thimpont J, Thimpont J, Germonpré P, Bosquée L, et al. Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. Am. J. Respir. Crit. Care Med. 2010; 181(6):620-625,
24. Creaney J, van Bruggen I, Hof M, Segal A, Musk AW, de Klerk N, et al. Combined CA125 and mesothelin levels for the diagnosis of malignant mesothelioma. Chest. 2007; 132(4):1239-46.
25. Van den Heuvel MM, Korse CM, Bonfrer JM, Baas P. Non-invasive diagnosis of pleural malignancies: The role of tumour markers. Lung Cancer 2008; 59(3):350-354.
26. Grigoriu B, Chahine B, Zerimech F, Grégoire M, Balduyck M, Copin MC, et al. Serum mesothelin has a higher diagnostic utility than hyaluronic acid in malignant mesothelioma. Clin. Biochem. 2009; 42(10-11):1046-1050.
27. Cristaudo A, Foddis R, Vivaldi A, Guglielmi G, Dipalma N, Filiberti R, et al. Clinical significance of serum mesothelin in patients with mesothelioma and lung cancer. Clin. Cancer Res. 2007; 13(17):5076-5081.
28. Park E, Sandrini A, Yates DH, Creaney J, Robinson BW, Thomas PS, et al. Soluble Mesothelin-Related Protein in an asbestos-exposed population: the dust diseases board cohort study. Am. J. Respir. Crit. Care Med. 2008;178(8):832-837.
29. Pass HI, Wali A, Tang N, Ivanova A, Ivanov S,

Harbut M, et al. Soluble mesothelin-related peptide level elevation in mesothelioma serum and pleural effusions. Ann. Thorac. Surg. 2008; 85(1);265-272.

30. Grigoriu B, Scherpereel A, Devos P, Chahine B, Letourneux M, Lebailly P, et al. Utility of osteopontin and serum mesothelin in malignant pleural mesothelioma diagnosis and prognosis assessment. Clin. Cancer Res. 2007; 13(10):2928-2935.
31. Shiomi K, Shiomi S, Ishinaga Y, Sakuraba M, Hagiwara Y, Miyashita K, et al. Impact of renal failure on the tumour markers of mesothelioma, N-ERC/mesothelin and osteopontin. Anticancer Res. 2011; 31(4)1427-1430.
32. Tsou JA, Shen LY, Siegmund KD, Long TI, Laird PW, Seneviratne CK, et al. Distinct DNA methylation profiles in malignant mesothelioma, lung adenocarcinoma, and non-tumour lung. Lung Cancer. 2005; 47(2):193-204.
33. Tsou JA, Galler JS, Wali A, Ye W, Siegmund KD, Groshen S, et al. DNA methylation profile of 28 potential marker loci in malignant mesothelioma. Lung Cancer. 2007; 58(2):220–230.
34. Christensen BC, Godleski JJ, Marsit CJ, Houseman EA, Lopez-Fagundo CY, Longacker JL, et al. Asbestos exposure predicts cell cycle control gene promoter methylation in pleural mesothelioma, Carcinogenesis. 2008; 29(8):1555-1559.
35. Hirao T, Bueno R, Chen C, Gordon GJ, Heilig E, Kelsey KT. Alterations of the p16INK4 locus in human malignant mesothelial tumours. Carcinogenesis. 2002; 23(7):1127-1130.
36. Christensen BC, Houseman EA, Godleski JJ, Marsit CJ, Longacker JL, Roelofs CR, et al. Epigenetic profiles distinguish pleural mesothelioma from normal pleura and predict lung asbestos burden and clinical outcome. Cancer Res. 2009; 69(1):227-234.
37. Goto Y, Shinjo K, Kondo Y, Shen L, Toyota M, Suzuki H, et al. Epigenetic profiles distinguish malignant pleural mesothelioma from lung adenocarcinoma. Cancer Res. 2009; 69(23):9073–9082.
38. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat. Cell. Biol. 2009; 11(3):228-234.
39. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med. 2012; 4(3):143-59.
40. Griffiths-Jones S, Saini HK, Dongen SV, Enright AJ. miRBase: tools for microRNA genomics. Nucleic Acids Res. 2008; 36 (Database issue), D154-158.
41. Bartel DP. MicroRNAs: Target Recognition and Regulation Functions. Cell. 2009; 136(2):215–233.
42. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc. Natl. Acad. Sci.USA 2004; 101(9):2999-3004.
43. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature. 2005; 435(7043):834-838.
44. Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet. 2009; 10(10):704–714.
45. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell. 2006; 9(3):189-198.
46. Chuang JC, Jones PA. Epigenetics and microRNAs. Ped. Res. 2007; 61 (5 Pt 2):24R-29R.
47. Lee YS, Dutta A. The tumour suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev. 2007; 21(9):1025–30.
48. Chang TS, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, et al. Widespread microRNA repression by Myc contributes to tumourigenesis. Nat. Genet. 2008; 40(1):43–50.
49. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. Nature 2007; 447(7148):1130–34.
50. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci USA 2002; 99(24):15524-15529.
51. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukaemia. N. Engl. J Med. 2005; 353(17):1793-1801.
52. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 2008; 299(4):425–36.

53. Singh R, Mo YY. Role of microRNAs in breast cancer. Cancer Biol Ther. 2013; 14(3):201-12.
54. Sittka A, Schmeck B. MicroRNAs in the lung. Adv Exp Med Biol. 2013; 774:121-34.
55. Di Leva G, Croce CM. The role of microRNAs in the tumorigenesis of ovarian cancer. Front Oncol. 2013; 13(3):1-10.
56. Bloomston M, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA 2007; 297(17):1901–8.
57. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008; 141(5):672-675.
58. Reid G, Kirschner MB, van Zandwijk N. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit. Rev. Oncol. Hematol. 2011; 80(2):193-208.
59. Ng EK, Li R, Shin VY, Jin HC, Leung CP, Ma ES. Circulating microRNAs as specific biomarkers for breast cancer detection. PLoS One. 2013; 8(1):e53141. doi: 10.1371/journal.pone.0053141.
60. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL. Circulating microRNAs as stable blood-based markers for cancer detection Proc Natl Acad Sci U S A. 2008; 105(30):10513–10518.
61. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K. et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell. Res. 2008; 18(10):997-1006.
62. Addis B, Roche H. Problems in mesothelioma diagnosis. Histopathology 2009; 54(1):55-68.
63. Gee GV, Koestler DC, Christensen BC, Sugarbaker DJ, Ugolini D, Ivaldi GP, et al. Downregulated microRNAs in the differential diagnosis of malignant pleural mesothelioma. Int. J. Cancer 2010; 127(12):2859-2869.
64. Baffa R, Fassan M, Volinia S, O'Hara B, Liu CG, Palazzo JP, et al. MicroRNA expression profiling of human metastatic cancers identifies cancer gene targets. J Pathol. 2009; 219(2):214-221.
65. Gregory PA. Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol. 2008; 10(5):593-601.
66. Guled M, Lahti L, Lindholm PM, Salmenkivi K, Bagwan I, Nicholson AG, et al. CDKN2A, NF2, and JUN are dysregulated among other genes by miRNAs in malignant mesothelioma - A miRNA microarray analysis. Genes Chromosomes Cancer 2009; 48(7):615-623.
67. Khodayari N, Mohammed KA, Goldberg EP, Nasreen N. EphrinA1 inhibits malignant mesothelioma tumour growth via let-7 microRNA-mediated repression of the RAS oncogene. Cancer Gene Ther. 2011; 18(11):806-816.
68. Busacca S, Germano S, De Cecco L, Rinaldi M, Comoglio F, Favero F, et al. MicroRNA signature of malignant mesothelioma with potential diagnostic and prognostic implications. Am J Respir Cell Mol Biol. 2010; 42(3):312-319.
69. Fassina A, Cappellesso R, Guzzardo V, Dalla Via L, Piccolo S, Ventura L, et al. Epithelial-mesenchymal transition in malignant mesothelioma. Mod Pathol. 2012; 25(1):86-99.
70. Balatti V, Maniero S, Ferracin M, Veronese A, Negrini M, Ferrocci G, et al. MicroRNAs dysregulation in human malignant pleural mesothelioma. J Thorac Oncol. 2011; 6(5):844-51.
71. Benjamin H, Lebanony D, Rosenwald S, Cohen L, Gibori H, Barabash N, et al. A diagnostic assay based on microRNA expression accurately identifies malignant pleural mesothelioma. J Mol Diagn. 2010; 12(6):771-779.
72. Pass HI, Goparaju C, Ivanov S, Donington J, Carbone M, Hoshen M, et al. Hsa-Mir-29c* is Linked to the Prognosis of Malignant Pleural Mesothelioma. Cancer Res. 2010; 70(5):1916-1924.
73. Ivanov SV, Goparaju CM, Lopez P, Zavadil J, Toren-Haritan G, Rosenwald S, et al. Pro-tumourigenic effects of miR-31 loss in mesothelioma. J. Biol. Chem. 2010; 285(30):22809-22817.
74. Hausler SF, Keller A, Chandran PA, Ziegler K, Zipp K, Heuer S, et al. Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. Br J Cancer 2010; 103(5):693–700.
75. Santarelli L, Strafella E, Staffolani S, Amati M, Emanuelli M, Sartini D, et al. Association of MiR-126 with Soluble Mesothelin-Related Peptides, a Marker for Malignant Mesothelioma. PLoS One 2011; 6(4): e18232.
76. Weber DG, Johnen G, Bryk O, Jockel KH, Bruning T. Identification of miRNA-103 in the Cellular Fraction of Human Peripheral Blood as a Potential

Biomarker for Malignant Mesothelioma. A Pilot Study. PLoS ONE. 2012; 7, (1): e30221.

77. Kirschner MB, Cheng YY, Badrian B, Kao SC, Creaney J, Edelman JJB, et al. Increased Circulating miR-625-3p: A Potential Biomarker for Patients With Malignant Pleural Mesothelioma. J Thorac Oncol. 2012; 7(7):1184-1191.

78. Mendell JT. MiRiad roles for the miR-17–92 cluster in development and disease. Cell 2008; 133(2): 217-222.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.