

MICRO-RNAs

AND

MALIGNANT PLEURAL MESOTHELIOMA

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a tumor associated with exposure to asbestos (1, 2). It is an aggressive disease with a poor prognosis whose standard treatment does not greatly improve survival (3-9), and it is the reason why many studies are currently investigating MPM to discover new findings that may help increase our knowledge about the disease and thus optimize treatment goals (10-26).

Emerging scientific evidence has shown that tumor aggressiveness may be associated with the genome and the aberrant expression of some genes. Several studies have therefore focused on the role of microRNAs (miRNAs) in the tumor genesis of MPM.

MiRNAs are small (17–22 nucleotides), single-stranded, non-coding RNAs involved in many cellular processes that regulate gene expression (7).

MiRNA are often aberrantly expressed in tumors. Specifically, multiple miRNA expression profiles have been documented in MPM cells as opposed to healthy mesothelial cells, suggesting a potential role for miRNAs as both oncogenes and tumor suppressors in tumor genesis.

We hope that new diagnostic methods will be found to improve the diagnosis and treatment of MPM (8,10), which is why research has focused on defining new clinical prognostic factors. New biomarkers are also being investigated because they can help predict the evolution of the disease.

Specific biomarkers that can diagnose MPM have not yet been validated, although studies are currently being conducted (27-31). Identifying new MPMP tumor markers could certainly help in the early diagnosis and treatment of this disease.

The purpose of this bibliography revision is to communicate the recent findings about the new class of microRNA markers.

DEFINITION OF miRNAs

Biogenesis of miRNAs

MicroRNA (miRNA) are short, single-stranded, non-coding RNAs acting as a new class of regulatory genes (32) that bind to the 3' untranslated region of their target mRNAs, thus regulating post-transcriptional gene expression.

The translation of miRNA in proteins is inhibited due to the partial complementary binding between the miRNA and its target. Conversely, full complementary binding leads to the degradation of the miRNA.

Function of miRNAs

A single miRNA can regulate hundreds of targets downstream, so these molecules play an important role in various cell processes, including proliferation, development, differentiation, apoptosis, and response to stress. They are also aberrantly expressed in various cancers and so could play a key role in tumor genesis.

Recent studies have shown that miRNAs are non-invasive biomarkers that may offer new pathways for early diagnosis and treatment of various cancers (33-35) due to their tumor-specific expression profiles and presence in peripheral blood (36-37). MiRNAs could be useful for defining profiles that identify different tumor subtypes (50-51).

MiRNAs are tissue-specific and can be investigated to identify cancer tissue origin (36-37).

The presence of miRNAs in bodily fluids such as serum, plasma, saliva and urine might be useful to predict the clinical outcome and response to cancer treatment (38-41).

Recent *in vitro* studies have shown that miRNA expression can be altered in various types of cancer, demonstrating their utility as cancer treatments (42).

They could also be used as prognostic markers, which has been documented in various cancer types (43-49).

Analysis of miRNAs

Microarray analysis and quantitative real-time polymerase chain reaction (qRT-PCR) are the most commonly used methods of investigating miRNAs.

Microarray profiling is a technique in which microscopic probes are attached to a solid surface such as glass, plastic or silicon chips to form an array (matrix) (52), permitting the simultaneous analysis of many genes within a sample.

Bibliography Review: December 2014

Microarrays use a technique known as inverse hybridization, which consists of attaching all the DNA segments (known as probes) onto a medium and marking the nucleic acid that we want to identify (known as the target). This method was developed in the 1990s and allows us to analyze gene expression by monitoring the RNA produced by thousands of genes in a single procedure.

MiRNAs are studied by first extracting them from the cells, converting them into cDNA using an enzyme known as reverse transcriptase and marking them with a fluorescent probe. When the probe in the matrix and the cDNA hybridize, the cDNA target binds to the probe and can be identified simply by looking at the position where it is bound.

The second method is qRT-PCR, which is a more sensitive, quantitative profiling instrument that analyzes the expression of a single cell, which could be applied in real time using primers together with specific samples (*or tests?*) [53].

These two methods should be used together to confirm the data and make them more credible, even though many studies have obtained results using a single technique.

Role of miRNAs

Few studies have actually evaluated the different expression of mRNAs with the aim of improving the diagnosis and treatment of MPM.

Early studies investigated the microarrays used to analyze how miRNAs are expressed differently in cancerous tissue versus normal tissue (54).

Biomarkers for the early identification of MPM

There is a growing desire to discover new biomarkers to identify MPM and a number of studies have been involved in defining the accuracy, feasibility and specificity of miRNAs as clinical biomarkers.

Abnormal increases in miRNA levels in different tumor types have been observed, and researchers suggest that these data could also be used to define the development and progression of MPM (55).

Vascular endothelial growth factor (VEGF) has also been described as one of the targets of a specific miRNA, miR-126, and MPM patients have very high VEGF levels in their blood (55).

Several studies have shown that miR-126 expression is reduced in cancerous cells, which are characterized instead by increased VEGF expression, suggesting that this miRNA may play a role in tumor suppression (56).

Bibliography Review: December 2014

A correlation between the levels of miR-126 found in serum and SMRP, a specific marker in patients at high risk of developing MPM, has been observed so it could presumably be used as a marker for the early diagnosis of MPM.

Recent research has shown that miR-126 can distinguish patients with MPM from patients with NSCLC (non-small cell lung cancer), and low levels of circulating miR-126 have in fact been found in MPM versus NSCLC samples (57).

This marker is not tumor-specific, however, and is often expressed in low levels in other tumor types so it should be used in combination with other markers such as mesothelin rather than alone (57).

Diagnosis

Up to now, it has been difficult to differentiate between MPM and adenocarcinomas or epithelial metastases of other cancers, and since there are no accurate markers miRNA expression could be an interesting option for obtaining a more differential diagnosis.

Several studies have shown that different patterns of miRNA expression can distinguish between MPM, lung adenocarcinoma or other cancers of the pleura (58-59).

Recent research has shown that some miRNA (miR-17-92) may be up-regulated in MPM cells, while other miRNA are down-regulated as typically occurs in other tumor types (miR-31, miR-221, miR-222) (60-61).

However, some statistical studies have shown that although specific miRNA (miR-17-5p, miR-30, miR-221, miR-222) are more characteristic of some histological tumor types, they cannot be considered as diagnostic markers to differentiate MPM from malignant mesothelial proliferations (58,59,61,63).

MiR-625-3p could be a promising marker, and some studies have shown that it is up-regulated in patients with MPM compared to the controls (65).

The miR-103 marker may be able to differentiate between the diagnosis of MPM and the controls who were exposed to asbestos (66).

Prognostic factors

MiRNAs have also been studied as prognostic factors.

Specifically, some researchers have observed that the patient population can be divided into two groups, namely those with a good prognosis and those with a bad prognosis based on the expression profile of the specific miRNAs (67-69).

Bibliography Review: December 2014

MiR-29 is considered a prognostic factor in terms of relapse and survival after cytoreductive surgery, and it does seem to be more highly expressed in patients with epithelial MPM as opposed to those with non-epithelial MPM.

High levels of miR-29 expression may be able to predict a more favorable prognosis compared to patients with lower levels of this miRNA. MiR-29 probably plays a role in inhibiting proliferation, migration, cell invasion and the formation of colonies.

On the other hand, other miRNAs such as miR-31 are associated with a worse prognosis.

Several miRNAs also appear to be specific to some histological subgroups of MPM, such as miR-17-5p, miR-29a, miR-30e-5p, miR-106a and miR-143 (64).

Other miRNAs such as mi-17-5p and miR-30c also have prognostic value, and might be able to identify sarcomatoid MPM with better outcomes.

It is important to remember that besides serving as prognostic markers, miRNAs have also been investigated for their potential role as predictive markers with promising results (54, 55, 58, 59, 61, 63).

Potential targets for anti-tumoral therapies

Several researchers have studied the role of miRNA biomarkers.

Various studies have shown that there is an actual pattern of miRNA that distinguishes between malignant mesothelioma and healthy mesothelial cell cultures (63). The genes involved in regulating the cell cycle could be targets of various mRNA, such as the members of the onco-miR miR-17-92 (miR 17-5p, 18a, 19b, 20a, 25, 92, 106a, 106b) cluster.

There are also specific miRNAs such as miR-31 that could be useful for defining new approaches to treating MPM.

Functional studies have shown that the forced re-expression of this miRNA may lead to the overexpression of the cell cycle and inhibit important factors involved in DNA replication and progression of the cell cycle.

It has been shown that miR-31 has tumor-suppression properties, suggesting the possibility of developing new therapeutic agents for MPM and other tumor types expressing the loss of chromosome 9p21.3 (71).

Another miRNA that has been studied at length is miR-34b/c, which appears to play a role in gene-silencing and suppression of some oncologic characteristics. Here too, the forced expression of this miRNA resulted in a significant anti-cancer effect, secondary to cell cycle arrest, suppression of

Bibliography Review: December 2014

migration, invasion and cell mobility. MiRNA may also have important therapeutic implications in the near future (72-73).

Cells transfected with miR-34 inhibitors are characterized by increased proliferation, migration and cell invasion (74).

It has been observed that methylation of miR-34b/c circulating in the blood is associated with MPM (75).

Treatment with ranpirnase (Onconase) induces an increase in miR-17 expression, which leads to a down-regulation of miR-30 and NF-kB expression, translating in turn to an increase in apoptosis and a decrease in the aggressiveness of the tumor (77).

MiR-1 appears to have a tumor-suppressive function in MPM treatment (80), and is in fact down-regulated in MPM cell lines versus normal mesothelium (78-79).

A decrease in miR-15 and miR-16 expression has been observed in mesothelioma cell lines. Strong expression of these miRNA is associated with an inhibition of the growth of MPM cells (81-85).

CONCLUSIONS AND FUTURE PROSPECTS

All the studies described in this bibliography revision show the importance of miRNAs in MPM due to their potential role as both diagnostic and prognostic markers and as antineoplastic agents for the treatment of this disease.

The Buzzi Foundation has also funded research in the miRNA field, and a report on the current project is available for consultation: “New targets in mesothelioma cells: hitting translational control and mirnas”. The project researchers have been able to describe a miRNA signature present in mesothelioma tissues. They have analyzed the level of expression of these and other miRNAs in 10 mesothelioma cell lines. The researchers have also demonstrated an extremely heterogeneous situation. In summary, it is very difficult at this stage to define if and which miRNAs have any diagnostic, prognostic or therapeutic significance. The results are in line with the heterogeneity seen in the literature about miRNAs in mesothelioma. However, a review of the data available points to not only heterogeneity in the tumor but also in the analytical methods. In this context, the researchers decided to develop an innovative method to unambiguously define which miRNA are expressed in human mesothelioma tissue, a necessary step in order to understand whether miRNAs are effective markers. This technology could be transferred to diagnostic laboratories in the near future.

Very few studies have actually focused on miRNA expression in MPM, so it is not surprising that there are discordant results among the different histotypes, the various sampling sources used in the

Bibliography Review: December 2014

in vitro and *in vivo* research, the control groups, the approaches, the standardization techniques and the qRT-PCR and microarray analyses.

Further standardized, multicenter studies are needed using proven and standardized methods.

In conclusion, all the results shown confirm the significance of miRNAs in the diagnosis, prognosis and treatment of MPM. All these data should be validated in a uniform manner to identify miRNAs as potential predictive and prognostic markers.

The recent progress in this field will certainly help define future prospects based on new treatment approaches and new opportunities for experimental treatment protocols.

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