Diagnostic and Therapeutic Implications of microRNAs in Non-Small Cell Lung Cancer

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Abstract: microRNAs (miRNAs), endogenous suppressors of target mRNAs, are deeply involved in every step of non-small cell lung cancer (NSCLC) development, from tumor initiation to progression and metastasis. They play roles in cell proliferation, apoptosis, angiogenesis, epithelial-to-mesenchymal transition, migration, invasion, and metastatic colonization, as well as immunosuppression. Due to their versatility, numerous attempts have been made to use miRNAs for clinical applications. miRNAs can be used as cancer subtype classifiers, diagnostic markers, drug-response predictors, prognostic markers, and therapeutic targets in NSCLC. Many challenges remain ahead of their actual clinical application; however, when achieved, the use of miRNAs in the clinic is expected to enable great progress in the diagnosis and treatment of patients with NSCLC.

Keywords: microRNA; non-small cell lung cancer; diagnosis; prognosis

1. Introduction

microRNAs (miRNAs) are small, non-coding RNAs that are 20–25 nucleotides in length [1,2], and are endogenous suppressors of target genes [3]. They have complementary sequences to 3′-untranslated regions (3′-UTRs) of target mRNAs and bind to these regions through Watson–Crick base pairing. Perfectly matched binding between miRNAs and 3′-UTRs leads to mRNA cleavage and degradation, while imperfectly matched binding leads to translational repression. This binding involves 7–8 nucleotides of the miRNAs, which is called the seed sequence; therefore, one miRNA can target multiple mRNAs, and one mRNA can be targeted by multiple miRNAs. Through suppressing target genes, miRNAs regulate diverse physiological and pathological conditions, including cancer. miRNAs can either promote or repress cancer development and progression according to their target genes. Numerous miRNAs can act as oncogenes by negatively regulating tumor suppressors. For example, miR-21 expression is up-regulated in colon cancer, and promotes cell growth and invasion by repressing the tumor suppressor PTEN [4]. Conversely, let-7 inhibits cellular proliferation by negatively regulating the KRAS oncogene in lung cancer [5].

Lung cancer manifests as a malignant tumor caused by uncontrolled cell growth in the bronchiolar and alveolar epithelium of the lungs. Lung cancer is the most common cancer worldwide in both incidence (11.6% of the total cases) and mortality (18.4% of the total cancer deaths) [6]. Long-term smoking is well-known to be the main cause of lung cancer, while environmental effects (e.g., air pollution and particulate matters) and genetic variations (e.g., KRAS and EGFR mutations) can
also cause lung cancer [7]. Lung cancer is classified into two main types depending on their histological phenotype: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [7]. SCLC accounts for 15% of lung cancer, and is mainly associated with smoking. NSCLC accounts for 85% of lung cancer, and is further divided into adenocarcinoma (LUAD), squamous cell carcinoma (LUSC), and large cell carcinoma on the basis of cellular pathology and sites of origin. This review focuses on NSCLC, and describes the latest research results and trends regarding the clinical implications of using miRNAs for the treatment of NSCLC.

2. Biogenesis of miRNAs

Since the first miRNA, lin-4, was discovered in *Caenorhabditis elegans* in 1993 [8,9], thousands of miRNAs have been identified in both animals and plants due to the development of molecular genetics and next-generation sequencing technology. According to miRBASE (Release 22.1, October 2018; http://www.mirbase.org), 2656 mature miRNAs have been identified in the human genome to date [10]. miRNAs are encoded in the genome as single genes (i.e., monocistronic) or as clusters that are transcribed together with other miRNAs (i.e., polycistronic). miRNAs are generally transcribed by RNA polymerase II in the nucleus in a form called primary miRNAs (pri-miRNAs), which can be longer than thousands of nucleotides. As RNA polymerase II is involved, the transcription of pri-miRNAs is regulated by general transcription factors and signal transduction pathways. The pri-miRNAs are also capped at the 5′-end and polyadenylated at the 3′-end, just like mRNA transcripts.

Like normal single-stranded RNA molecules, pri-miRNA can form secondary structures in which part(s) of the pri-miRNA strand forms hairpin loops, stabilized by intramolecular hydrogen bonds. The mature miRNAs, comprised of 20–25 nucleotides, are contained in the stem of the hairpin structures. Once transcription is completed, pri-miRNAs are processed into premature miRNAs (pre-miRNAs) which are stem–loop structures of about 80 nucleotides in length. The processing of pri-miRNAs to pre-miRNAs is fulfilled by a microprocessor complex, which consists of Drosha, a double-stranded RNA-specific ribonuclease (RNase) III [1,2,11,12], and DGCR8, a molecular anchor recognizing the double-strand/single-strand junction of pri-miRNAs [13]. With the help of other additional factors, such as DDX5 and DDX17 RNA helicases [14], the Drosha:DGCR8 complex cuts out the stem–loop structure (pre-miRNA) from the pri-miRNA at the precise positions on the 5′ and 3′ sides.

This processing of pri-miRNAs to pre-miRNAs occurs in the nucleus. Processed pre-miRNAs are exported to the cytoplasm by exportin 5, a double-stranded RNA-binding protein, and RanGTP, a GTP-binding nuclear protein [1,15–17]. Exportin 5 binds to stem–loop pre-miRNAs, and RanGTP binding triggers nuclear export of the pre-miRNA:exportin 5 complex. Pre-miRNAs are transported through the nuclear pore and then released into the cytoplasm upon the hydrolysis of RanGTP to RanGDP. Intriguingly, exportin 5 has also been reported to promote pri-miRNA processing, which is independent of RanGTP [18].

Once released into the cytoplasm, the stem of the pre-miRNA is further processed to a small RNA duplex (20–25 nucleotides) by another RNase III-type endonuclease, Dicer. The N-terminal helicase domain of Dicer recognizes pre-miRNAs at their terminal loop, and the internal PAZ domain binds to the termini of pre-miRNAs. The recognition of pre-miRNAs by the helicase and PAZ domains may function as “a molecular ruler” which guarantees precise cleavage of pre-miRNAs [1]. Dicer cooperates with the RNA-binding protein TRBP, which facilitates pre-miRNA processing and determines the exact length of mature miRNAs [19].

Processed miRNA duplexes bind to Ago proteins, which are main components of the RNA-induced silencing complex (RISC). During this process, only one of the two miRNA strands is specifically selected (guide strand) to function as a mature miRNA, while the other is degraded (passenger strand). The exact mechanism of strand selection has not been fully elucidated; however, it has been shown that stability of duplex ends and types of 5′-terminal nucleotides can determine strand fate [20]. In some cases, both the 5′- (5p) and 3′-sides (3p) of a pre-miRNA can be functional [21]. For example, both miR-142-5p and miR-142-3p are downregulated in liver cancer possibly through promoter hypermethylation [22].
3. Functional Mechanisms of miRNAs

In most cases, miRNAs recognize mRNA targets via binding to specific sequences at their 3′-UTRs; however, some miRNAs can bind to 5′-UTRs or coding sequences of mRNA targets [24]. For example, miR-1254 interacts with the 5′-UTR of CCAR1 and enhances its stability [25], while miR-20a represses DAPK3 by binding to its protein coding sequences (exon 2) [26]. Even promoter regions can be targeted by miRNAs; let-7i binds to the core promoter region of IL2 and up-regulates IL2 promoter activity [27]. Recently, numerous studies have shown that long non-coding RNAs are also binding partners of miRNAs [28].

Mature miRNAs loaded onto the RISC mediate gene silencing via two mechanisms: mRNA decay and translational repression [29,30]. Ago proteins have an RNase H-like domain; thus, RISC can cleave mRNA targets when they are perfectly complementary to miRNAs, which is common in plants [31]. In contrast, animal miRNAs bind to their target mRNAs at seed sequences of 7–8 nucleotides in length thorough partial complementarity, which instead induces translational repression and mRNA decay. miRNA-loaded Ago proteins bind to GW182, a scaffolding protein [32] that recruits poly(A)-binding protein (PABP) and deadenylase complexes (CCR4-NOT and PAN2-PAN3). Deadenylation then causes degradation of target mRNAs. In addition, RISC recruits decapping factors, such as DCP1, DCP2, and DDX6, which promotes decapping and degradation of target mRNAs [29,30].

GW182 binding to RISC represses initiation of translation by breaking the interaction between PABP and eIF4G, which stimulates ribosome recruitment [29]. GW182 also recruits translation repressors working at the inhibition step, such as DDX6 [33] and eIF4E-binding protein 4E-T [34]. In addition, miRNAs have been shown to dissociate the eIF4A complex, thus inhibiting ribosome binding and scanning [35]. Translational repression and mRNA decay are both crucial mechanisms of miRNA-mediated gene silencing; however, these two mechanisms do not always work synergistically [36], and the preference between the two mechanisms may depend on cellular context or miRNA:mRNA binding characteristics [29,37]. The biogenesis and functional mechanisms of select miRNAs are summarized in Figure 1.

4. Regulation of miRNA Expression

miRNA expression can be regulated through transcriptional and post-transcriptional mechanisms [38]. Like mRNAs transcribed by RNA polymerase II complex, miRNAs are also under the transcription control of various signaling pathway components and transcription factors [39]. Promoter methylation
is also one of major mechanisms for regulating miRNA expression. miR-34a is downregulated in colon cancers with liver metastases, which is due to hypermethylation on the promoter region [40]. Upregulation of oncogenic miR-21 in various types of cancer is associated with hypomethylation on the promoter region [41]. In addition, histone modification on the promoter of miR-200b/200a/429 cluster causes silencing of miR-200 family members and promotes stemness of breast cancer cells [42].

After transcription and processing/maturation, miRNA levels are further regulated by diverse endogenous factors. Ago2 protein increases miRNA abundance by promoting miRNA processing and enhancing miRNA stability, which is independent of its RNase function [43]. RNA binding protein FXR1 stabilizes miR-301a-3p and facilitates p21 targeting in oral cancer [44]. QKI-5 directly interacts with miR-196b-5p and reduces its stability [45]. Numerous competing endogenous RNAs (ceRNAs) are also involved in the regulation of miRNA levels. ITGA1 mRNA functions as a sponge against miR-181b and relieves ADCY9 targeting [46]. FN1 mRNA acts as a ceRNA for miR-200c and modulates epithelial-to-mesenchymal transition (EMT) in breast cancer cells [47]. Moreover, one of the main features of long non-coding RNAs [48] and circular RNAs [49] is their functioning as ceRNAs, thus preventing the interaction between miRNAs and their target genes.

5. Roles of miRNAs in Progression and Metastasis of NSCLC

From the moment of tumor initiation to distant metastasis, epithelial cancer cells, such as NSCLC cells, must undergo a series of steps that are necessary for the acquisition of their pathological properties [50]. First, certain epithelial cells gain a selective growth advantage via genetic or epigenetic events. Through the EMT, highly proliferative cancer cells lose cell-to-cell and cell-to-matrix adhesion, and separate from the primary tumor. These cells acquire additional invasive abilities, and invade into basement membranes and stromal extracellular matrix (ECM). The invasive cancer cells further invade into surrounding blood or lymphatic vessels (intravasation). Then, they are transported via the circulatory system to distant sites and exit from blood vessels (extravasation). Once at the new site, the cancer cells reinvade, adapt to the new environment, and, finally, proliferate to form secondary metastatic tumors. miRNAs are closely involved in every step of NSCLC progression and metastasis, and precisely control the expression and activity of key factors during these processes. Select miRNAs that are known to regulate NSCLC development are shown in Table 1.

5.1. Primary Tumor Growth

Abnormal and uncontrolled cell growth beyond defined boundaries is the initial and essential step of tumor progression. Both enhanced proliferation and suppressed cell death (apoptosis) increase cancer cell growth and are controlled by numerous miRNAs targeting oncogenes or tumor suppressors. let-7 family members inhibit proliferation of NSCLC cells by directly targeting K-Ras [51] and cyclin D1 [52]. miR-34 family members (miR-34a, 34b, and 34c) also suppress NSCLC cell proliferation by targeting cyclin E1 [53], CDK4 [54], and c-Myc [55]. In contrast, miR-224 promotes NSCLC growth by targeting TNFα-induced protein 1, which is involved in DNA synthesis and apoptosis [56]. miR-212 also exerts tumor-promoting effects in NSCLC cells via suppression of the hedgehog signaling pathway receptor PTCH1 [57]. miR-21, a well-known oncogenic miRNA, promotes NSCLC progression by targeting the tumor suppressor PTEN [58]. Apoptosis is also modulated by miRNAs. miR-34a and miR-7 target BCL-2 [59,60], and miR-195 targets survivin [61], both of which are anti-apoptotic proteins. In contrast, miR-484 targets Apaf-1 [62], and miR-182 and miR-494 target caspase-2, both of which are key promoters of apoptosis [63,64].

5.2. Angiogenesis and Hypoxia

The formation of a network of new blood vessels that can supply enough oxygen and nutrients to the tumor tissues is essential for optimal tumor outgrowth. Cancer cells facilitate growth of vascular endothelial cells and promote angiogenesis by secreting vascular endothelial growth factors (VEGFs) [50]. miR-128 and miR-195 directly target VEGFs thereby suppressing tumorigenesis and
angiogenesis [65,66]. miR-200b targets the VEGF receptors Flt-1 and KDR, and suppresses cancer cell invasion and metastasis [67,68]. On the opposite side, miR-130b promotes tumorigenesis by targeting TIMP-2, an inhibitor of metalloproteinase-2 and angiogenesis [69]. An inadequate supply of oxygen around the tumor microenvironment causes hypoxia. HIF1α, which promotes angiogenesis, is induced under hypoxic conditions. Several studies have reported that HIF1α is also targeted by several miRNAs, such as miR-130a, miR-199a, and miR-200c [70–72].

5.3. EMT, Migration, and Invasion

Epithelial cancer cells lose apical–basal polarity during EMT, and turn into mesenchymal-like cells with enhanced migratory and invasive capacities [73]. EMT is regulated by various EMT-inducing transcription factors (e.g., ZEB1/2, Snail, Slug, Twist), cell adhesion molecules (e.g., E-cadherin, N-cadherin), and tight junction proteins (e.g., Crumbs, Claudins), as well as numerous miRNAs. miR-200 family members that target ZEB1/2 are well-known suppressors of EMT [74]. Moreover, Snail and Twist are direct targets of miR-34a [75] and miR-98 [76], respectively. miR-544a promotes invasion by targeting E-cadherin [77], and miR-124 suppresses EMT by targeting N-cadherin [78]. Matrix metalloproteinases (MMPs) also play key roles during cancer cell invasion and metastasis [79]. Among them, MMP2 is a target of miR-29b [80], and MMP14 is a target of miR-584 [81]. As mentioned above, TIMP-2, which regulates MMPs’ activity, is a target of miR-130b [69]. In addition, regulators of Rho GTPases, which are involved in actin cytoskeleton remodeling, filopodia and lamellipodia formation, adhesion, migration, and invasion of cancer cells, are also targeted by various miRNAs [82].

5.4. Survival and Immune Escape

Lack of attachment to the ECM triggers cell death (anoikis) in epithelial cells. Once they invade the vascular or lymphatic system, cancer cells need to acquire anoikis resistance [83]. miRNAs promote or inhibit anoikis by targeting key molecules involved in anoikis signaling pathways [84]. miR-34a and miR-451 enhance the susceptibility of lung cancer cells to anoikis [85,86]. miR-148a inhibits anchorage-independent growth by targeting MMP15 and ROCK1 in NSCLC [87]. In contrast, exosomal miR-222 promotes cell survival under anchorage-independent conditions by directly targeting SOCS3 [88]. Cancer cells must survive the attacks of the immune system to progress, and miRNAs are involved in both immune attacks on tumors and immune escape [89,90]. miR-451 suppresses cell proliferation and metastasis in lung cancer cells by directly targeting PSMB8, which is one subunit of an immunoproteasome and modulates inflammatory responses [91]. miR-200 family members target PD-L1 and control immunosuppression in NSCLC cells [92]. miR-138, miR-140, and miR-142 have also been reported to target PD-L1 [93–95]. On the contrary, miR-197 enhances PD-L1 expression through the regulation of the cyclin-dependent kinase CKS1B and STAT3 pathway [96].

5.5. MET and Metastatic Colonization

A subset of cancer cells that survive within the systemic circulation will ultimately colonize at a distant metastatic site. In contrast to the initial stages of cancer development, these cancer cells lose their mobility and invasiveness and regain cell-to-cell and cell-to-matrix adhesiveness, which is a reverse process of EMT known as the mesenchymal-to-epithelial transition (MET) [97]. Although metastasis occurs independently of MET in some cases [98], various miRNAs associated with EMT also regulate MET. Ectopic expression of miR-200 family members induces MET in highly metastatic lung cancer cells [99]. Additionally, miR-147 induces MET and reverses drug resistance [100]. miR-29b promotes MET and prevents lung fibrosis [101]. Selection of the final metastatic destination and colonization at various organ sites, such as the bones, brain, and lymph nodes, is also affected by a variety of miRNAs [102].
Table 1. miRNAs regulating progression and metastasis of non-small cell lung cancer.

| miRNA            | Type | Effect                            | Target Gene          | References   |
|------------------|------|-----------------------------------|----------------------|--------------|
| **Tumor Growth and Apoptosis**                                                                                      |
| let-7 family     | tsmiR| Inhibits cell proliferation       | KRAS, CCND1          | [51,52]      |
| mir-34 family    | tsmiR| Inhibits cell proliferation,      | CCNE1, CDK4          | [53–55]      |
| mir-7            | tsmiR| Promotes apoptosis                | MYC, BCL2            | [59]         |
| mir-195          | tsmiR| Promotes apoptosis                | BIRC5                | [60]         |
| mir-224          | oncomiR| Promotes cell growth             | TNEAIP1              | [56]         |
| mir-212          | oncomiR| Promotes cell growth             | PTCH1                | [57]         |
| mir-21           | oncomiR| Promotes tumor progression       | PTEN                 | [58]         |
| mir-484          | oncomiR| Suppresses apoptosis             | APAF1                | [62]         |
| mir-182          | oncomiR| Suppresses apoptosis             | CASP2                | [63]         |
| mir-494          | oncomiR| Suppresses apoptosis             | CASP2                | [64]         |
| **Angiogenesis**                                                                                                    |
| mir-128          | tsmiR| Suppresses angiogenesis           | VEGFA                | [65]         |
| mir-195          | tsmiR| Suppresses angiogenesis           | VEGFA                | [66]         |
| mir-200b         | tsmiR| Suppresses angiogenesis, invasion, and metastasis | FLT1, KDR | [67,68]      |
| mir-130a         | tsmiR| Suppresses angiogenesis           | HIF1A                | [70]         |
| mir-199a         | tsmiR| Suppresses angiogenesis           | HIF1A                | [71]         |
| mir-200c         | tsmiR| Suppresses angiogenesis           | HIF1A                | [72]         |
| mir-130b         | oncomiR| Promotes angiogenesis            | TIMP2                | [69]         |
| **Epithelial-to-Mesenchymal Transition (EMT), Migration, and Invasion**                                             |
| mir-200 family   | tsmiR| Inhibits EMT                      | ZEB1, ZEB2           | [74]         |
| mir-34a          | tsmiR| Inhibits EMT                      | SNAI1                | [75]         |
| mir-98           | tsmiR| Inhibits EMT                      | TWIST1               | [76]         |
| mir-544a         | oncomiR| Promotes EMT                     | CDH1                 | [77]         |
| mir-124          | tsmiR| Inhibits EMT                      | CDH2                 | [78]         |
| mir-29b          | tsmiR| Inhibits invasion                 | MMP2                 | [80]         |
| mir-584          | tsmiR| Inhibits invasion                 | MMP14                | [81]         |
| mir-130b         | oncomiR| Promotes invasion                | TIMP2                | [69]         |
| **Anchorage-Independent Survival and Immune Escape**                                                              |
| mir-148a         | tsmiR| Inhibits cell survival            | MMP15, ROCK1         | [87]         |
| mir-222          | oncomiR| Promotes cell survival           | SOCS3                | [88]         |
| mir-451          | tsmiR| Promotes immune escape            | PSMB8                | [91]         |
| mir-200 family   | tsmiR| Promotes immune escape            | CD274                | [92]         |
| mir-138          | tsmiR| Promotes immune escape            | CD274                | [93]         |
| mir-140          | tsmiR| Promotes immune escape            | CD274                | [94]         |
| mir-142          | tsmiR| Promotes immune escape            | CD274                | [94]         |
| mir-197          | oncomiR| Promotes immune escape            | CSK1B                | [96]         |
| **Mesenchymal-to-Epithelial Transition (MET) and Colonization**                                                     |
| mir-200 family   | tsmiR| Promotes MET                      | ZEB1, ZEB2           | [99]         |
| mir-29b          | tsmiR| Promotes MET                      | TGFB1                | [101]        |

1 tsmiR: tumor suppressive miRNA, oncomiR: oncogenic miRNA.

6. Clinical Implications of miRNAs in NSCLC

Considering that multiple miRNAs have functions similar to their target genes and that a single miRNA can regulate several mRNAs, a panel of miRNAs is considered a better biomarker than individual miRNAs for clinical applications [74]. Since cancer-associated miRNA biomarkers can be easily detected in tissue, blood, or other bodily fluids, circulating miRNAs grant several potential advantages for clinical application, including high stability in serum, ease of non-invasive detection
in circulation, and a convenient screening method [103]. Circulating miRNAs also afford a chance to overcome the problem of tumor heterogeneity by allowing for the collection of all pathological signals from many disparate portions of primary tumors and metastatic sites.

6.1. NSCLC Subtype Classifiers

LUAD and LUSC are the major histological subtypes of NSCLC. LUSC is most commonly associated with tobacco use, while LUAD is commonly associated with non-smokers and women [104], suggesting the existence of several underlying major differences not only in biological patterns, but also in molecular characteristics, between histological subtypes. For example, activating mutations in epidermal growth factor receptor (EGFR) and mutations in anaplastic lymphoma kinase (ALK) fusion proteins usually occur in LUAD, but not in LUSC, rendering therapy targeted at these genes ineffective for LUSC [105]. MiRNAs can be used to distinguish subtypes among NSCLC. In a previous report, four miRNAs (miR-205, miR-93, miR-221, and miR-30e) were shown to be highly expressed in LUSC, and five miRNAs (miR-29b, miR-29c, let-7e, miR-100, and miR-125a-5p) were highly expressed in LUAD [106]. Through an analysis of three miRNome profiling datasets, Hu et al. identified that miR-375, miR-203, and miR-205 were differentially expressed miRNAs that could be used to distinguish LUSC from other NSCLC subtypes [107]. A recent study using machine learning approaches identified miR-944 and miR-205 as useful for classifying tumors into the LUAD and LUSC subtypes [108].

6.2. Diagnostic Markers

To date, serum tumor markers have not been employed for early lung cancer screening due to limitations in their effectiveness, sensitivity, and specificity. However, circulating miRNAs have demonstrated potential advantages for use in clinical screening methods [103]. Currently, several articles have shown that many kinds of circulating miRNAs can be used to detect lung cancer [109–112]. Recently, in an analysis of serum samples from 1566 lung cancer and 2178 non-cancer participants, the diagnostic accuracy, sensitivity, and specificity of the combined expression levels of two miRNAs (miR-1268b and miR-6075) were all 99%, regardless of the histological type and pathological Tumor, Node, Metastasis (TNM) stage of the NSCLC [110]. In another study of 2856 participants, a 14-miRNA signature distinguished patients with lung cancer from patients with non-tumor lung diseases with an accuracy of 92.5%, sensitivity of 96.4%, and specificity of 88.6%. In addition, the expression level of miR-17-3p distinguished patients with lung cancer from those with non-tumor lung diseases with the highest significance and an Area Under the Receiver Operating Characteristics (AUROC) value of 0.899 [112].

6.3. Drug-Response Predictors

Furthermore, miRNAs could be useful for predicting tumor responsiveness to chemotherapy or different therapeutic approaches. In a study with drug-resistant NSCLC cell lines, several markers were identified as being predictive of the degree of responsiveness to therapy, with miR-192, miR-194, miR-205, miR-30a, and miR-30c demonstrated to be predictive factors for a positive response to chemotherapy [113]. In an analysis of 148 LUAD patients who were negative for EGFR mutations or ALK translocations and who received maintenance treatment with pemetrexed, progression-free survival duration for patients expressing different levels of circulating miR-25, miR-145, and miR-210 were significantly different in the pemetrexed-treated group, suggesting these three miRNAs are predictors for the efficacy of maintenance treatment [114]. Recently, with the advent of immune checkpoint blockade therapy, immunotherapy has shown promising results in various types of cancer including lung cancer [115]. In a comparative analysis between responders and non-responders to PD-1/PD-L1 inhibitors, miR-320 family members, such as miR-320d, miR-320c, and miR-320b, were identified as potential biomarkers for predicting the efficacy of immunotherapy in advanced NSCLC. In addition, the level of exosomal miR-125b-5p was dramatically downregulated in the partial response-post samples, indicating that miR-125b-5p levels might be useful for monitoring the efficacy...
of anti-PD-1/PD-L1 treatment [116]. When the T-cell suppressor miR-125b-5p is downregulated during immunotherapy, patients may achieve increased T-cell function and respond well to immunotherapy. Of note, plasma-derived exosomes detected in patients are mainly released from tumor cells, which can more accurately and dynamically reflect the state and function of tumor cells.

6.4. Prognostic Markers

A prognostic biomarker should ideally provide information on the overall disease outcome in patients, such as disease recurrence or disease progression, independent of the treatment regimen. The discovery of prognostic factors could contribute to classifying patients by prognosis and identifying high-risk cases requiring aggressive approaches [117]. Similar to oncogenes and tumor suppressor genes, oncogenic miRNAs and tumor suppressive miRNAs are playing major roles in accurately identifying lung cancer prognoses. Numerous studies have shown that individual miRNAs play prognostic roles in NSCLC patients [52,54,58,61,74,76–78,80,91,96,99]. In a recent analysis using the TCGA database, two different prognostic miRNA signatures (a four-miRNA signature for LUAD: miR-375, miR-148a, miR-29b-1, and miR-584; and a four-miRNA signature for LUSC: miR-4746, miR-326, miR-93, and miR-671) were found to be independent prognostic factors in LUAD and LUSC patients [118]. Machine learning algorithms are indeed useful methods for analyzing large volumes of data, such as genetic information produced by next-generation sequencing technologies. We previously applied a neural network-based algorithm called Cascaded Wx framework to extract miRNA markers most highly associated with LUAD patient survival [119]. Through subsequent profiling of miRNA expression levels in LUAD patient samples, miR-374a and miR-374b, both EMT-related miRNAs, were identified as potential prognostic markers associated with poor survival in LUAD patients [120].

6.5. Therapeutic Targets

MiRNAs have various functions within cancer cells, with some having a high specificity for cancer-associated pathways, and they are one of the most promising therapeutic targets with well-characterized expressions. Biologically, the attractiveness of using miRNAs for cancer treatment comes from their ability to target multiple genes involved in multiple cancer-related pathways. Adding tumor suppressive miRNAs or reducing oncogenic miRNAs in cancer cells could be effective as a therapeutic strategy. In in vivo lung cancer models for therapeutic applications, miR-15/16 [121], miR-29b [122], miR-7 [123], miR-34a [124], let-7 [125], miR-200c [126], and miR-145 [127] were tested using different delivery systems. Two phase I clinical trials have been conducted for advanced solid cancers, including NSCLC (Table 2) [128–130]. The first-in-human, phase I study of a microRNA-based cancer therapy using MRX34, a liposomal mimic of miR-34a, was conducted but closed early due to unexpectedly severe immune-mediated toxicities with a modest overall response rate of 4% [129]. In contrast, in a phase I MesomiR-1 trial, TargomiRs, comprised of a miR-16-based microRNA mimic packaged in EDV™ nanocells that are targeted with an anti-EGFR-specific antibody, was tested in patients with advanced NSCLC or malignant pleural mesothelioma. Overall, TargomiR treatment has been well tolerated and shown to be safe in patients. Interim data indicated that disease control was achieved in five of six patients after 8 weeks of protocol treatment [128,130].
Table 2. miRNA-based Therapy Phase I Clinical Trials in NSCLC.

| miRNA                      | Target | Population                  | N  | DLT                                        | Safety (≥G3, %)                                      | Efficacy | Status      | References |
|----------------------------|--------|-----------------------------|----|-------------------------------------------|-----------------------------------------------------|----------|-------------|------------|
| MRX34 (NCT01829971)       | miR-34 | HCC, Melanoma, RCC, NSCLC, SCLC, GIST | 85 | Hypoxia, thrombocytopenia, neutropenia, thrombocytopenia | SAEs (35), deaths (9), fever (4), chills (14), fatigue (9), back/neck pain (5), dyspnea (5), lymphopenia (18), thrombocytopenia (6), neutropenia (8) | ORR: 4%, SD for ≥4 cycles: 24% | Early closed | [129]      |
| TargomiRs, MesomiR-1 trial (NCT02369198) | miR-16 | MPM, NSCLC                  | 27 | Infusion-related inflammatory symptoms, coronary ischemia, anaphylaxis, cardiomyopathy, non-cardiac pain | lymphopenia (42), temporal hypophosphatemia (15), increased AST or ALT (19), cardiomyopathy (4), infusion-related inflammatory symptoms (8) | ORR: 5%, SD: 68%, DOR: 32 weeks | Completed | [128,130] |

DLT: dose-limiting toxicities; HCC: hepatocellular carcinoma; RCC: renal cell carcinoma; NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; GIST: gastrointestinal stromal tumor; SAEs: severe adverse events; ORR: objective response rate; SD: stable disease; MPM: malignant pleural mesothelioma; AST: aspartate aminotransferase; ALT: alanine aminotransferase; DOR: duration of the objective response.
7. Pitfalls and Challenges for Clinical Application of miRNAs

Two phase I clinical trials have provided early data elucidating the efficacy of novel miRNA-based treatment approaches [128,129]. Considering the fact that phase I studies are predominantly comprised of previously heavily treated patients who have partially or fully failed other treatment modalities, the potential of partial treatment response can be high (Table 2). However, in a MRX34 trial, treatment-attributed serious adverse events tended to occur late after the completion of daily MRX34 infusions. The serious adverse events included sepsis, hypoxia, cytokine release syndrome, and hepatic failure, which is a pattern suggestive of immune-mediated toxicity. These adverse events were not observed in the pre-clinical tests with MRX34 in animal models, including non-human primates [129]. These findings suggest that the development of appropriate drug delivery systems for these miRNA-based therapies is essential. The potentially wide-ranging impacts of miRNAs on the regulation of gene expression may result in unexpected side effects in normal cells through nontargeted delivery or unintentional targeting due to the expression of cancer-related antigens. Thus, to avoid “off target” effects, such as systemic immune activation, the effective and specific targeting of miRNA therapeutics to cancer tissues which spares normal tissues is an essential, but as yet unresolved, challenge to overcome.

8. Conclusions

To date, miRNAs have been widely known for their key roles in both tumorigenesis and tumor suppression, and have been extensively studied in the field of NSCLC. miRNAs represent a powerful tool to help in the diagnosis, prognosis, and prediction of response to various treatments of NSCLC to improve patient survival rates. miRNA mimics of tumor suppressive miRNAs or miRNA inhibitors (such as Antagomirs) against oncogenic miRNAs may have therapeutic potential. However, despite the potential clinical benefits of miRNA mimics, recent phase I trials have shown unexpected adverse events. Thus, the future development of innovative methods of miRNA delivery will be required to avoid evoking an undesirable immune response.

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Abbreviations

| miRNAs       | microRNAs          |
|--------------|--------------------|
| NSCLC        | non-small cell lung cancer |
| 3′-UTRs      | 3′-untranslated regions |
| SCLC         | small cell lung cancer |
| LUAD         | lung adenocarcinoma |
| LUSC         | lung squamous cell carcinoma |
| pri-miRNAs   | primary miRNAs     |
| pre-miRNAs   | premature miRNAs   |
| RNase        | ribonuclease        |
| RISC         | RNA-induced silencing complex |
| PABP         | poly(A)-binding protein |
| ceRNAs       | competing endogenous RNAs |
| EMT          | epithelial-to-mesenchymal transition |
| ECM          | extracellular matrix |
| VEGFs        | vascular endothelial growth factors |
| MMPs         | matrix metalloproteinases |
| MET          | mesenchymal-to-epithelial transition |
tsmiR tumor suppressive miRNA
oncomiR oncogenic miRNA
EGFR epidermal growth factor receptor
ALK anaplastic lymphoma kinase
TNM tumor, node, metastasis
AUROC area under the receiver operating characteristics

References
1. Ha, M.; Kim, V.N. Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* 2014, 15, 509–524. [CrossRef] [PubMed]
2. Treiber, T.; Treiber, N.; Meister, G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. *Nat. Rev. Mol. Cell Biol.* 2019, 20, 5–20. [CrossRef] [PubMed]
3. Peng, Y.; Croce, C.M. The role of MicroRNAs in human cancer. *Signal Transduct. Target. Ther.* 2016, 1, 15004. [CrossRef] [PubMed]
4. Wu, Y.; Song, Y.; Xiong, Y.; Wang, X.; Xu, K.; Han, B.; Bai, Y.; Li, L.; Zhang, Y.; Zhou, L.-M. MicroRNA-21 (Mir-21) promotes cell growth and invasion by repressing tumor suppressor PTEN in colorectal cancer. *Cell Physiol. Biochem.* 2017, 43, 945–958. [CrossRef] [PubMed]
5. Johnson, S.M.; Grosshans, H.; Shingara, J.; Byrom, M.; Jarvis, R.; Cheng, A.; Labourier, E.; Reinert, K.L.; Brown, D.; Slack, F.J. RAS is regulated by the let-7 microRNA family. *Cell* 2005, 120, 635–647. [CrossRef] [PubMed]
6. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J. Clin.* 2018, 68, 394–424. [CrossRef]
7. Lemjabbar-Alaoui, H.; Hassan, O.U.; Yang, Y.-W.; Buchanan, P. Lung cancer: Biology and treatment options. *Biochim. Biophys. Acta (BBA)-Rev. Cancer* 2015, 1856, 189–210. [CrossRef]
8. Lee, R.C.; Feinbaum, R.L.; Ambros, V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993, 75, 843–854. [CrossRef]
9. Wightman, B.; Ha, I.; Ruvkun, G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell* 1993, 75, 855–862. [CrossRef]
10. Kozomara, A.; Birgaoanu, M.; Griffiths-Jones, S. miRBase: From microRNA sequences to function. *Nucleic Acids Res.* 2019, 47, D155–D162. [CrossRef]
11. Lee, Y.; Ahn, C.; Han, J.; Choi, H.; Kim, J.; Yim, J.; Lee, J.; Provost, P.; Rådmark, O.; Kim, S.; et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003, 425, 415–419. [CrossRef] [PubMed]
12. Cho, S.J.; Lee, M.; Stout-Delgado, H.W.; Moon, J.S. DROSHA-dependent miRNA and AIM2 inflammasome activation in idiopathic pulmonary fibrosis. *Int. J. Mol. Sci.* 2020, 21, 1668. [CrossRef] [PubMed]
13. Guo, W.T.; Wang, Y. Dgcr8 knockout approaches to understand microRNA functions in vitro and in vivo. *Cell. Mol. Life Sci.* 2019, 76, 1697–1711. [CrossRef] [PubMed]
14. Fuller-Pace, F.V.; Moore, H.C. RNA helicases p68 and p72: Multifunctional proteins with important implications for cancer development. *Future Oncol.* 2011, 7, 239–251. [CrossRef]
15. Bohnsack, M.T.; Czaplinski, K.; Gorlich, D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 2004, 10, 185–191. [CrossRef]
16. Wu, K.; He, J.; Pu, W.; Peng, Y. The role of exportin-5 in MicroRNA biogenesis and cancer. *Genom. Proteom. Bioinform.* 2018, 16, 120–126. [CrossRef]
17. Yi, R.; Qin, Y.; Macara, I.G.; Cullen, B.R. Exportin-5 mediates the nuclear export of pre-miRNAs and short hairpin RNAs. *Genes Dev.* 2005, 17, 3011–3016. [CrossRef]
18. Wang, J.; Lee, J.E.; Riemondy, K.; Yu, Y.; Marquez, S.M.; Lai, E.C.; Yi, R. XPO5 promotes primary miRNA processing independently of RanGTP. *Nat. Commun.* 2020, 11, 1845. [CrossRef]
19. Fareh, M.; Yeom, K.H.; Haagsma, A.C.; Chauhan, S.; Heo, I.; Joo, C. TRBP ensures efficient Dicer processing of precursor microRNA in RNA-crowded environments. *Nat. Commun.* 2016, 7, 13694. [CrossRef]
20. Chen, L.; Sun, H.; Wang, C.; Yang, Y.; Zhang, M.; Wong, G. miRNA arm switching identifies novel tumour biomarkers. *EBioMedicine* 2018, 38, 37–46. [CrossRef]
21. Guo, L.; Lu, Z. The fate of miRNA* strand through evolutionary analysis: Implication for degradation as merely carrier strand or potential regulatory molecule? PLoS ONE 2010, 5, e11387. [CrossRef] [PubMed]

22. Godfrey, J.D.; Morton, J.P.; Wilczynska, A.; Sansom, O.J.; Bushell, M.D. MiR-142-3p is downregulated in aggressive p53 mutant mouse models of pancreatic ductal adenocarcinoma by hypermethylation of its locus. Cell Death Dis. 2018, 9, 643. [CrossRef] [PubMed]

23. Tsang, F.H.-C.; Au, S.L.-K.; Wei, L.; Fan, D.N.-Y.; Lee, J.M.-F.; Wong, C.C.-L.; Ng, I.O.-L.; Wong, C.-M. MicroRNA-142-3p and microRNA-142-5p are downregulated in hepatocellular carcinoma and exhibit synergistic effects on cell motility. Front. Med. 2015, 9, 331–343. [CrossRef] [PubMed]

24. Xu, W.; San Lucas, A.; Wang, Z.; Liu, Y. Identifying microRNA targets in distant metastasis of colon cancer. Int. J. Mol. Sci. 2018, 20, 56, 12024. [CrossRef] [PubMed]

25. Zhang, Y.; Fan, M.; Zhang, X.; Huang, F.; Wu, K.; Zhang, J.; Liu, J.; Huang, Z.; Luo, H.; Tao, L.; et al. Cellular microRNAs up-regulate translation interaction with promoter TATA-box motifs. RNA 2014, 20, 1878–1889. [CrossRef]

26. López-Urrutia, E.; Montes, L.P.B.; Cervantes, D.L.D.G.; Pérez-Plasencia, C.; Campos-Parra, A.D. Cross-talk between long non-coding RNAs, micro-RNAs and mRNAs: Deciphering molecular mechanisms of master regulators in cancer. Front. Oncol. 2019, 9, 669. [CrossRef]

27. O’Brien, J.; Hayder, H.; Zayed, Y.; Peng, C. Overview of microRNA Biogenesis, mechanisms of actions, and circulation. Front. Endocrinol. 2018, 9, 402. [CrossRef]

28. Hauptmann, J.; Schraivogel, D.; Bruckmann, A.; Manickavel, S.; Jakob, L.; Eichner, N.; Urban, M.; Sprunck, S.; Hafner, M.; et al. Biochemical isolation of argonaute protein complexes by Ago-APP. Biochemistry 2018, 57, 5247–5256. [CrossRef] [PubMed]

29. Kamenska, A.; Lu, W.-T.; Kubacka, D.; Broomhead, H.; Minshall, N.; Bushell, M.; Standart, N. Human 4E-T represses translation of bound mRNAs and enhances microRNA-mediated silencing. Nucleic Acids Res. 2013, 42, 3298–3313. [CrossRef]

30. Fukao, A.; Mishima, Y.; Takizawa, N.; Oka, S.; Imataka, H.; Pelletier, J.; Sonenberg, N.; Thoma, C.; Fujiiwara, T. MicroRNAs trigger dissociation of eIF4AI and eIF4AII from target mRNAs in humans. Mol. Cell 2014, 56, 79–89. [CrossRef]

31. Freimer, J.W.; Hu, T.J.; Bleloch, R. Decoupling the impact of microRNAs on translational repression versus RNA degradation in embryonic stem cells. Elife 2018, 7, e38014. [CrossRef]

32. Mayya, V.K.; Duchaine, T.F. Ciphers and executioners: How 3′-untranslated regions determine the fate of messenger RNAs. Front. Genet. 2019, 10, 6. [CrossRef]

33. Gulyaeva, L.F.; Kushliniskiy, N.E. Regulatory mechanisms of microRNA expression. J. Transl. Med. 2016, 14, 143. [CrossRef]

34. Schanen, B.C.; Li, X. Transcriptional regulation of mammalian miRNA genes. Genomics 2011, 97, 1–6. [CrossRef]

35. Siemens, H.; Neumann, J.; Jackstadt, R.; Mansmann, U.; Horst, D.; Kirchner, T.; Hermeking, H. Detection of miR-34a promoter methylation in combination with elevated expression of c-Met and β-catenin predicts distant metastasis of colon cancer. Clin. Cancer Res. 2013, 19, 710–720. [CrossRef]

36. Lu, J.; Tan, T.; Zhu, L.; Hong, H.; Xian, R. Hypomethylation causes MIR21 overexpression in tumors. Mol. Ther. Oncolytics 2020, 18, 47–57. [CrossRef] [PubMed]
63. Yang, L.; Dou, Y.; Sui, Z.; Cheng, H.; Liu, X.; Wang, Q.; Gao, P.; Qu, Y.; Xu, M. Upregulated miRNA-182-5p expression in tumor tissue and peripheral blood samples from patients with non-small cell lung cancer is associated with downregulated Caspase 2 expression. *Exp. Ther. Med.* 2020, 19, 603–610. [CrossRef] [PubMed]

64. Zhang, Q.; Li, Y.; Zhao, M.; Lin, H.; Wang, W.; Li, D.; Cui, W.; Zhou, C.; Zhong, J.; Huang, C. MiR-494 acts as a tumor promoter by targeting CASP2 in non-small cell lung cancer. *Sci. Rep.* 2019, 9, 3008. [CrossRef]

65. Hu, J.; Cheng, Y.; Li, Y.; Jin, Z.; Pan, Y.; Liu, G.; Fu, S.; Zhang, Y.; Feng, K.; Feng, Y. microRNA-128 plays a critical role in human non-small cell lung cancer tumorigenesis, angiogenesis and lymphangiogenesis by directly targeting vascular endothelial growth factor-C. *Eur. J. Cancer* 2014, 50, 2336–2350. [CrossRef]

66. Liu, H.; Chen, Y.; Li, Y.; Li, C.; Qin, T.; Bai, M.; Zhang, Z.; Jia, R.; Su, Y.; Wang, C. miR-195 suppresses metastasis and angiogenesis of squamous cell lung cancer by inhibiting the expression of VEGF. *Mol. Med. Rep.* 2019, 20, 2625–2632. [CrossRef] [PubMed]

67. Choi, Y.C.; Yoon, S.; Jeong, Y.; Yoon, J.; Baek, K. Regulation of vascular endothelial growth factor signaling by miR-200b. *Mol. Cells* 2011, 32, 77–82. [CrossRef]

68. Roybal, J.D.; Zang, Y.; Ahn, Y.H.; Yang, Y.; Gibbons, D.L.; Baird, B.N.; Alvarez, C.; Thilaganathan, N.; Liu, D.D.; Saintigny, P.; et al. MiR-200 Inhibits lung adenocarcinoma cell invasion and metastasis by targeting Flt1/VEGFR1. *Mol. Cancer Res.* 2011, 9, 25–35. [CrossRef]

69. Hiroto, T.; Jingushi, K.; Nagata, T.; Sato, M.; Minami, K.; Aoki, M.; Takeda, A.H.; Umehara, T.; Egawa, H.; Nakatsuji, Y.; et al. MicroRNA-130b functions as an oncomiRNA in non-small cell lung cancer by targeting tissue inhibitor of metalloproteinase-2. *Sci. Rep.* 2019, 9, 6956. [CrossRef]

70. Shi, J.; Wang, H.; Feng, W.; Huang, S.; An, J.; Qiu, Y.; Wu, K. MicroRNA-130a targeting hypoxia-inducible factor 1 alpha suppresses cell metastasis and Warburg effect of NSCLC cells under hypoxia. *Life Sci.* 2020, 255, 117826. [CrossRef] [PubMed]

71. Ding, G.; Huang, G.; Liu, H.D.; Liang, H.X.; Ni, Y.F.; Ding, Z.H.; Ni, G.Y.; Hua, H.W. MiR-199a suppresses the hypoxia-induced proliferation of non-small cell lung cancer cells through targeting HIF1α. *Mol. Cell. Biochem.* 2013, 384, 173–180. [CrossRef]

72. Byun, Y.; Choi, Y.C.; Jeong, Y.; Lee, G.; Yoon, S.; Jeong, Y.; Yoon, J.; Baek, K. MiR-200c downregulates HIF-1α and inhibits migration of lung cancer cells. *Cell. Mol. Biol. Lett.* 2019, 24, 28. [CrossRef] [PubMed]

73. Kalluri, R.; Weinberg, R.A. The basics of epithelial-mesenchymal transition. *J. Clin. Investig.* 2009, 119, 1420–1428. [CrossRef] [PubMed]

74. Lee, J.S.; Ahn, Y.H.; Won, H.S.; Sun, S.; Kim, Y.H.; Ko, Y.H. Prognostic role of the microRNA-200 family in various carcinomas: A systematic review and meta-analysis. *BioMed Res. Int.* 2017, 2017, 1928021. [CrossRef] [PubMed]

75. Nie, D.; Fu, J.; Chen, H.; Cheng, J.; Fu, J. Roles of MicroRNA-34a in epithelial to mesenchymal transition, competing endogenous RNA sponging and its therapeutic potential. *Int. J. Mol. Sci.* 2019, 20, 861. [CrossRef]

76. Zhou, H.; Huang, Z.; Chen, X.; Chen, S. miR-98 inhibits expression of TWIST to prevent progression of non-small cell lung cancers. *Biomed. Pharmacother.* 2017, 89, 1453–1461. [CrossRef] [PubMed]

77. Mo, X.; Zhang, F.; Liang, H.; Liu, M.; Li, H.; Xie, H. miR-544a promotes the invasion of lung cancer cells by targeting cadherin 1 in vitro. *OncoTargets Ther.* 2014, 7, 895–900. [CrossRef]

78. Ma, T.; Zhao, Y.; Wei, K.; Yao, G.; Pan, C.; Liu, B.; Xia, Y.; He, Z.; Qi, X.; Li, Z.; et al. MicroRNA-124 functions as a tumor suppressor by regulating CDH2 and epithelial-mesenchymal transition in non-small cell lung cancer. *Cell. Physiol. Biochem.* 2016, 38, 1563–1574. [CrossRef]

79. Merchant, N.; Nagaraju, G.P.; Rajitha, B.; Lammata, S.; Jella, K.K.; Buchwald, Z.S.; Lakka, S.S.; Ali, A.N. Matrix metalloproteinases: Their functional role in lung cancer. *Carcinogenesis* 2017, 38, 766–780. [CrossRef]

80. Wang, H.; Guan, X.; Tu, Y.; Zheng, S.; Long, J.; Li, S.; Qi, C.; Xie, X.; Zhang, H.; Zhang, Y. MicroRNA-29b attenuates non-small cell lung cancer metastasis by targeting matrix metalloproteinase 2 and PTEN. *J. Exp. Clin. Cancer Res.* 2015, 34, 59. [CrossRef]

81. Guo, T.; Zheng, C.; Wang, Z.; Zheng, X. miR-584-5p regulates migration and invasion in non-small cell lung cancer cell lines through regulation of MMP-14. *Mol. Med. Rep.* 2019, 19, 1747–1752. [CrossRef] [PubMed]

82. Humphries, B.A.; Wang, Z.; Yang, C. MicroRNA regulation of the small rho gtpase regulators-complexities and opportunities in targeting cancer metastasis. *Cancers* 2020, 12, 1092. [CrossRef] [PubMed]

83. Strilic, B.; Offermanns, S. Intravascular survival and extravasation of tumor cells. *Cancer Cell* 2017, 32, 282–293. [CrossRef] [PubMed]
84. Malagobadan, S.; Nagoor, N.H. Evaluation of microRNAs regulating anoikis pathways and its therapeutic potential. BioMed Res. Int. 2015, 2015, 716816. [CrossRef] [PubMed]

85. Ahn, Y.H.; Gibbons, D.L.; Chakravarti, D.; Creighton, C.J.; Rizvi, Z.H.; Adams, H.P.; Pertsemidis, A.; Gregory, P.A.; Wright, J.A.; Goodall, G.J.; et al. ZEB1 drives prometastatic actin cytoskeletal remodeling by downregulating miR-34a expression. J. Clin. Invest. 2012, 122, 3170–3183. [CrossRef] [PubMed]

86. Wang, X.C.; Tian, L.L.; Jiang, X.Y.; Wang, Y.Y.; Li, D.G.; She, Y.; Chang, J.H.; Meng, A.M. The expression and function of miR-451 in non-small cell lung cancer. Cancer Lett. 2011, 311, 203–209. [CrossRef]

87. Joshi, P.; Jeon, Y.J.; Lagana, A.; Middleton, J.; Secchiero, P.; Garofalo, M.; Croce, C.M. MicroRNA-148a reduces tumorigenesis and increases TRAIL-induced apoptosis in NSCLC. Proc. Natl. Acad. Sci. USA 2015, 112, 8650–8655. [CrossRef]

88. Wei, F.; Ma, C.; Zhou, T.; Dong, X.; Luo, Q.; Geng, L.; Ding, L.; Zhang, Y.; Zhang, L.; Li, N.; et al. Exosomes derived from gemcitabine-resistant cells transfer malignant phenotypic traits via delivery of miRNA-222-3p. Mol. Cancer 2017, 16, 132. [CrossRef]

89. Eichmüller, S.B.; Olsen, W.; Mandelboim, O.; Seliger, B. Immune modulatory microRNAs involved in tumor attack and tumor immune escape. J. Natl. Cancer Inst. 2017, 109, dxj034. [CrossRef]

90. Wang, Q.; Lin, W.; Tang, X.; Li, S.; Guo, L.; Lin, Y.; Kwok, H.F. The roles of microRNAs in regulating the expression of PD-1/PD-L1 immune checkpoint. Int. J. Mol. Sci. 2017, 18, 2540. [CrossRef]

91. Yin, P.; Peng, R.; Peng, H.; Yao, L.; Sun, Y.; Wen, L.; Wu, T.; Zhou, J.; Zhang, Z. MiR-451 suppresses cell proliferation and metastasis in A549 lung cancer cells. Mol Biotechnol. 2015, 57, 1–11. [CrossRef] [PubMed]

92. Chen, L.; Gibbons, D.L.; Goswami, S.; Cortez, M.A.; Ahn, Y.H.; Byers, L.A.; Zhang, X.; Yi, X.; Dwyer, D.; Lin, W.; et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat. Commun. 2014, 5, 5241. [CrossRef] [PubMed]

93. Xie, W.B.; Liang, L.H.; Wu, K.G.; Wang, L.X.; He, X.; Song, C.; Wang, Y.Q.; Li, Y.H. MiR-140 expression regulates cell proliferation and targets PD-L1 in NSCLC. Cell. Physiol. Biochem. 2018, 46, 654–663. [CrossRef] [PubMed]

94. Wan, J.; Ling, X.; Peng, B.; Ding, G. miR-142-5p regulates CD4+ T cells in human non-small cell lung cancer through PD-L1 expression via the PTEN pathway. Oncol. Rep. 2018, 40, 272–282. [CrossRef]

95. Song, N.; Li, P.; Song, P.; Li, Y.; Zhou, S.; Su, Q.; Li, X.; Yu, Y.; Li, P.; Peng, M.; et al. MicroRNA-138-5p suppresses non-small cell lung cancer cells by targeting PD-L1/PD-1 to regulate tumor microenvironment. Front. Cell Dev. Biol. 2020, 8, 540. [CrossRef]

96. Fujita, Y.; Yagishita, S.; Hagiwara, K.; Yoshioka, Y.; Kosaka, N.; Takeshita, F.; Fujiwara, T.; Tsuta, K.; Nokihara, H.; Tamura, T.; et al. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. Mol. Ther. 2015, 23, 717–727. [CrossRef]

97. Banyard, J.; Bielenberg, D.R. The role of EMT and MET in cancer dissemination. Connect. Tissue Res. 2015, 56, 403–413. [CrossRef]

98. Somarelli, J.A.; Schaeffer, D.; Marengo, M.S.; Bepler, T.; Rouse, D.; Ware, K.E.; Hish, A.J.; Zhao, Y.; Buckley, A.F.; Epstein, J.I.; et al. Distinct routes to metastasis: Plasticity-dependent and plasticity-independent pathways. Oncogene 2016, 35, 4302–4311. [CrossRef]

99. Gibbons, D.L.; Lin, W.; Creighton, C.J.; Rizvi, Z.H.; Gregory, P.A.; Goodall, G.J.; Thilaganathan, N.; Du, L.; Zhang, Y.; Pertsemidis, A.; et al. Contextual extracellular cues promote tumor cell EMT and metastasis by regulating miR-200 family expression. Genes Dev. 2009, 23, 2140–2151. [CrossRef]

100. Lee, C.G.; McCarthy, S.; Gruidl, M.; Timme, C.; Yeatman, T.J. MicroRNA-147 induces a mesenchymal-to-epithelial transition (MET) and reverses EGFR inhibitor resistance. PLoS ONE 2014, 9, e84597. [CrossRef]

101. Sun, J.; Li, Q.; Lian, X.; Zhu, Z.; Chen, X.; Pei, W.; Li, S.; Abbas, A.; Wang, Y.; Tian, L. MicroRNA-29b mediates lung mesenchymal-epithelial transition and prevents lung fibrosis in the silicosis model. Mol. Ther. Nucleic. Acids 2019, 14, 20–31. [CrossRef] [PubMed]

102. Wu, S.G.; Chang, T.H.; Liu, Y.N.; Shih, J.Y. MicroRNA in lung cancer metastasis. Cancers 2019, 11, 265. [CrossRef] [PubMed]

103. Li, Y.; Sarkar, F.H. MicroRNA targeted therapeutic approach for pancreatic cancer. Int. J. Biol. Sci. 2016, 12, 326–337. [CrossRef] [PubMed]

104. Subramanian, J.; Govindan, R. Lung cancer in never smokers: A review. J. Clin. Oncol. 2007, 25, 561–570. [CrossRef] [PubMed]
105. Rekhtman, N.; Paik, P.K.; Arcila, M.E.; Tafe, L.J.; Oxnard, G.R.; Moreira, A.L.; Travis, W.D.; Zakowski, M.F.; Kris, M.G.; Ladanyi, M. Clarifying the spectrum of driver oncogene mutations in biomarker-verified squamous carcinoma of lung: Lack of EGFR/KRAS and presence of PIK3CA/AKT1 mutations. *Clin. Cancer Res.* **2012**, *18*, 1167–1176. [CrossRef]

106. Zhang, Y.K.; Zhu, W.Y.; He, J.Y.; Chen, D.D.; Huang, Y.Y.; Le, H.B.; Liu, X.G. miRNAs expression profiling to distinguish lung squamous-cell carcinoma from adenocarcinoma subtypes. *J. Cancer Res. Clin. Oncol.* **2012**, *138*, 1641–1650. [CrossRef]

107. Hu, Y.; Wang, L.; Gu, J.; Qu, K.; Wang, Y. Identification of microRNA differentially expressed in three subtypes of non-small cell lung cancer and in silico functional analysis. *OncoTarget* **2017**, *8*, 74554–74566. [CrossRef]

108. Sherafatian, M.; Arjmand, F. Decision tree-based classifiers for lung cancer diagnosis and subtyping using TCGA miRNA expression data. *Oncol. Lett.* **2019**, *18*, 2125–2131. [CrossRef]

109. Montani, F.; Marzi, M.J.; Dezi, F.; Dama, E.; Carletti, R.M.; Bonizzi, G.; Bertolotti, R.; Bellomi, M.; Rampinelli, C.; et al. Identification of microRNA diagnostic signature in NSCLC patients who are negative for epidermal growth factor receptor mutations or anaplastic lymphoma kinase translocations. *Transl. Res.* **2016**, *170*, 1–7.

110. Johnson, D.B.; Rieth, M.J.; Horn, L. Immune checkpoint inhibitors in NSCLC. *Curr. Treat. Options Oncol.* **2014**, *15*, 658–669. [CrossRef]

111. Peng, X.X.; Yu, R.; Wu, X.; Wu, S.Y.; Pi, C.; Chen, Z.H.; Zhang, X.C.; Gao, C.Y.; Shao, Y.W.; Liu, L.; et al. Correlation of plasma exosomal miRNAs with the efficacy of immunochemotherapy in EGFR/ALK wild-type advanced non-small cell lung cancer. *J. Investig. Cancer* **2020**, *8*, e00376. [CrossRef]

112. Hines, M.C.; Dickerson, K.; Klein, P.; Mayer, M.; Noss, K.; Slamon, D.; Sledge, G.; Visco, F.M. The future of biomarker research in breast cancer to ensure clinical relevance. *Nat. Rev. Cancer* **2007**, *7*, 309–315. [CrossRef]

113. Chen, B.; Gao, T.; Yuan, W.; Zhao, W.; Wang, T.H.; Wu, J. Prognostic value of survival of microRNAs signatures in non-small cell lung cancer. *J. Cancer* **2019**, *10*, 5793–5804. [CrossRef]

114. Shin, B.; Park, S.; Hong, J.H.; An, H.J.; Chun, S.H.; Kang, K.; Ahn, Y.H.; Ko, Y.H.; Kang, K. Cascaded wx: A Novel prognosis-related feature selection framework in human lung adenocarcinoma transcriptomes. *Front. Genet.* **2019**, *10*, 662. [CrossRef]

115. Kim, J.S.; Chun, S.H.; Park, S.; Lee, S.; Kim, S.E.; Hong, J.H.; Kang, K.; Ko, Y.H.; Ahn, Y.H. Identification of novel microRNA prognostic markers using cascaded wx, a neural network-based framework, in lung adenocarcinoma patients. *Cancers* **2020**, *12*, 1890. [CrossRef]

116. Finnerty, J.R.; Wang, W.X.; Hébert, S.S.; Wilfred, B.R.; Mao, G.; Nelson, P.T. The miR-15/107 group of microRNA genes: Evolutionary biology, cellular functions, and roles in human diseases. *J. Mol. Biol.* **2010**, *402*, 491–509. [CrossRef] [PubMed]

117. Wu, Y.; Crawford, M.; Mao, Y.; Lee, R.J.; Davis, I.C.; Elton, T.S.; Lee, L.J.; Nana-Sinkam, S.P. Therapeutic delivery of microRNA-29b by cationic lipoplexes for lung cancer. *Mol. Ther. Nucleic Acids* **2013**, *2*, e84. [CrossRef] [PubMed]
123. Rai, K.; Takigawa, N.; Ito, S.; Kashihara, H.; Ichihara, E.; Yasuda, T.; Shimizu, K.; Tanimoto, M.; Kiura, K. Liposomal delivery of MicroRNA-7-expressing plasmid overcomes epidermal growth factor receptor tyrosine kinase inhibitor-resistance in lung cancer cells. *Mol. Cancer Ther.* 2011, 10, 1720–1727. [CrossRef] [PubMed]

124. Wiggins, J.F.; Ruffino, L.; Kelhar, K.; Omotola, M.; Patrawala, L.; Brown, D.; Bader, A.G. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. *Cancer Res.* 2010, 70, 5923–5930. [CrossRef] [PubMed]

125. Trang, P.; Wiggins, J.F.; Daige, C.L.; Cho, C.; Omotola, M.; Brown, D.; Weidhaas, J.B.; Bader, A.G.; Slack, F.J. Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. *Mol. Ther.* 2011, 19, 1116–1122. [CrossRef]

126. Cortez, M.A.; Valdecanas, D.; Zhang, X.; Zhan, Y.; Bhardwaj, V.; Calin, G.A.; Komaki, R.; Giri, D.K.; Quini, C.C.; Wolfe, T.; et al. Therapeutic delivery of miR-200c enhances radiosensitivity in lung cancer. *Mol. Ther.* 2014, 22, 1494–1503. [CrossRef]

127. Vázquez-Ríos, A.J.; Molina-Crespo, Á.; Bouzo, B.L.; López-López, R.; Moreno-Bueno, G.; de la Fuente, M. Exosome-mimetic nanoplatforms for targeted cancer drug delivery. *J. Nanobiotechnology* 2019, 17, 85. [CrossRef]

128. Reid, G.; Kao, S.C.; Pavlakis, N.; Brahmbhatt, H.; MacDiarmid, J.; Clarke, S.; Boyer, M.; van Zandwijk, N. Clinical development of TargomiRs, a miRNA mimic-based treatment for patients with recurrent thoracic cancer. *Epigenomics* 2016, 8, 1079–1085. [CrossRef]

129. Hong, D.S.; Kang, Y.K.; Borad, M.; Sachdev, J.; Ejadi, S.; Lim, H.Y.; Brenner, A.J.; Park, K.; Lee, J.L.; Kim, T.Y.; et al. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *Br. J. Cancer* 2020, 122, 1630–1637. [CrossRef]

130. Van Zandwijk, N.; Pavlakis, N.; Kao, S.C.; Linton, A.; Boyer, M.J.; Clarke, S.; Huynh, Y.; Chrzanowska, A.; Fulham, M.J.; Bailey, D.L.; et al. Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: A first-in-man, phase 1, open-label, dose-escalation study. *Lancet. Oncol.* 2017, 18, 1386–1396. [CrossRef]

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