REVIEW

The miRNA Response after Exposure to Particulate Matter: a Potential Epigenetic Mechanism

Carsten Knutsen

Abstract

Particulate matter, one form of air pollution, is a dangerous environmental toxin. Increasing numbers of cars on the road and further industrialization will continue to exacerbate this problem into the future. Particulate matter inhalation has been connected with cancer onset, especially in the respiratory and cardiovascular systems. Alterations in the microRNA (miRNA) pathway may be one factor contributing to the resulting cancer. miRNAs are small non-coding RNAs that act as post-transcriptional gene regulators. They bind to the 3' UTR of an mRNA through nitrogenous base pairing, leading to repression of the target gene. Methylation of the DNA loci encoding miRNAs may explain different levels of miRNA output seen in cancerous cells. Understanding this link is important in determining how cancer can start and in potential therapeutic approaches. However, methylation of miRNA template DNA is only one facet in a very complex epigenetic pathway.

*Biology Department, University of Minnesota Duluth,

fine particulate matter ($PM_{2.5}$) particles under 2.5 micrometers such as those found in smog and haze. Due to their size they can bypass mucus membrane and deposit into the lung

Alveolar macrophages a type of white blood cell found in the pulmonary system. They are on the boundary between the body and the outside world, and are responsible for neutralizing any potential toxin.

Corresponding Author:
Carsten Knutsen
knuts635@d.umn.edu

Introduction

Particulate matter, a mixture of small solid particles and liquid droplets, is a dangerous environmental toxin. It is classified into two distinct types based on the diameter of the particles: coarse (<10 μm) and fine (<2.5 μm). The air pollution of industrializing cities is a growing problem in developing countries. Heavy traffic of large cities has a drastic effect on the concentration of **fine particulate matter ($PM_{2.5}$)**, which is made up of diesel exhaust particles (DEPs) and industrial pollution. The number of motor vehicles in the world is expected to reach 1.3 billion by 2020 (Sperling and Clausen 2004), which would double the number of vehicles currently on the road. Air pollution and particulate matter related diseases will continue to be a challenge in the future.

Particulate matter inhalation has been associated with many adverse health effects including lung cancer and cardiovascular disease, and high levels of exposure can even lead to death (WHO 2005). Chronic low-level exposure, even below the WHO guidelines, has been linked to increased pulmonary disease and bronchial irritation (Pope et al 2011). Particulate matter has also been linked to oncogenesis in tracheal epithelial cells (Hornberg and Seemayer 1995). One explanation for this may lay in alterations in the miRNA pathway.

Particulate matter exposure primarily affects the respiratory and cardiovascular systems. First Pulmonary epithelial cells and **alveolar macrophages** are exposed to particulate matter first after inhalation. These cells represent a first line of defense against potentially harmful compounds. However, fine particulate matter can bypass the alveolar defense and deposit in the lungs. Expression analyses have shown that pulmonary epithelial cells trigger a cascading inflammatory response, which is altered in individuals exposed to particulate matter (Wang et al 2005). This indicates that particulate matter has an effect on molecular signaling in the pulmonary and cardiovascular systems.

Oncogenesis
onset or formation of cancer

UTR
acronym for untranslated region. These are regions on the mRNA transcript that are not translated

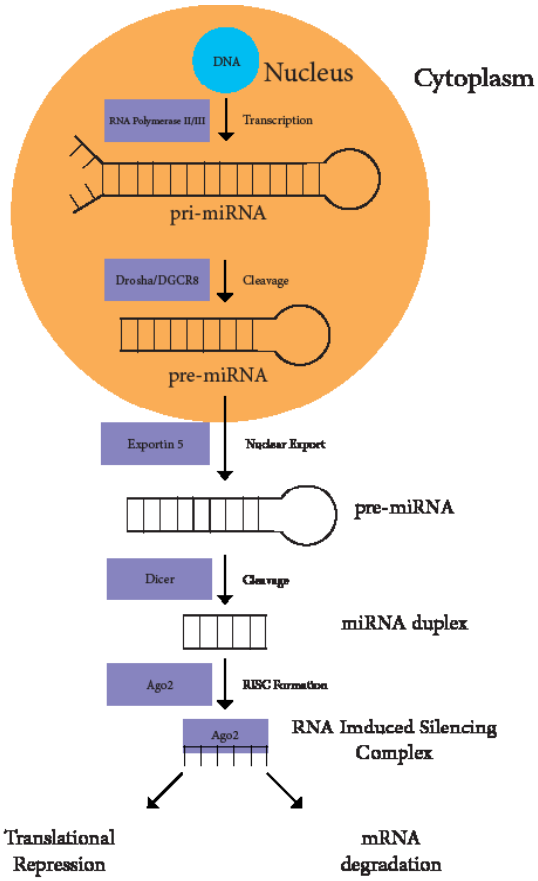

Figure 1 miRNA synthesis pathway. RNA polymerase I or III transcribes the primary miRNa transcript. The pri-miRNA is cleaved by the protein complex Drosha–DGCR8 in the nucleus; resulting in the pre-miRNA which is exported into the cytoplasm. The RNase Dicer complex cleaves the pre-miRNA hairpin to its mature length. The functional strand of the mature miRNA is loaded with Argonaute (Ago2) proteins into the RNA-induced silencing complex (RISC), where it acts as a binding template to silence target mRNAs through mRNA cleavage or translational repression.

Alterations in the miRNA pathway may be one factor in oncogenesis. Cells change in response to their external environments and in order to have this plasticity they must be able to repress or activate certain protein-coding genes. As miRNAs are translational regulators, they play a role in this plasticity. Target genes of miRNA are preferentially regulated after an environmental stress, pointing to miRNA playing a role in cellular plasticity (Wu and Song 2011). In addition, environmental toxins have been connected with altered profiles of miRNA in many different reports (Rager et al 2014; Surichio et al 2014; Zhang et al 2014). These altered profiles are associated with oncogenesis.

Aberrant methylation of miRNA genes has been noted numerous times in lung cancer cells (Khodyrev et al 2012, Rykov et al 2013, Watanabe et al 2012), and this is hypothesized to be a mechanism involved in the initiation of cancer. Lung cancer is linked to particulate matter exposure, so finding the connection between this methylation and altered miRNA profiles is worthy of exploration. Understanding how particulate matter causes **oncogenesis** is key in diagnosis and treatment. This review will examine several different miRNA expression profiles after exposure to particulate matter and how the miRNA genes are epigenetically regulated in response to this exposure

miRNA

miRNAs are small (typically 22bp) non-coding RNAs that act as post-transcriptional gene regulatory factors by binding to the 3' **UTR** of an mRNA through nitrogenous base pairing. This results in the repression of a target gene, which can occur in two distinct ways: mRNA cleavage or translational repression. Given sufficient complementarity to the mRNA, the miRNA will direct the RNA Induced Silencing Complex (RISC) to cleave the mRNA. If there is not sufficient complementarity, the mRNA will go through translation, but not as effectively because the miRNA will disrupt normal ribosome activity. The recognition sequence between miRNA-mRNA pairings is only about 8 base pairs long. This allows for a single miRNA to potentially target hundreds of genes. miRNAs have a number of diverse functions, including roles in cell apoptosis, differentiation and oncogenesis. These

miRNAs also seem to be highly conserved throughout the eukaryotic genome, supporting the fact they are vital in the cell cycle (Bartel 2009).

miRNA synthesis Pathway

To begin miRNA synthesis, large, self-complementary RNA transcripts are produced. These transcripts, known as pri-miRNA, form a double stranded hairpin structure that is then cleaved by the ribonuclease Drosha into the smaller pre-miRNA reducing the length to 70bp. This cleavage determines the 5' and 3' ends of pre-miRNA. Pre-miRNA is exported out of the nucleus into the cytoplasm where Dicer protein cuts the pre-miRNA down to 22bp miRNA. Argonaute proteins and RNA form the RISC which pairs to the 3' UTR of their target mRNA and represses expression of the target gene (Figure 1).

Oncogenes
gene that has the potential to cause cancer

Tumor suppressor
a gene that protects the cell from one step on the path to cancer

miRNA role in oncogenesis

Every malignancy seems to be a combination of processes involving changes in **oncogenes** and **tumor suppressors** (Croce 2009). In a healthy cell, one function of miRNA is to repress proto-oncogenes before their transcripts can be translated to proteins. This decreases the expression of the proto-oncogene (Croce 2009). Tumor suppressors are not inhibited by miRNA in normal healthy cells. Alterations of the methylation of miRNA loci affect miRNA expression leading to changes in translation and expression of proto-oncogenes and tumor suppressors (Chen et. al 2013). When expression of miRNAs is altered, their role in regulating oncogenes and tumor suppressors is changed, increasing the likelihood of oncogenesis.

DNA methyltransferase
family of enzymes that catalyze the transfer of a methyl group to DNA.

miRNA response to particulate matter

The two primary anthropogenic sources of particulate matter are automobile exhaust, rich in hydrocarbons, and industrial emissions, containing heavy metals. These distinct chemicals affect miRNA profiles in distinct ways.

Diesel Exhaust Particles (DEPs), found in automobile exhaust, are the largest source of emitted particulate matter, and they alter miRNA expression profiles (Jardim et al. 2009). In cultured bronchial epithelial cells exposed to DEPs, roughly 63% of cultured bronchial epithelial cells had detectable changes to miRNA profiles as compared to unexposed cells (Jardim et. al 2009). The miRNAs with the greatest changes targeted genes involved in the inflammatory response with strong tumorigenic signatures. Inflammation contributes to cancer by promoting cell division of mutated cells. These results suggest that the miRNA response to particulate matter, DEPs in particular, are involved in oncogenesis (Jardim et al 2009).

Heavy metals also contribute to particulate matter that alters miRNA profiles. Blood samples of foundry workers were examined to find the acute effect on miRNA by the heavy metal rich particulate matter inhaled by the workers (Motta et. Al 2013). By comparing blood samples taken at the beginning and end of the workweek, they found that four miRNAs were differentially expressed. These four miRNAs are apart of 11 mRNA-miRNA pairs that regulate inflammatory genes (Motta et al 2013).

Jardim et al (2009) and Motta et al. (2013) highlight the effect that particulate matter has on miRNA expression. However comparing these two studies demonstrates the challenges in identifying miRNAs role in cancer. The miRNA response is highly dependent on tissue type and the environmental stress. There is not just one set of miRNAs involved in all cancer. Instead, there can be many different sets depending

on a multitude of factors, illustrating the complexity of this system.

Mechanisms of altered miRNA profiles

Epigenetic effects, such as differential methylation of the DNA template for miRNAs, may explain the different miRNA expression levels (Chhabra 2015). Hypermethylation of promoters may inhibit miRNA response. The aberrant methylation of the CpG islands in the promoter of miRNA loci has been implicated in pathophysiology of various diseases, such as cancer, diabetes, and neurodegenerative diseases (Chhabra 2015).

Hypermethylaton of miRNA promoters was first observed when studying a drug treating bladder carcinoma (Saito et al 2006).Cancerous cells were treated with a DNA methyltransferase inhibitor.
The upregulation of miRNAs in the group receiving the drug points to methylation of miRNA genes as the cause of down regulation of miRNAs in cancerous cells. This study was a landmark in looking at the effect of DNA modifications on miRNA.

Methylation of miRNA loci effects the expression of their target genes. In lung cancer, the methylation status of four miRNA families (miR-9-1, miR-9-3, miR-34b/c, miR-193a) correlates with the expression of the RARbeta2 target gene, a gene involved in cell growth and differentiation (Khoydrev et al 2012). In this case, hypermethylation led to different miRNA and mRNA profiles within the cancerous cells, leading to differential expression of the target genes. This work suggests a connection between the differential expression of miRNA and lung cancer. Particulate matter exposure is known to alter miRNA expression, as stated previously, and is also known to increase risk of lung cancer, so it is possible that particulate matter exposure affects methylation of miRNA loci.

Methylation of DNA has been observed after exposure to particulate matter. Methylation of **tandem repeats** was observed in the DNA sequences of truck drivers and office workers in Beijing (Guo et al 2014). This study found that these repeats were hypomethylated after exposure. The effect was notably stronger in the truck drivers compared to the office workers, suggesting that an increased environmental exposure reduces the methylation on these repeats (Guo et al 2014). Hypomethylation of tandem repeats is common in tissues of cancer patients (Guo et al 2014).This alteration in methylation status provides an example of particulate matter's effect on gene expression. As particulate matter as altered methylation status in this case, it is not unreasonable to hypothesize that it could have similar effects on miRNA loci.

Particulate matter is just one of many potential environmental toxins, exploring the relationship between miRNA loci methylation and particulate matter exposure could shed a light on the cellular response to environmental stresses.

tandem repeats
a sequence of DNA that is repeated over and over directly adjacent to each other. These sequences are commonly examined to detect inheritance patterns.

Methylation

Methylation, the addition of a $-CH_3$group, of DNA is a critical factor in gene regulation. Typically, methylation near the promoter of a gene inhibits the transcription of the gene. CpG (5' C-phosphate-G 3') islands are critical regions of methylation in vertebrates. Methylation of a binding site may prevent some transcription factors from binding. Certain repressors may also require a methyl-group to bind to the specific site on the DNA strand. Methyl-groups may be added by a class of enzymes called DNA methyltransferases and, removed by demethylases. Controlling the methylation of CpG islands is one way the cell can control its gene regulation.

Challenges and Future directions

There is much to learn about the extent miRNA plays a role in the cells response to environmental stresses (Chhabra 2015).

However there is some contentionon miRNAs role in oncogenesis(Gielen et al 2012). Looking into the chemical pathway regulating miRNAs expression in relation to target genes is an important research step.

miRNA have been heavily researched as a potential biomarker for disease. Early diagnosis is key in treating many diseases. Different miRNA profiles would have to be associated with target genes connected with cancer. Adopting a single cohesive database of miRNAs and their target mRNAs would be a good start. Integrating a stepwise process of identifying certain miRNAs altered in certain tissues after environmental stress would be key in miRNAs future use as a biomarker. One example of research into miRNA as a biomarker is in aflatoxin, a naturally occurring fungal toxin, exposure has been promising, connecting specific alterations in miRNA profiles to aflatoxin exposure (Valencia Quintana et al 2014). Continued research into miRNA profiles of environmental toxins could prove a useful tool for medical research into the future.

Conclusion

Methylation of miRNA coding genes is a possible explanation to different levels of miRNA expression in cells exposed to particulate matter. By studying the miRNA response to particulate matter, diseases associated with air pollution can be better understood. Understanding the underlying mechanism to these different miRNA profiles is an important research step. Elucidating the pathways in which the environment effects methylation is key to our understanding of epigenetics and the cellular response. The different combinations of particles and chemicals affect the cellular response to particulate matter, resulting in a very complicated system. Future studies looking at methylation status of miRNA genes after particulate matter could explain how the different miRNA output is regulated. Finding the reasons why these mechanisms occur after environmental exposure, especially to particulate matter, would shed a light on oncogenesis as a whole.

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Author Biography

Carsten Knutsen is a senior pursuing his B.S. in Biology with a minor in Chemistry. He has a wide range of research interests including epigenetics, biogeochemistry, and biotechnology. In his free time he enjoys hiking, mountain biking, rock climbing, and playing music.

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