Literature Review

The Role of miRNA in Non-Small Cell Lung Carcinoma

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Abstract

Micro Ribonucleic Acids, or miRNAs, are short non-coding RNAs. Their length, on average, is 22 nucleotides, and they function in post-transcriptional regulation of gene expression. MiRNAs have their own nomenclature, and the same miRNAs can be found across different species. MiRNAs play important roles in many physiological and pathological processes in the human body. Regarding cancer, miRNAs are involved in carcinogenesis. MiRNAs work by binding completely or incompletely with messenger RNAs (mRNAs). Complete binding with mRNA produces mRNA degradation, while incomplete binding produces translational inhibition. Eventually, miRNAs decreased gene expression. In cancer, miRNAs show unique and different expression profiles. Based on their role in activating or deactivating intracellular signaling pathways, miRNAs can be classified as oncomiR or tumor suppressor miRNA. In non-small cell lung cancer (NSCLC), miRNAs are associated with gene mutation status, and they have important roles as diagnostic, prognostic, and predictive biomarkers.

Keywords: microRNA, NSCLC, biomarkers

INTRODUCTION

As shown by Globocan 2018 statistics, the incidence of lung cancer was 2,093,876 cases, or approximately 11.6% of all cases, while the mortality rate was 1,761,007 cases, or 18.4% of all cancer deaths. According to this data, lung cancer has the highest incidence and fatality rates worldwide when compared to other types of cancer.[1,2]

According to data from 2018, the incidence of lung cancer is the third highest (30,023 or 8.6% of total new cancer cases in Indonesia), while lung cancer mortality is the highest, with 26,095 fatalities or 12% of total cancer mortality in Indonesia.[3] Non-small cell lung cancer (NSCLC) is the most frequent histological type detected in the population.[4]

Micro Ribonucleic Acid (microRNA/ miRNA) is a small RNA that plays an essential role in various physiological and pathological processes in the human body, including NSCLC.[5–7] MicroRNAs can also be used as biomarkers for diagnosis, prognosis, and prediction. MicroRNAs can also be used as biomarkers for diagnosis, prognosis, and prediction.[8,9] This literature

review will describe the role of miRNAs in lung cancer, especially NSCLC.

microRNA

Based on human genome data, it is known that 2/3 of the human genome is non-coding ribonucleic acid *(*ncRNA) genes or RNAs that do not contain a code that plays a role in transcription and translation processes. These non-coding RNAs are divided into two major groups based on their function. The first group is housekeeping ncRNAs, which are consistently produced and play a role in regulating intracellular physiological processes. Ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA) are examples of housekeeping ncRNAs.[10]

The first two housekeeping RNAs mentioned are involved in gene expression processes, especially in translating messenger RNA (mRNA) sequences on the ribosomes to produce proteins. The second group of ncRNAs is the regulatory ncRNAs. This group of ncRNAs regulates gene expression at the post-transcriptional stage. This second group is further subdivided according to the length of the RNA strand. Regulatory ncRNAs with less than 200 nucleotides in length are referred to as short ncRNAs (short or small ncRNAs), whereas those with more than 200 nucleotides in length are referred to as long ncRNAs.[10]

MiRNAs belong to the group of short ncRNAs with an average length of 22 nucleotides and play an essential role in regulating gene expression. Besides miRNAs, other short ncRNAs include small interfering RNA (siRNA) and PIWI-interacting RNA (piRNA). Both types of short ncRNAs also play a role in influencing the translation process of messenger RNA into a protein, as is the case with miRNAs, but this literature review will not discuss their position further. A more concise classification of the division of non-coding RNA can be seen in Figure 1.[10]

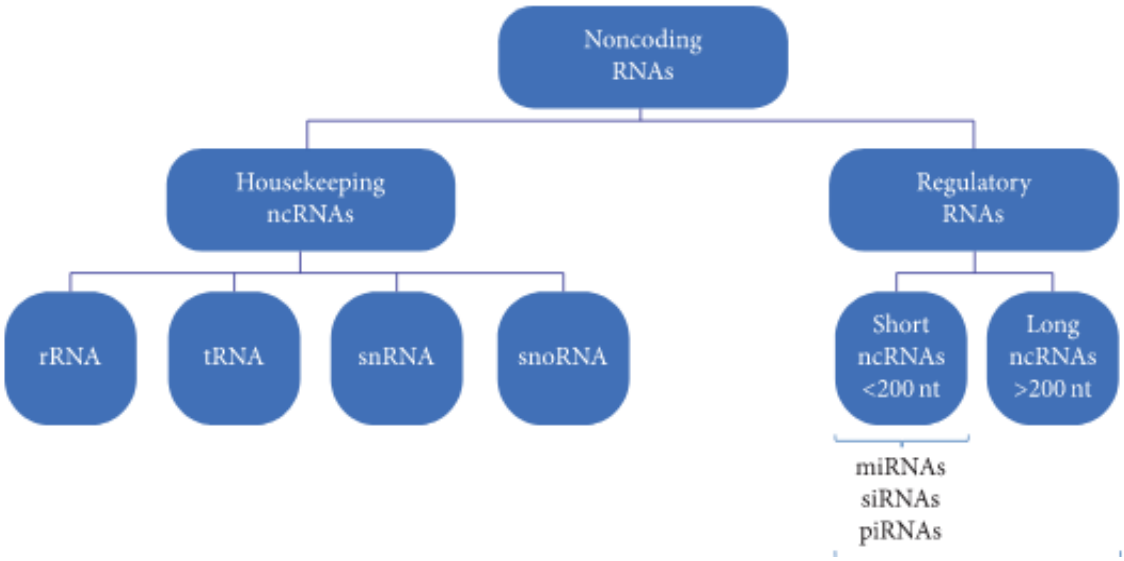

Figure 1. Types of non-coding RNA[10]

In 1993, Lee et al. discovered the first miRNA, lin-4, in Caenorhabditis elegans, a transparent soil-living nematode species, and subsequently, many other miRNAs with homologous sequences were found in different species.[7,11] Most miRNAs are located intracellularly. However, it can also be found in extracellular compartments such as breast milk, saliva, serum, or plasma.[12–15] This extracellular microRNA is stable and resistant to RNAse activity in the blood through several mechanisms, for example, being encased in membrane-bound vesicles (exosomes, apoptotic bodies, and microparticles) bound to proteins.[13,14,16,17]

microRNA NOMENCLATURE

The name miRNA consists of the species abbreviation and a number. A miRNA naming formula can be seen at the bottom of this paragraph. On the part of the organism are the 3-letter initials of the Latin name of a species from which the miRNA in question was obtained. For example, humans are written as hsa (from *Homo sapiens*), the mouse is written as mmu (from *Mus musculus*), and rice is written as osa (from *Oryza sativa*). The inscription "miR" indicates that this is a mature miRNA. The part after the words "miR" is a number that indicates the family or family of the miRNA. There are only 2 precursor arms, namely 3' or 5' arms.[18]

For example, hsa-miR-99a-5p shows that this mature miRNA is found in humans, belongs to the miR 99a family, and originates from the 5p component. Another example of osa-miR-169-3p shows that this mature miRNA is from Oryza sativa, of the 3p component 169 miRNA family. Below is the miRNA naming formula.[18]

Organism - miRnx - precursor arm and/or y

n = sequential number representing the miR family
x = letter describing a member of the miRNA family
y = number indicating the occurrence of more than 1 mature sequence of the same precursor.

POSITION miRNA BIOSYNTHESIS AND MECHANISM OF ACTION

The process of miRNA biosynthesis begins in the cell nucleus and ends in the cytoplasm. First, the RNA polymerase II enzyme transcribed the primary miRNA (pri-miRNA) gene. Then, the pri-miRNA formed will be processed by Drosha, an RNAse III enzyme in the cell nucleus, and the cofactor of DiGeorge syndrome critical region 8 (DGCR 8). Through the action of these enzymes and cofactors, pri-miRNAs are transformed into pre-miRNAs, which are 70 nucleotides long.[7,19]

Furthermore, the Exportin-5 protein will transport the pre-miRNA from the cell nucleus to the cytoplasm. Finally, in the cytoplasm, a circular strand (stem-loop) at one end of the pre-miRNA is cut by the Dicer enzyme (RNAase III enzyme) to form a miRNA/MIRNA* duplex with a length of about 22 nucleotides. Ultimately, one strand of the duplex, or the so-called

mature miRNA, is loaded onto the Argonaute protein complex (AGO) to form an RNA-induced silencing complex (RISC). In contrast, the partner RNA strand is degraded.[7,19]

This RISC complex will bind to the target messenger RNA in the seed sequence area, which is the area with the nucleotide sequence paired with the nucleotide sequence of the miRNA.[7] Perfect binding between RISC and messenger RNA will cause the messenger RNA to unravel, while the nearly perfect bond inhibits the translation process.[7,11,19] The action of miRNA in influencing this translation process causes the expression of a gene to be reduced. Another term for that miRNA action is gene silencing, although it does not entirely halt total expression of a gene.[20] An overview of miRNA biosynthesis can be seen in Figure 2.

THE ROLE OF miRNA IN PHYSIOLOGICAL PROCESSES

Animal and human studies have shown that miRNAs play a role in the homeostasis of physiological processes in the body. Especially in the lungs, miRNAs play an essential role in the immune control process.[6] For example, miR-155 plays a role in differentiating naive T cells into Th1 and Th2 cells in the lung. MicroRNA 26a is highly expressed in alveolar and bronchial epithelial cells and is essential in vascular remodeling and lung development.[21] let-7, miR-29, miR-15, and miR-16 act as tumor suppressor miRNAs in lung cells. MicroRNA 146a/b plays an essential role in the regulation of negative feedback IL-1β-induced inflammation.[6]

THE ROLE OF miRNA IN LUNG CANCER

Several reports indicated that there was abnormal miRNA expression in cancer, and there were differences in miRNA expression patterns between one cancer and another. The exact cause is unknown but may be due to chromosomal abnormalities, gene mutations, epigenetic changes, or other reasons. In addition, several miRNA genes are often found in cancer-associated gene areas, so that changes in miRNA expression are directly related to changes in genes or chromosomes that occur due to cancer by the previously described mechanism.[22]

Regarding the cancer phenotype, miRNAs can be classified as "oncomyR" or "*tumor-suppressor* miRNAs". This classification was based on its increased or decreased expression in certain malignancies, as well as its impact on intracellular signaling pathways.[22] In lung cancer, let-7 is a tumor suppressor miRNA and the first miRNA known to be dysregulated in lung cancer.[23,24]

Other tumor suppressor miRNAs besides let-7 are miR-34 and miR-200, all of which have decreased expression *(*down-regulation*)* in lung cancer. Oncogenic miRNAs that had increased expression in lung cancer were miR-21, miR-17-92, and miR-221/222. These two types of miRNAs are involved in cell proliferation, apoptosis, and cell migration.[23]

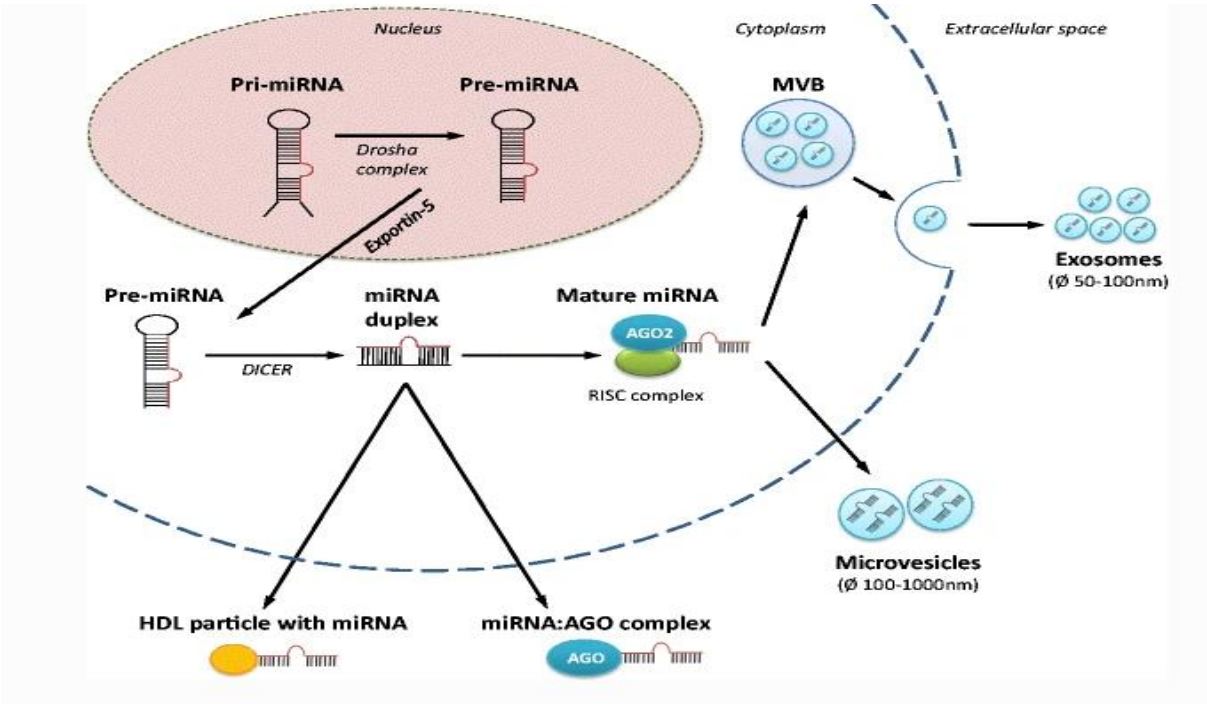

Figure 2. Biosynthesis of miRNA[25]

Table 1. MicroRNA in Physiological Processes in the Lung[6]

miRNA	Function
miR-155	T cell differentiation in the lung
miR-26a	It is highly expressed on alveolar and bronchial epithelial cells. Essential for lung development.
Let-7	Widely expressed in normal lung tissue. As tumor suppressor miRNA in lung cells
miR-29	As tumor suppressor miRNA in lung cells
miR-15, miR-16	As tumor suppressor miRNA in lung cells
miR-146a/b	Negative feedback regulation of IL-1β -induced inflammation

Table 2. OncomiR and tumor suppressor miRNA in lung cancer[23]

Micro RNA	Gene Target	Biological Process
Tumor suppressor miRNAs are reduced in expression in lung cancer		
Family Let-7	RAS, HMGA 2, CDK6, MYC, DICER1	Cell proliferation (RAS, MYC, HMGA2) Cell cycle regulation (CDK6) MiRNA maturation (DICER1)
Family miR-34	MET, BCL2, PDGFRA, PDGFRB, ZEB1, ZEB2, E- Cadherin, vimentin (VIM)	Ligand-induced cell proliferation and apoptosis TNF
Family miR-200		Promotion of epithelial-mesenchymal transition and metastasis
OncomiR whose expression is increased in lung cancer		
miR-21	PTEN, PDCD4, TPM1	Apoptosis, proliferation and migration of cells
miR-17-92 Cluster	E2F1, PTEN, HIF1A	Cell proliferation and carcinogenesis
miR-221/222	PTEN, TIMP3	Apoptosis and migration cell

Table 2 describes in more detail some of the oncomiRs and tumor suppressor miRNAs in lung cancer. Due to the role of miRNAs in carcinogenesis and their varying levels of expression, miRNAs have the potential to act as biomarkers related to diagnosis, prognosis, and predictive response to cancer therapy.[8,9]

miRNA AND GENE MUTATIONS IN NSCLC

Several studies have shown that miRNA profiles in NSCLC are associated with common gene mutation status in NSCLC. Pak et al. studied 103 lung adenocarcinoma cancer tissue samples and divided them into 3 groups based on EGFR mutations in exon-19, exon-21, and wild type. MicroRNA profiles were obtained using a microarray platform, and 3 significant miRNAs were accepted and validated by qRT-PCR. The three miRNAs were miR-34c, miR-183, and miR-210. They found decreased expression of miR-34c and increased expression of miR-183 and miR-210 in cancer tissues with positive EGFR mutations. MiR-183 expression was significantly increased in cancer tissue with exon-19 EGFR mutation.[26]

Kim et al. examined lung adenocarcinoma tissue specimens with different mutation profiles, namely positive EGFR mutations, positive KRAS mutations, positive ALK mutations, and those that did not contain the three mutations (triple-negative/TN). They found different miRNA expressions in each type of gene mutation studied. The let-7e and miR-342-3p microRNAs were increased in the EGFR and KRAS groups and decreased in the ALK and TN groups.[20]

Analysis of miRNA expression in samples between different groups of mutations showed different results. For example, in the ALK group, the expression of miR-1343-3p was more increased, and miR-671-3p, miR-103a-3p, let-7e, and miR-342-3p were more decreased than in the EGFR/KRAS group. Meanwhile, in the EGFR group, miR-647, miR-200b-5p, miR-361-5p, miR-23b-3p, and miR-27b-3p increased more, and miR-23a-3p decreased more than the expression of these miRNAs in the KRAS group.[20]

miRNA AS DIAGNOSTIC BIOMARKER

Some miRNAs can be diagnostic markers of certain cancers. Zhao et al. compared the expression levels of miR-21 in the serum of NSCLC patients and healthy people. They discovered that miR-21 expression levels in the serum of NSCLC patients were considerably higher.[27]

Hamamoto et al. investigated miRNA profiles in lung cancer tissue samples from NSCLC patients. They discovered a significant increase in miR-205 expression in tissue samples from squamous cell carcinoma (SCC) lung cancer, which can be employed as a diagnostic marker for SCC.[21,28] Another study found that a panel of 4 miRNAs in plasma (miR-21, miR-126, miR-210, and miR-486-5p) can be used to identify stage I NSCLC with a sensitivity of 73% and a specificity of 97%.[23,29]

miRNA AS PROGNOSTIC BIOMARKER

Another role of miRNA in cancer-related research is as a prognostic biomarker, that is, a biomarker associated with the outcome of a disease. MiRNA-21 is an oncomiR that acts as a diagnostic and prognostic biomarker. MiR-21 expression was significantly increased in the serum of patients with advanced-stage NSCLC with low survival rates.[24,27,30]

The effect of miRNA-21 on lung cancer may be explained by a study in mice showing that overexpression of miR-21 activates the Raf-MAPK signaling pathway and *phosphatidylinositol 3-kinase* (PI3K) in cells and modulates the number, incidence, and size of lung tumors induced by the K-Ras oncogene in mice.[30]

In Indonesia, Hanafi et al. examined serum miRNA profiles in 52 NSCLC patients using a Real-Time quantitative PCR (qRT-PCR) platform. They found that the expression levels of miR-34 (tumor suppressor miRNA) and miR-148 were low and the expression of 2 oncomiRs was low. High miR-222 and miR-155 are associated with a poor prognosis. High expression of miR-222 and miR-155 was associated with poor prognosis in stage IV M1b adenocarcinoma patients with positive EFGR mutations. In contrast, high expression of miR-34 was found in multiple metastatic adenocarcinomas with negative EGFR mutations.[31] The oncogenic nature of miR-155 is related to its role in signaling pathways in cell proliferation and apoptosis.[32]

miRNA AS A PREDICTIVE BIOMARKER

Predictive biomarkers are biomarkers associated with response to therapy. Several miRNAs with aberrant expression patterns are associated with therapy resistance in NSCLC. For example, the expression of miR-214 was increased in radioresistant NSCLC cells and could protect radiosensitive cells against the effects of radiation.[25,32]

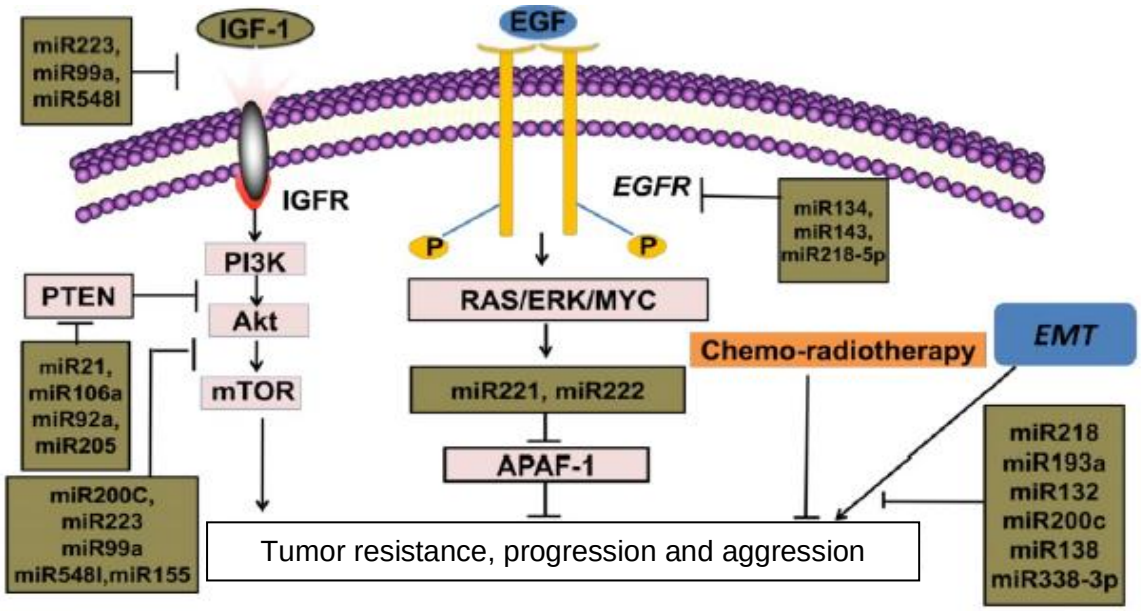

Figure 3. Micro RNA that plays a role in modulating the effects of chemotherapy, radiotherapy, or targeted therapy.[33]

In studies of human lung cancer cell lines, increased expression of miR-9 and let-7g was associated with sensitivity to radiotherapy. In addition, increased expression of these two genes reduces the expression of the NFkB1 gene in cancer cells, and this NFKb1 gene plays a role in repairing DNA damage and increasing cell survival.[24]

There is a relationship between resistance to chemotherapy and miRNA expression. In NSCLC cell lines, miR-135a knockdown increased expression of the *adenomatous polyposis coli* (APC) tumor suppressor gene that regulates mitotic checkpoints during mitosis and plays a role in apoptosis of paclitaxel-resistant cells. Wang et al. found that miR-214 increased its expression in gefitinib-resistant adenocarcinoma cell lines, and *knockdown* of this miRNA led to resensitization of that cell strain to gefitinib.[34] MiR-21 and miR-210 were decreased in the serum of patients with NSCLC who responded well to platinum-based chemotherapy.[24] Cui et al. studied 260 patients with advanced-stage NSCLC and found that high expression of miR-125b in serum was associated with poor treatment response. The results of Cui et al. study showed that miR-125b is a predictive biomarker of cisplatin chemotherapy response.[33] Other miRNAs associated with therapeutic success in NSCLC can be seen in Figure 3.

CONCLUSION

MicroRNA is a non-coding RNA, which is 22 nucleotides long and plays a role in regulating post-transcriptional gene expression. MiRNA works by binding to messenger RNA and can cause degradation of messenger RNA or inhibition of the translation process. In addition to physiological processes, miRNAs also play an essential role in pathological processes in the human body. MiRNAs play an important role in NSCLC. Some miRNAs serve as oncogenes (oncomiR) or tumor suppressor miRNAs. miRNA expression is associated with gene mutations in NSCLC. MiRNA can serve as a diagnostic, prognostic, and predictive biomarker in NSCLC.

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