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REVIEW ARTICLE

MicroRNAs in Lung Cancer Oncogenesis and Tumor Suppression: How it Can Improve the Clinical Practice?

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Abstract: ***Background*:** Lung cancer (LC) development is a process that depends on genetic mutations. The DNA methylation, an important epigenetic modification, is associated with the expression of non-coding RNAs, such as microRNAs. MicroRNAs are particularly essential for cell physiology, since they play a critical role in tumor suppressor gene activity. Furthermore, epigenetic disruptions are the primary event in cell modification, being related to tumorigenesis. In this context, microRNAs can be a useful tool in the LC suppression, consequently improving prognosis and predicting treatment.

***Conclusion*:** This manuscript reviews the main microRNAs involved in LC and its potential clinical applications to improve outcomes, such as survival and better quality of life.

Keywords: Lung cancer, squamous cell carcinoma, adenocarcinoma, epigenetic modification, DNA methylation, microRNA.

1. INTRODUCTION

Lung cancer (LC) presents high rates of morbidity and mortality, being the most devastating malignancy in the world. In this context, finding early screening methods, such as microRNAs (miRNA), will increase hope about LC early diagnosis, pathogenesis understanding, more curative-intent treatment and prognosis [1, 2]. LC development is a process that depends on many genetic mutations. These alterations promote the dysfunction of critical genes, including tumor suppressor genes. In addition, genes can be silenced through epigenetic changes, appearing aberrant methylation, and contributing to the initiation or malignant progression of tumors [2, 3].

2. DNA METHYLATION IN TUMORIGENESIS

Methylation is an important epigenetic modification where a methyl group addition generally occurs in cytosine within CpG dinucleotides in CpG islands, covering regions of 0.2 to several kilobases. CpG islands are typically non-methylated in healthy adult tissues, is associated with the expression of the adjacent gene. This mechanism is essential for development and proper cell physiology. Thus, it plays a critical role in the transcriptional gene expression regulation, including human tumor suppressor gene activity, and is crucial to regulate the genome by changing chromatin architecture. Importantly, epigenetic disruptions are the primary event in the initiation of tumorigenic cell modification [3-6].

Tumorigenesis is a process linked to genes silenced by DNA methylations, including genes involved in DNA repair, apoptosis, and metastasis. DNA methylation facilitates metastasis by adapting tumor cell behavior to the new environment. Methylation can be divided into two processes: hypermethylation that inactivates certain tumor-suppressor genes, and hypomethylation that also acts on cells genome. Interestingly, DNA methylation is a reversible process [3, 4, 7] and some alterations in DNA methylation patterns are hallmarks and potential targets for cancer treatment [8].

Hypomethylation was reported to be related to breast, ovarian, melanoma, gastric and colorectal cancers. In cancer cells, it is quite unusual to find individual genes with DNA hypomethylation, being more common in tissue-specific genes. The hypomethylation mechanisms include disruption and loss of DNA methylation affecting genomic integrity and stability [4]. Some genes, such as GORASP2, ZYG11A, and SFN, are significantly hypomethylated in LC adenocarcinoma [9].

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Hypermethylation of CpG islands (CGIs) can cause inactivation of certain genes involved in crucial cellular processes, that are unmethylated in normal tissues, generating epigenetic silence and facilitating human tumorigenesis or malignant progression. Genes, such as MLH1, BRCA1, O-6-methylguanine- DNMT, p15, p 16, von Hippel-Lindau (VHL), death-associated protein kinase 1 (DAPK1) are hypermethylated in tumor tissues promoting cancer cell survival. Consequently, hypermethylation can also be found in gliomas, leukemias gastric, renal, colorectal, and ovarian cancers [3, 4, 10]. In LC, there are tumor suppressor genes hypermethylated, including CDKN2A, CDH13, FHIT, WWOX, CDH1, RASSF1A, and many histone gene loci, particularly HIST1H4F [8, 11].

The processes of DNA methylation alterations and repetitive DNA sequences are also associated with the regulation of expression of non-coding RNAs such as miRNAs, frequently located in fragile chromosomal sites. Thus, DNA methylation affects not only protein-coding genes, but also miRNAs, playing an important role in tumor formation and progression due to genomic instability. DNA hypermethylation of CGIs can silence miRNAs leading to carcinogenesis. In this context, miRNAs may be upregulated/overexpressed or downregulated/under-expressed in cancer cells [4, 12]. Additionally, miRNA upregulation of DNA methyltransferases DNMT1, DNMT3a, and DNMT3b were found in LC, mainly in smokers, leading to a poorer prognosis [8, 13].

3. MicroRNAs

The miRNAs are a family of small non-coding endogenous RNA molecules, with approximately 23 nucleotides (20-25), that regulates the expression of genes associated with various biological processes. The biogenesis of miRNAs involves long primary miRNA transcript or precursor miRNA molecules, and its expression is regulated in the nucleus at the transcription level, being dependent on RNA polymerase II. In the following cellular process, the primary miRNA matures through regulatory proteins, including RNase III Dicer, Argonaute 2, and trans-activation-responsive RNA-binding protein. At the simplest level, miRNAs are encoded by specific genes, and their function is repressing miRNA translation or promoting miRNA degradation, altering gene expression post-transcriptionally. In addition, many miRNA targets are themselves noncoding RNAs. miRNAs comprise one of the more abundant classes of gene regulatory molecules, being part of cellular communication and playing important roles in some biological processes, such as inflammation, cell growth, apoptosis, development, differentiation, endocrine homeostasis, and even tumorigenesis. Approximately one-third of human genes are putative targets of miRNAs, and depending on the gene they are targeting, can function as negative or positive regulators. A single miRNA has the ability to regulate the expression of multiple genes and one gene can be modulated by several miRNAs [2, 14-21].

A recent study about miRBase (v22) was published and cataloged names and distributes miRNA gene sequences reports miRNA sequences from 271 organisms: 38 589 hairpin precursors and 48 860 mature miRNAs [18]. The miRNAs are related to many fundamental biological processes, including human genome functioning [17]. The miRNAs function within the cell and guides a functional ribonucleoprotein to the target RNA of interest. The decrease in target protein levels usually occurs if the target is a protein-coding mRNA. The expression of a miRNA is influenced in different levels in the cell, including transcription, processing and function. This expression is also influenced by biding with many other types of RNAs, such as ncRNAs. On the other hand, the expression of the target RNA is regulated at numerous levels by miRNAs, including epigenetic effects, promoter regulation, RNA processing and stability, and translation. In addition, this mechanism is not limited to the cell where it began, having a positive or negative impact on others cells of the environment. Furthermore, DNA methyltransferases, histone deacetylases and histone methyltransferases can be repressed by miRNAs [19, 21].

Carcinogenesis is a complex and multistep mechanism that involves genetic, epigenetic, biochemical, and histological changes. miRNAs play an important role in tumorigenesis because of their deregulated levels of expression in normal and cancer cells, acting as tumor suppressors, oncogenes, metastasis suppressors or activators [17, 21, 22]. Oncogenic signaling pathways, such as Notch, wingless /ß-Catenin, Janus-activated kinase/signal transducer and activator of transcription, phosphatidylinositol 3-kinase/ protein kinase B, and nuclear factor kappa-light-chain-enhancer of activated B, are miRNAs targets involved in carcinogenesis. In this context, miRNAs are considered an important player in human cancer initiation, progression, and prognosis. Moreover, oncogenic miRNAs, that can be amplified, leading to its upregulation and silencing of the tumor repressors genes, are located in fragile regions. Thus, mutations or deletion of oncogenic miRNAs can lead to overexpressing target oncogenes. Besides the involvement in tumorigenesis, abnormal expression of miRNAs is also related with tissue invasion, metastasis, drug resistance, and stimulating antiapoptotic activity. These mechanisms can be a consequence of cancer stem cells activation and regulation (maintenance, reprogramming, pluripotency, and differentiation) through miRNAs [17].

However, miRNAs communication is context-dependent, having both oncogenic and tumor-suppressive ability in different systems. Both genetic and epigenetic alterations are vital for the initiation and progression of human malignancies as they act as tumor suppressors and oncogenes. For example, miR-125b is downregulated in hepatocellular, breast, and lung cancers, but overexpressed in others such as colorectal, pancreatic, gastric and some leukemias [17, 19]. Furthermore, miRNAs can act together being grouped by families, overlapping targets, consequently amplifying the repression of target pathways. For example, the miR-17~92 cluster is a polycistronic miRNA overexpressed in multiple cancers [19].

4. KEY miRNAs IN SUPPRESSION AND ONCOGENESIS MECHANISMS IN LUNG CANCER

The miRNAs can be classified into proto-oncogenes that are normally low expressed in normal tissues, but overexpressed in LC. On the other hand, some miRNAs underexpressed in LC can be considered as “anti-oncogenes”. In

addition, there is a mutual regulation of multiple target genes by some miRNAs and different miRNAs can target the same gene creating a delicate regulatory net [20]. Some of the major carcinogenesis mechanisms including sustaining proliferative signaling; evading growth suppressors, enabling replicative immortality; activating invasion and metastasis; inducing angiogenesis; resisting apoptosis; deregulating cellular energetics; avoiding immune destruction and tumors promoting inflammation; genome instability and mutation [2]. The basic mechanisms including the involved miRNAs, are summarized in Table **1**.

Epidermal growth factor (EGF) and its receptor (EGFR) are among the main kinases/kinases receptor involved in the signalize proliferation and cell cycle progression for lung cancer cells through downstream RAS/ERK and PI3K/AKT/mTOR pathways. This mechanism, called sustaining proliferative signaling, is one of the fundamental characteristics of cancer. The main miRNAs involved in this process by targeting EGFR are miR-7, miR-27a-3p, miR-30, miR-34, miR-128, miR-133, miR-134, miR-145, miR-146, miR-149, miR-205, miR-218, and miR-542-5p [2, 23, 24].

Cancer stem cells (CSC) are a small tumorigenic population related to cancer development, resistance, metastasis, and relapse leading to poor clinical outcomes due to self-renewal ability and potential to redevelop the entire tumor heterogeneities. In this context, aberrant or dysregulated functioning miRNAs regulate and maintain CSCs through targeting various oncogenic signaling pathways, including Notch, WNT/β-Catenin, JAK/STAT, PI3K/AKT-mTOR leading to carcinogenesis with poor prognosis and drug resistance [17].

Instead, MiR-153 targets ADAM19 with consequent inhibition of cellular migration and invasion, producing an anti-tumorigenic activity through AKT suppression [25, 26]. Likewise, miR-451, the most downregulated miRNA in LC, can decreases phosphorylation of AKT in LC when it is overexpressed [27]. Additionally, miR-205 has a positive correlation with AKT gene expression in LC with an impact on the patient´s survival [24].

Let-7 is a miRNA involved in the suppressive tumorigenic process that usually reduces cancer aggressiveness and treatment resistance. However, when its expression in LC is reduced, the consequences are poor prognosis with a shorter survival rate. Only in very few situations, let-7 can be oncogenic by promoting cancer progression, invasion, metastasis, and treatment resistance. Moreover, let-7 targets cell cycle regulation proto-oncogenes, such as RAS, CDC25A, CDK6 and cyclin D [2, 28, 29].

Additionally, ROS1 gene rearrangements, mutant EGFR kinases, HER2 and BRAF mutations, RET gene rearrangements, high-level MET amplification, and anaplasic lymphoma kinase (ALK) fusion oncogene are among the most predictive biomarkers in advanced diagnostic, being recommended in patients with non-squamous histology whenever possible [30, 31]. In a similar way, echinoderm microtubule-associated protein-like 4 (EML4) and ALK fusion proteins (EML4-ALK) and ROS1 also induce tumorigenesis, through cell survival and proliferation. Likewise, mutations in the Kirsten rat sarcoma 2 viral oncogene homolog (KRAS - a common downstream reactor of receptor tyrosine kinases) drives DNA methylation with consequent silence of specific tumor suppressor genes leading to LC development and poor prognosis [2, 32-34].

Other emerging carcinogenic therapeutic targets include ALK, ROS1, EGFR, HER2, KRAS that are among the most predictive biomarkers in LC. Thus, LC treatment should include tumor molecular profiling with targeted therapies, being considered the first treatment choice whenever possible [32, 35, 36]. The main miRNAs involved in this process are: miR-96 by suppressing ALK; and miR-760 by suppressing ROS1; miR-193a-3p and miR-181a-5p that targets KRAS; miR-148a-3p and miR-1258 that target Rat sarcoma protein [2].

Cell proliferation is potentiated through deregulations of retinoblastoma (RB) and p53 protein, which are, in healthy subjects, cell growth suppressors through senescence or apoptosis. Furthermore, the silencing of some miRNAs such as miR-296, miR-299, miR-491, miR-512 and miR-1182 that target the telomerase reverse transcriptase can lead to unlimited cancer cells proliferative potential by maintaining telomere function and protecting from apoptosis [2, 37, 38]. Likewise, DNA methyltransferases (DNMT) are also affected by miRNAs. DNMTs are close related to miRNAs, where the miR-29 family (29a, 29b, and 29c) was found to directly target DNMT3A and -3B, presenting inversely correlated expression in LC and a tumor-suppressive effect by restoring normal patterns of DNA methylation and inducing apoptosis. DNA methylation can be induced by KRAS activation through miR-29b with consequent repression in TET1 expression. Thus, the development of epigenetic therapies that use synthetic miR-29s could be used in LC by reducing aberrant patterns of methylation and restoring hydroxymethylation [8, 34]. Similarly, miR-15a/miR-16 and miR-449a were found downregulated in LC, being targets for emerging therapeutics. Also, miR-16 and miR-301b target proteins related to cellular resistance to apoptosis; and miR-641 and miR-660 could be also used to enhance apoptosis of LC cells [2].

The tumor metastasis and invasion process are related to Snail, Slug, and Wnt, which are key players in epithelial to mesenchymal transition (EMT). EMT is an embryonic biochemical process, rarely observed in adult human cells, that increases migratory cell capacity, resistance to apoptosis, production of extracellular matrix components, loss of E-cadherin-mediated cell adhesion, with increased invasiveness, leading to cancer metastasis [2, 39-41]. Among the several miRNAs that target the balance between EMT and the reverse process, the downregulation of miR-153 induced by TGF-β mesenchymal phenotype of epithelial cancer cells consequently influences the expression of SNAI1 (Snail Family Zinc Finger 1) and ZEB2 (Zinc Finger E-Box Binding Homeobox 2) protein levels. Additionally, SNAI1 and ZEB2 are direct targets for miR-153, acting as pro-metastatic factor by inducing EMT [39, 40, 42].

There are two other important miRNAs, EMT-related, in LC: miR-200 and miR-218. The first one is a cell-autonomous suppressor of EMT and metastasis, targeting transcriptional factors, such as ZEB, being related to the promotion of EMT and the development of LC cells by

Table 1. Main microRNAs, targets, functions and effects in lung cancer.

microRNAs	Targets	Function	Effect	References
let-7	RAS, CDC25A, CDK6, cyclinD, LIN28, MYC, HMGA2,HOXA9, TGFBR1, BCL-XL, MAP4K3	represses cell proliferation, regulates cell cycle, cell signaling, and maintenance of differentiation	- ↓	[16, 28, 29]
mir-21	PTEN, PDCD4, TPM1	proliferative and anti-apoptotic function	- ↑	[77, 79, 83]
miR-23a	VEGF	decreases angiogenesis	- ↑	[2, 47]
miR-29	DNMT3A and -3B and TETs	restores normal patterns of DNA methylation and induces apoptosis	- ↓ + ↑	[8, 34]
miR-31-5p	HIF-1	increases glycolysis and ATP production	+ ↓ - ↑	[53]
miR-33b	lactate dehydrogenase A (LDHA)	glucose metabolism attenuation	- ↓ + ↑	[50]
miR-34	PD-L1, MET, BCL2, PDGFRA, PDGFRB	tumor immune evasion (apoptosis, DNA damage, and cell cycling)	- ↓ + ↑	[61, 83]
miR-101	COX-2, Lin28B, EZH2, IL-1β	inhibits cell proliferation and inflammation; dysregulation of cell cycle	+ ↑ - ↓	[66-69]
miR-130b	Peroxisome proliferator-activated receptor γ (PPARγ) /VEGF-A	facilitates proliferation, invasion and metastasis	- ↑	[49]
miR-144	GLUT1	increases glucose uptake and lactate production	- ↓	[51]
miR-155	hexokinase 2, APAf-1	promotes glucose metabolism, modulates cellular apoptosis and DNA damage	- ↑	[84-86]
miR-153	ADAM19, AKT, SNAI1 and ZEB2	inhibits migration and invasion of human non-small-cell lung cancer; inhibits the proliferation and migration, and promotes apoptosis of cultured lung cancer cells. Pro-metastatic factor by inducing EMT	- ↓ + ↑	[25, 26, 39, 40]
miR-197	Bcl-2, c-Myc, cyclin D1 and PD-L1	promotion of chemoresistance, tumorigenicity, and pulmonary metastasis	- ↓	[62]
miR-199a	HIF-1a	suppresses the hypoxia-induced proliferation	- ↓	[52]
miR-200	ZEB, E-cadherin, vimentin, VEGF	inhibits angiogenesis, suppresses EMT and metastasis	+ ↑	[2, 43, 44, 87]
miR-205	DOK4 gene	cell invasion	- ↑	[24, 81]
miR-210	SDHD, subunit D of succinate dehydrogenase complex (SDH)	major influence on mitochondrial function, cell survival and homeostasis in advanced cancer	- ↑	[54]
miR-218	Slug/ZEB2, tumor protein D52	inhibits cell migration, invasion and metastasis	- ↓	[45, 88]
miR-487b-5p	LAMP2	tumor activator in lung cancer tissues	- ↑	[89]
miR-494	VEGF, PTEN, AKT	promotes angiogenesis and tumor growth under hypoxic condition	- ↑	[2, 90]
miR-708	TMEM88, Wnt signaling pathway	increases cell proliferation, migration, and invasion	- ↑	[91]

Legend: - negatively/pro-oncogenic/facilitates cancer development; + positively/anti-oncogenic/inhibits cancer development; ↑ when upregulated/overexpressed, ↓ when downregulated/under-expressed.

influencing its motility [43, 44]. The second one, the miR-218, modulates Slug/ZEB2 signal [45]. There are many other miRNAs that act as suppressors of EMT: miR-124, miR-135a, miR-148a and miR-193a-3p/5p, but they are often downregulated in LC [46] Some miRNAs are involved in tumor angiogenesis through vascular endothelial growth factors (VEGFs), such as miR-126, miR128 and miR-200.

Regarding neovascularization, which is essential to cancer cells proliferation, miR-494 and miR-23a promote angiogenesis by activation of VEGF pathway. The inhibition of miR-23a has the potential to decrease angiogenesis and, consequently, tumor growth representing an emergent therapy for LC cancer-derived exosomal miR-23a [2, 47]. By presenting antagonist behavior, miR-126, miR-128, miR-200, and miR-497 downregulate VEGF, consequently inhibiting angiogenesis in LC. Those miRNAs can be used as biomarkers in LC, and possibly new therapeutic targets in the LC treatment [2, 48]. However, these miRNAs also present a tumorigenic effect. The downregulation of miR-128 was significantly correlated with LC differentiation, aggression and metastization [23]. On the other hand, the up-regulation of miR-130b facilitates proliferation, invasion and metastasis, with consequently unfavorable prognosis of LC patients [49].

The following miRNAs, miR-144, miR-33b, miR-199a, and miR-31-5p, can promote deregulations in LC cellular energetics, being important regulators of cancer glucose metabolism [2, 50]. MiR-144 was found downregulated in LC cells, with consequently increased glucose uptake and lactate production. Thus, aerobic metabolism is enhanced in these cells, with consequently negative altered metabolism, leading to the rapid proliferation of tumor cells. A future potential therapy should consider the possible stimulation of the miR-144 expression through GLUT1, a direct target of this miRNA, with inverse expression [51]. LC cell proliferation is also increased when miR-33b is downregulated. In this context, miR-33b has an anti-oncogenic potential since its overexpression inhibits LC cells proliferation through glucose metabolism attenuation [50]. Additionally, the decreased levels of miR-199a, or overexpression of miR-31-5p were reported to be related to LC progression through hypoxia inducible factor 1 (HIF-1), by affecting glycolysis and ATP production [2, 52, 53]. MiR-210 is also related to the hypoxia through HIF-1a, mainly in late stages of non-small cell LC, with consequently poor outcomes [54].

Cancer cells can evade immune destruction through programmed death-ligand 1 (PD-L1) and its receptor (PD-1) [2]. Apart from the imagology, pathological tests, including PD-L1 immunohistochemistry, is recommended in the initial LC workup [30]. Furthermore, PD-L1 blockade immunotherapy, with or without chemotherapy, is one of the best current LC treatment choices due to survival benefits and less adverse events [55-60]. This process is target related to miR-34, miR-138, miR-197, miR-200 and miR-513. Taking in consideration that PDL1 is regulated by p53 *via* miR-34, or by miR-197-mediated CKS1B/STAT3 axis, these miRNAs could be used as novel immunotherapy treatment, associated with standard protocols, to improve LC treatment and consequently prognosis [61, 62].

One of the main promotors and maintainers of LC is chronic inflammation mediated by tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), IL-6, IL-8 and cyclooxygenase-2 (COX-2) [63, 64]. High levels of IL-1β in the cancer microenvironment inhibits miR-101, a tumor-suppressive miRNA, *via* COX-2-HIF1α pathway, being directly associated with bad prognosis due to the invasiveness promotion [63, 65, 66]. Thus, the downregulation of miR-101 by IL-1β promotes the development of the malignant process [64, 67]. Additionally, the upregulation of the Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2), in LC IL-1β-miR-101-EZH2 axe, promotes tumor development and progression by cell cycle dysregulation [68, 69]. Let-7 expression is also related to the tumorigenic inflammatory process. The positive feedback happens when NFκB reduces let-7 levels, inhibiting IL-6 expression, and consequently activating NFκB [28, 29]. In this perspective, miRNAs control cancer cellular behaviors and dysregulates their interaction with surrounding cells, being the cancer microenvironment an important aspect in diagnosis and treatment [2].

5. CLINICAL ROLE OF miRNAs IN DIAGNOSIS, PROGNOSIS AND TREATMENT OF LUNG CANCERS

As already reported, DNA methylations can be used as biomarkers to detect premalignant and malignant stages of a disease or to assess the risk of progression to malignancy. The identification of selective, specific and minimally invasive biomarkers is essential in order to facilitate early LC diagnosis and to improve clinical outcomes including prognosis, prediction of therapeutic response, monitoring therapy, or assessment of risk of recurrence after curative surgery in precision medicine. The discovery that miRNAs are present and very stables in the blood flow circulation; and that many of them are significantly and independently associated with LC development, opens a new perspective for diagnosis and prognosis [4, 20, 70-73]. Some plasma levels of miRNAs are already being tested, including miRNA-195, miR-223, and let-7, that could be used as a biomarker for the early LC detection, as well as, a potential monitoring system for cancer treatment. It was demonstrated that low expression of let-7 after surgery leads to a poor prognosis [29, 74, 75] and that the miR-374a low expression (biopsied from tumor or normal lung tissue) in early NSCLC (stages I or II) was related to poor survival rates [76]. However, the combination of multiple serum miRNAs, instead of only one, allows a more accurate cancer diagnosis [70]. Furthermore, plasma miRNA signatures can more accurately predict cancer development and prognosis than yearly spiral-CT [77].

Besides the plasma, miRNAs are also stably and found in the cytological analysis of sputum, being a potentially non-invasive diagnostic tool for LC with a sensitivity of 73% and a specificity of 96%. It was demonstrated that the best prediction for lung squamous cell carcinoma where obtained through miR-205 (sensitivity of 96% and specificity of 90%) in comparison with miR-210 and miR-708 [78]. The sputum analysis of the combination among miR-21, miR-486, miR-375 and miR-200b demonstrated 80.6% of sensitivity and 91.7% of specificity for early detection of lung adenocarcinoma [79].

The sputum markers for LC showed a potential method of minimal invasive examination for LC diagnosis includes a

cytological examination. However, the DNA methylation markers have low sensitivity. On the other hand, the DNA methylation alteration in histone genes of HIST1H4F analysis can reach 96.7% of specificity and 87.0% of sensitivity, having the potential to improve LC screening [11].

Table 2. Main microRNAs in some lung cancer subtypes according to histology.

MicroRNAs	Histologic Subtype	References
miR-124a ↓ **miR-27a** ↑ **miR-212** ↑ **miR-132** ↑	adenocarcinoma	[2]
miR-375 ↑	adenocarcinoma	[79]
miR-133B ↓ **miR-145** ↓ **miR-155** ↑	adenocarcinoma	[82, 92-94]
miR-486↓ ***miR-126***↓ ***miR-145***↓ ***miR-21***↑ ***miR-182***↑ ***miR-375***↑ ***miR-200b***↑	adenocarcinoma	[77, 79, 95]
miR-487b-5p ↑	resistant lung carcinoma	[89]
miR-205 ↑	squamous cell carcinoma	[78, 80]
miR-17-92 ↑ **miR-106a-363** ↑ **miR-93-106b** ↑	squamous cell carcinoma	[96]
miR-205 ↑ **miR-210** ↑ **miR-708** ↑	squamous cell carcinoma	[77, 78]
miR-126↓ **miR-193a-3p**↓ **miR-30d**↓ **miR-30a**↓ **miR-101**↓ **let-7i**↓ **miR-15a**↓ **miR-185** ↑ **miR-125a-5p**↑	squamous cell carcinoma	[92, 97]
miR-708 ↑	adenocarcinoma and squamous cell carcinoma	[91]

Legend: ↑ upregulated/overexpressed, ↓ downregulated/under-expressed.

The differentiation between under or overexpressed miRNAs is important for treatment planning (Table **1**). A meta-analysis of miRNA expressions in LC found that seven miRNAs were statistically significant upregulated (miR-21, miR-210, miR-182, miR-31, miR-200b, miR-205 and miR-183) and eight downregulated (miR-126-3p, miR-30a, miR-30d, miR-486-5p, miR-451a, miR-126-5p, miR-143 and miR-145) [80]. Additionally, the upregulated miR-7, miR-21, miR-200b, miR-210, miR-219-1, miR-324 and downregulated miR-126, miR-451, miR-30a, and miR-486, are deregulated in accordance to CT identifiable LCs [77]. Asbestos-related LC also presents some overexpressed miRNAs (miR-148b, miR-374a, miR-24-1) and other under-expressed (miR-939, miR-671-5p, miR-605, miR-1224-5p, miR-202) with a predictive accuracy of 100% in most cases. Additionally, miR-934, miR-500, miR-892b were found overexpressed in smokers [81]. Some miRNAs are more specific for different LC histologic subtypes, as depicted in Table **2**.

The precision medicine to early diagnosis could change the unfavorable clinical prognosis by allowing the more adequate and less aggressive treatment choice. Furthermore, the personalized, targeted medicine will allow the reduction in side effects of the conventional treatments. However, large scale studies are required in order to confirm the value of DNA methylation and miRNAs markers, together with transcriptome and proteome biomarkers applied as powerful targets to effective therapy strategy [4, 20]. Most of the LC are related to DNA hypermethylation, however, some genes (GORASP2 and ZYG11A) are hypomethylated and overexpressed in invasive adenocarcinoma. Thus, there is a large field for the development of new prognostic indicators and potential novel target molecules for clinical applications [9].

Another potential important application of miRNAs is in the LCs that are resistant to conventional treatments. It is known that unstable miRNAs can influence in the LC therapeutics due to differential expressions, of some miRNAs, in drug-sensitive and drug-resistant patients. In this context, the expression levels of miRNAs, mainly those with high sensitivity and specificity, can predict effects, guide therapies, or even be used in the therapies. The direct targeting of TP53 by miR-155, involved in the resistance of many chemotherapy modalities, could lead to the development of new therapies oriented to reverse chemoresistance by using anti-miR-155, for example [20, 82].

CONCLUSION

Despite the reduction of LC mortality through computed tomography, the image screening method presents high false-positive rates, leading to overdiagnosis and negative related factors. The reversibility of DNA methylation and the capacity of miRNAs to regulate multiple target genes, open new possibilities for personalized and less invasive therapeutic possibilities. Thus, the development of other diagnostic methods and prognostic prediction, based on the genetic and epigenetic expression levels of miRNAs, for example, can potentially serve as targets for cancer therapy in precision medicine. This is very important to allow early diagnosis in order to improve survival, but also to overcome drug resistance in some LC treatments [2, 4, 21, 23, 82, 98].

However, in spite of the promising applications in diagnosis, prognosis and even treatment that are still in the phase of its clinical trials, there is still inconsistency in miRNA

research being hard to compare results and to find an ideal diagnostic model. In addition, the delivery of miRNA-based drugs, the immunogenic and toxic effects are still under investigation. Thus, the first step is to move towards consistency in the miRNAs studies to allows a more secure use in clinical practice [17, 73]. Finally, new comprehensive large-scale investigations will bring light for powerful and efficient protocols in improved LC diagnosis, prognosis and specific challenge therapeutics, leading to minimal toxicity and improving survival and quality of life.

CONSENT FOR PUBLICATION

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