

MicroRNA-21: A potential therapeutic target in lung cancer (Review)

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Received May 20, 2025; Accepted June 30, 2025

DOI: 10.3892/ijo.2025.5773

Abstract. In this review, the role of microRNA-21 (miRNA-21) as an oncogene in lung cancer was investigated. Studies have shown that miRNA-21 can promote the progression of lung cancer by targeting downstream target genes, and its expression can be modulated by transcription factors, DNA methylation or competitive endogenous RNA as an upstream regulator. This review highlights that miRNA-21 can promote the progression of lung cancer through multiple signaling pathways, with a focus on the PI3K/AKT, MEK/ERK, TGF- β /SMAD, Hippo, NF- κ B and STAT3 signaling pathways. Mechanistically, miRNA-21 plays an important role in the progression of lung cancer by regulating multiple biological processes, such as proliferation, invasion, metastasis, apoptosis and angiogenesis in lung cancer cells. Higher expression of miRNA-21 is associated with chemotherapy, radiotherapy and immune resistance in lung cancer. Targeting these molecular pathways may be a novel therapeutic strategy for treating lung cancer. Additionally, miRNA-21 can serve as a biomarker for lung cancer diagnosis, prognosis and treatment response. This review also summarized the following: i) Current methods employed to inhibit the expression of miRNA-21 in lung cancer, including CRISPR/Cas9 technology; ii) the application of natural anticancer agents, oligonucleotides, small molecules and miRNA sponges; and iii) the nano-delivery systems developed for miRNA-21 inhibitors. Finally, the advancements in research on miRNA mimics and inhibitors in clinical trials, which may promote the application of miRNA-21 in clinical trials in lung cancer, were discussed. Given that lung cancer is a considerable public health challenge, these studies provide new ways of treating patients with lung cancer.

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1. Introduction

Lung cancer is a common malignant tumor of the respiratory system. According to global cancer statistics, there were 2.5 million new cases of lung cancer worldwide in 2022 (1). An estimated 1.8 million deaths due to lung cancer occurred in the same year, accounting for ~18.7% of all cancer-related deaths (1). Lung cancer is a great challenge for public health, and new and effective means of prevention and treatment need to be developed. Based on histological features, the pathological stage of lung cancer can be divided into small cell lung cancer and non-small cell lung cancer (NSCLC), with NSCLC accounting for ~80-85% of all cases (2). Owing to the insidious nature of the disease, most patients with lung cancer have already developed metastases at the time of diagnosis (3). Although the use of low-dose computed tomography increases the diagnostic rate of early lung cancer, overdiagnosis due to the high false-positive rate of CT and radiation exposure is also a burden to patients (4). Therefore, more sensitive and highly accurate diagnostic methods need to be developed. Early-stage lung cancer is treated mainly via surgery. For intermediate and advanced lung cancer, chemotherapy, radiotherapy, immunotherapy and targeted therapy are the main treatments, but despite these treatments, the three-year survival rate of patients with lung cancer is only ~20% (5), whereas the five-year overall survival (OS) rate is 37% (3). Therefore, new therapeutic approaches need to be developed to improve the prognosis of patients with lung cancer.

MicroRNAs (miRNAs) are small non-coding RNAs found in eukaryotes and are ~22 nucleotides in length. They can negatively regulate gene expression by binding to the

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Key words: miRNA-21, lung cancer, target, proliferation, chemotherapy, biomarker

3'-untranslated region (3'-UTR) of the mRNA of target genes, resulting in degradation and/or translational inhibition of the target genes and thus regulating biological processes such as cell proliferation, growth, aging and migration (6). This in turn regulates biological processes such as cell proliferation, growth, senescence and migration (6). Researchers have found an association between miRNA and the development of cancer. Additionally, miRNA has attracted much attention in the diagnosis, prognosis and treatment of cancer (7).

There are broadly two types of miRNA: Oncogenes and tumor suppressors (8). When miRNAs act as tumor suppressors, the downstream target genes are oncogenes; when miRNAs act as oncogenes, the downstream target genes are tumor suppressors. However, because each miRNA has multiple downstream target genes, it may act as both an oncogene and tumor suppressor in the same cancer (9). As an oncogene, miRNA-21 is associated with the development of various types of cancer, such as lung cancer (10), oesophageal cancer (11), prostate cancer (12), oral cancer (13), breast cancer (14), ovarian cancer (15), cervical cancer (16), colorectal cancer (17), liver cancer (18), bladder cancer (19), renal cancer (20), osteosarcoma (21) and glioblastoma (22). Targeting and inhibiting the expression of downstream target genes, miRNA-21 is highly expressed in lung cancer and promotes processes such as the growth, metastasis and invasion of tumors. High expression of miRNA-21 promotes lung cancer cell proliferation, metastasis, invasion and angiogenesis, inhibits apoptosis and contributes to lung cancer progression through multiple signaling pathways. Additionally, miRNA-21 is associated with the therapeutic efficacy of chemotherapy, radiotherapy and targeted therapy in lung cancer. Targeting these molecular pathways may be a novel therapeutic strategy for treating lung cancer. Furthermore, miRNA-21 can serve as a biomarker for early diagnosis, prognosis and therapeutic response in patients with lung cancer, which is beneficial for early diagnosis and personalized treatment. Complementing or inhibiting the function and activity of dysregulated miRNA in cancer is a hotspot for assessing new strategies to treat cancer. Examples include the use of miRNA mimics and antagonists, which show potential for cancer phenotype reversal and improved efficacy. Therefore, analyzing methods to inhibit the expression of miRNA-21 in lung cancer may help prevent cancer from spreading and prolong the survival of patients with advanced lung cancer. This review also summarized the current research progress in the use of miRNA mimics and inhibitors in clinical trials, which may promote the application of miRNA-21 in clinical trials in lung cancer, were discussed. Overall, these insights highlight the therapeutic potential of miRNA-21 and its promising role in the development of novel treatments for cancer.

2. Biosynthesis and regulation of the expression of miRNA-21

With a length of ~22 nucleotides, miRNA-21 is a single-stranded ncRNA that is located in the intronic region of the transmembrane protein 49 locus of human chromosome 17 (23). Obtaining mature miRNA-21 is a complex process. First, miRNA-21 is generated by RNA polymerase II to produce primary miRNA-21 (pri-miRNA-21) with a 5' end cap

and a 3' poly A tail. Pri-miRNA-21 is cleaved and processed into a precursor miRNA (pre-miRNA-21) by the Drosha enzyme-Dgcr8 complex. Subsequently, pre-miRNA-21 is transported to the cytoplasm by Exportin-5 and further cleaved by the Dicer enzyme into a mature double-stranded miRNA-21 molecule of ~22 nucleotides in length. The double-stranded miRNA-21 molecule unfolds to form two single-stranded miRNA-21 molecules, and one of the single-stranded miRNA-21 molecules (the guiding strand) interacts with the Argonaute protein to form an RNA-induced silencer in the cytoplasm. This forms an RNA-induced silencing complex, which can bind to the 3'-UTR of the target gene (24). This interaction leads to degradation or translational repression of the target gene, which in turn regulates biological processes (Fig. 1).

The expression of miRNA-21 is regulated by several mechanisms, including DNA methylation, transcription factors, competitive endogenous RNA (ceRNA) and hypoxic conditions. DNA methylation occurs in CpG islands in the promoter region and is an important epigenetic modification. Deletion of DNA methylation in thyroid cancer leads to the overexpression of miRNA-21 (25). Lu *et al* (26) found a hypomethylation pattern of miRNA-21 in eight tumour types and hypomethylation of the promoter led to the overexpression of miRNA-21 in tumours. Additionally, miRNA-21 is under the regulation of multiple transcription factors. For instance, the transcription factor nuclear factor- κ B (NF- κ B) positively regulates miRNA-21 expression (27). However, peroxisome proliferator-activated receptor- γ negatively regulates miRNA-21 expression (28). Long chain ncRNA (lncRNA) and circular RNA (circRNA) can act as ceRNA and sponges for miRNA-21, which in turn regulates the expression of miRNA-21 downstream target genes. The lncRNA cancer susceptibility candidate 2 can bind to miRNA-21 and inhibit its expression, leading to an increase in the expression of the miRNA-21 downstream target gene p53 protein, which in turn inhibits lung cancer progression (29). In addition, circ-SLC7A6 acts as a sponge for miRNA-21 and significantly reduces its expression (30). The artificial synthesis of miRNA-21 ceRNA may be an effective method for treating lung cancer (31). In addition, miRNA-21 expression is higher in tumors in a hypoxic environment (32). These four mechanisms that can regulate miRNA-21 expression may be effective way to target miRNA-21 as a therapeutic target against cancer in the future.

3. Multiple signalling pathways in lung cancer progression are regulated by miRNA-21

By targeting downstream target genes and influencing multiple pathways, including PI3K/AKT, MEK/ERK, TGF- β /SMAD, Hippo, NF- κ B and STAT3, miRNA-21 can promote the progression of lung cancer (Fig. 2).

PI3K/AKT signalling pathway. Dysregulation of the PI3K/Akt signalling pathway is characteristic of carcinogenesis, and activation of this signalling pathway enhances cancer cell proliferation, angiogenesis and resistance to chemotherapy and immunotherapy (33). PI3K can be activated by tyrosine kinases to produce phosphatidylinositol-3,4,5-trisphosphate (PIP3) in the plasma membrane. Phosphatase and tensin homolog

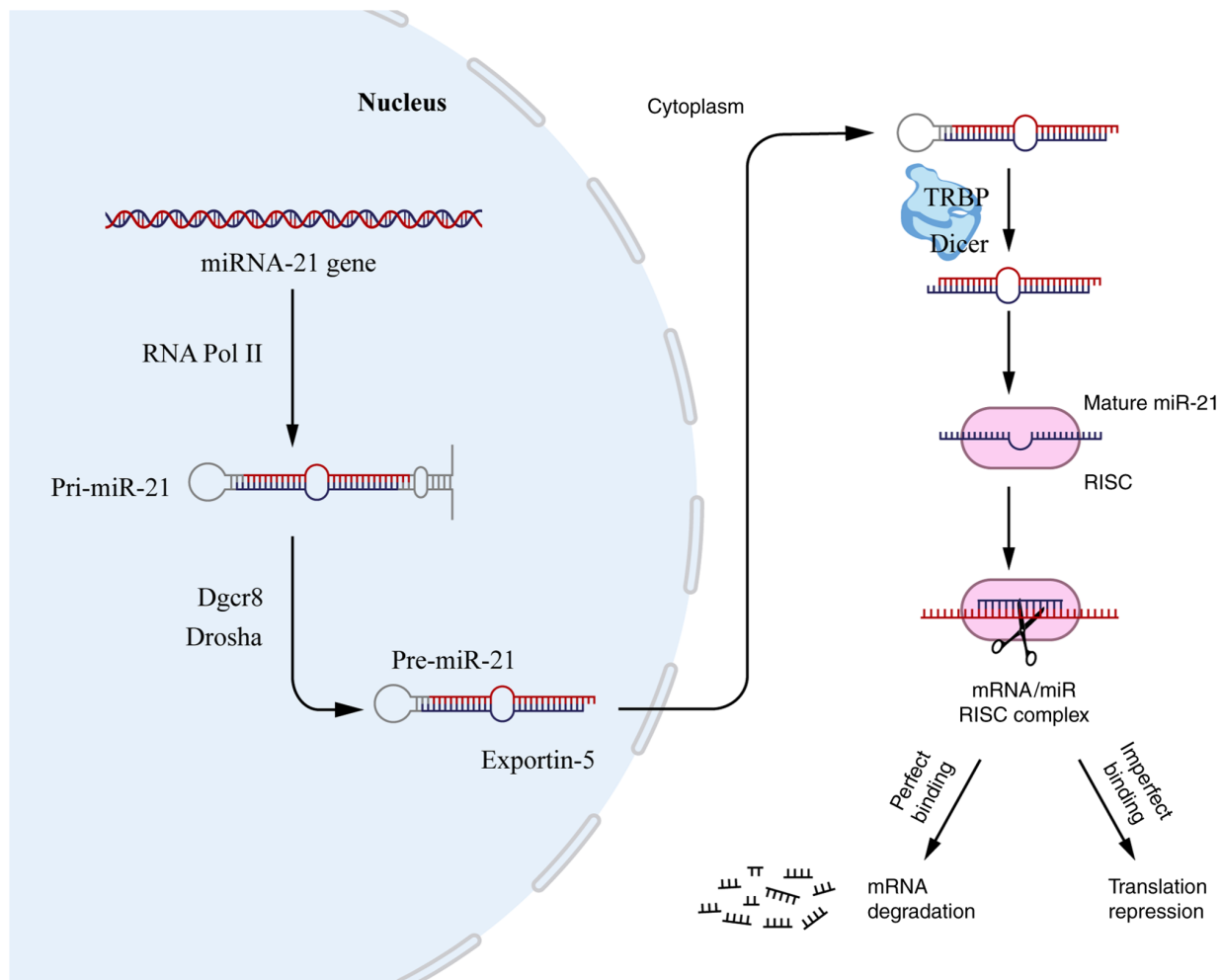


Figure 1. Biosynthesis and regulation of the expression of miRNA-21. First, in the nucleus, the miRNA-21 gene is transcribed into pri-miRNA-21 in response to RNA Pol II. Pri-miRNA-21 is further processed into Pre-miRNA-21 via the Drosha enzyme-Dgcr8 complex. Pre-miRNA-21 is transported to the cytoplasm by Exportin-5. In the cytoplasm, Pre-miRNA-21 is discern and cleaved by the TRBP and Dicer enzyme into mature double-stranded miRNA-21, which is ~22 nucleotides in length. One strand (the guide strand) in the mature double-stranded miRNA-21 is subsequently loaded into the Argonaute protein to form an RISC that binds to the 3'-UTR of the target gene mRNA through sequence complementation. The target gene mRNA degrades if perfectly bound, whereas translational repression occurs if it is bound imperfectly. Pri-miRNA-21, primary microRNA-21; Pre-miRNA-21, precursor miRNA-21; RISC, RNA-induced silencing complex; RNA Pol, RNA polymerase; TRBP, trans-activation response RNA-binding protein.

(PTEN) is a tumour suppressor, and PIP3 is dephosphorylated by PTEN and reduced to PIP2, thus preventing the activation of Akt. If the inhibition of PTEN expression leads to Akt activation, it causes the activation of mTOR signalling and the inhibition of pro-apoptotic gene expression, which promotes tumour growth and proliferation and inhibits apoptosis (34).

By targeting PTEN and activating the PI3K/Akt signalling pathway, miRNA-21 can lead to uncontrolled proliferation of tumour cells and promote tumour cell migration, metastasis, inhibition of apoptosis and drug resistance (35). This approach can make the treatment of lung cancer more challenging. Inhibitors of miRNA-21 can inhibit PI3K/Akt and thus prevent metastasis and invasion, and promote apoptosis in lung cancer. The underlying mechanism involves miRNA-21 targeting the apoptosis-stimulating protein 2 of p53 (ASPP2) (36). The inhibition of miRNA-21 expression can inhibit PI3K/Akt signaling and thus make lung cancer cells more sensitive to radiotherapy (37). Additionally, the lncRNA CASC2, in combination with miRNA-21, can increase PTEN expression, inhibit the PI3K/Akt signalling pathway and promote

cisplatin-induced apoptosis in lung cancer cells (38). Overall, miRNA-21 can activate the PI3K/Akt pathway to promote lung cancer progression.

MEK/ERK signalling pathway. The MEK/ERK signalling pathway is a classical MAPK signalling pathway that involves the classical Ras/Raf/MEK/ERK pathway with Ras/Raf proteins and the activation of ERK upon phosphorylation of MEK, which is involved in mitotic signalling and regulates cell proliferation, differentiation, migration, apoptosis and drug resistance (39). Upregulation of MEK/ERK can lead to the progression of different types of cancers (40). Researchers have also investigated the targeting of other components of the MEK/ERK signalling pathway for therapeutic intervention. Examples include targeting upstream EGFR family members and the RAF/MEK/ERK pathway regulators Src homologous protein tyrosine phosphatase 2 and son of sevenless homolog 1 (41).

Trametinib is a Food and Drug Administration (FDA)-approved ERK inhibitor and is also approved in combination with dabrafenib for treating patients with BRAF

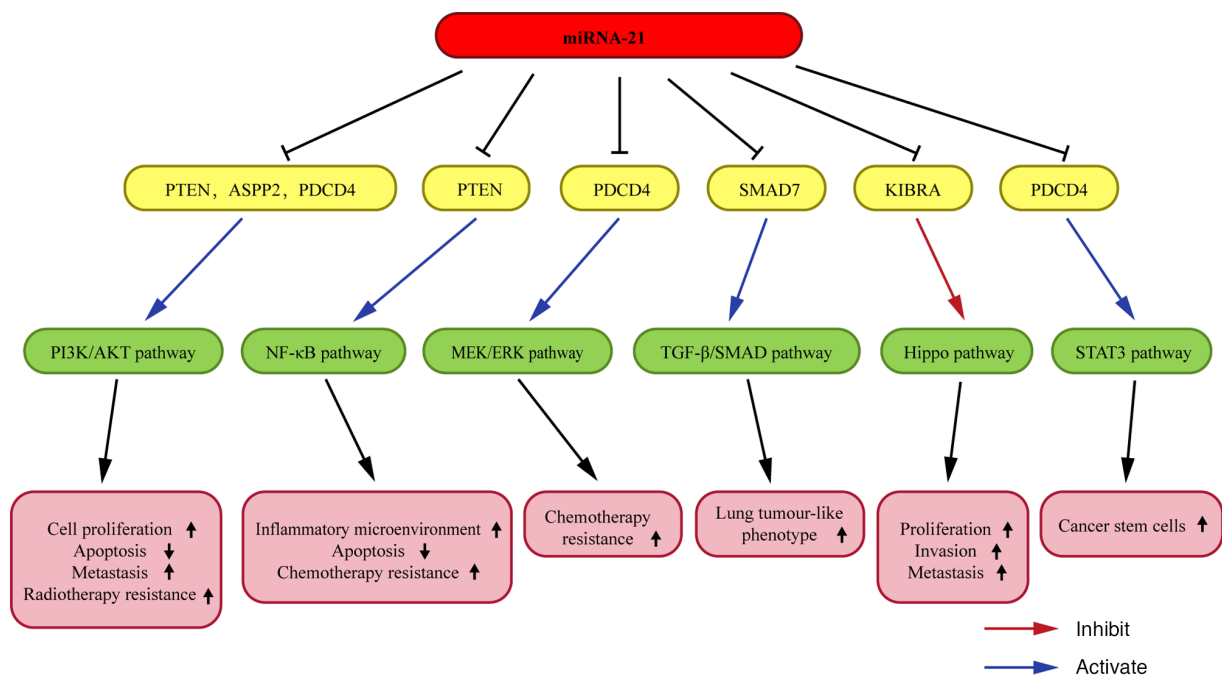


Figure 2. MiRNA-21 targets downstream target genes to regulate PI3K/AKT, MEK/ERK, TGF- β /SMAD, Hippo, NF- κ B and STAT3 pathways, which in turn promote the progression of lung cancer. MiRNA-21, microRNA-21. PTEN, phosphatase and tensin homolog; ASPP2, apoptosis stimulating protein 2 of p53; PDCD4, programmed cell death factor 4; SMAD7, Sma and Mad-related protein 7; KIBRA, kidney and brain expressed protein.

V600E-mutated NSCLC (42). Trametinib inhibits miRNA-21 expression, promotes the expression of the downstream target gene programmed cell death factor 4 (PDCD4), downregulates MEK1/2 and ERK1/2 expression, inhibits the MEK/ERK pathway and restores the sensitivity of ositinib-resistant NSCLC cells to ositinib (43).

TGF- β /SMAD signalling pathway. The cytokine TGF- β has three isoforms: TGF- β 1, TGF- β 2 and TGF- β 3. SMAD is an intracellular protein and downstream target of TGF- β that transduces extracellular signals from cell surface receptors and participates in the regulation of cellular processes such as proliferation, motility and differentiation (44). The SMAD family has eight members, which include receptor activated Smad1, 2, 3, 5 and 8, co-mediator Smad 4, and inhibitory SMAD6 and SMAD7. Inhibitory SMAD7 can negatively regulate the TGF- β /SMAD signalling pathway via feedback, which in turn blocks the biological effects of TGF- β (45).

Activation of the TGF- β /SMAD signalling pathway often promotes angiogenesis and triggers epithelial-to-mesenchymal transition (EMT), which leads to a loss of polarity and adhesion properties of cells, thereby promoting cell invasion and metastasis (46). Studies have reported that miRNA-21 can activate the TGF- β /SMAD signalling pathway by inhibiting the expression of SMAD7, leading to the upregulation of the expression of the mesenchymal markers Nanog, CD44, Sox2 and Snail, triggering EMT, which allows human bronchial cells to acquire tumour-initiating stem cell-like features, which in turn promotes transformation into a lung tumor-like phenotype (45).

Hippo signalling pathway. The group members of the Hippo signaling pathway include transcriptional coactivators containing PDZ-binding motifs (TAZ), large tumor

suppressor genes 1/2 (LATS1/2), mammalian sterile lineage 20-like kinase 1/2 and Yes-associated proteins (YAP). Among them, activating YAP/TAZ phosphorylation plays a major role in various functions of the Hippo/YAP signalling pathway. When the Hippo signalling pathway is activated, YAP and TAZ are phosphorylated by LATS1/2 and then restricted to the cytoplasm and degraded by 14-3-3 proteins; these changes inhibit the oncogenic effects of YAP/TAZ. However, when the Hippo signalling pathway is inhibited, unphosphorylated YAP/TAZ is transferred to the nucleus and binds to the TEA structural domains 1-4 to regulate the transcription of relevant target genes, thus exerting a cancer-promoting effect. In NSCLC, activation of the Hippo signalling pathway and a reduction in YAP phosphorylation can inhibit the proliferation and migration of NSCLC cells (47). Notably, miRNA-21 can target kidney and brain expressed protein to inhibit the Hippo signalling pathway and promote lung cancer proliferation, invasion and metastasis (48).

NF- κ B signalling pathway. The protein complex NF- κ B is an important regulator of cellular inflammation, metabolism, immunity and cell survival (49). It contains dimeric proteins formed by two subunits, p50 and p65. Normally, NF- κ B is bound to its inhibitor I κ B and is enclosed in the cytoplasm. When stimulated by intracellular and extracellular stimuli, I κ B kinase is activated, leading to phosphorylation and degradation of I κ B, which promotes the release of NF- κ B and its entry into the nucleus to play a gene-regulatory role and participate in the induction of inflammation, cell proliferation, and apoptosis (50). In cancer, NF- κ B is abnormally activated, leading to a sustained inflammatory response, promotion of angiogenesis and immune evasion, creating a sustained favourable tumor microenvironment for tumour cells (51).

Notably, miRNA-21 can activate the NF- κ B pathway by targeting negative regulators of the NF- κ B pathway, thereby activating the NF- κ B pathway. For instance, miRNA-21 targets PTEN, activates the NF- κ B pathway, promotes the expression of the proinflammatory factors interleukin (IL)-6 and IL-8, and inhibits the expression of cleaved caspase-3, which provides lung cancer cells with a sustained inflammatory microenvironment and inhibits apoptosis (52). Studies have shown that miRNA-21 can target PTEN, promote NF- κ B and promote cisplatin resistance in lung cancer cells (53). Thus, miRNA-21 can promote lung cancer progression by activating the NF- κ B pathway.

STAT3 signalling pathway. STAT3 is a member of the STAT family of proteins that are implicated in physiological processes such as cell proliferation, differentiation, apoptosis and angiogenesis. The Janus protein tyrosine kinase (JAK)/STAT signalling pathway mediates STAT3 activation (54). The JAK/STAT signalling pathway comprises mainly the JAK family, STAT and receptor tyrosine kinases. The JAK family contains JAK1, JAK2, JAK3 and selective tyrosine kinase 2, which binds noncovalently to cytokine receptors, triggering the phosphorylation of resting STAT monomers in the cytoplasm and leading to homodimerization or heterodimerisation of STAT. The altered STAT translocates to the nucleus and acts as a transcription factor to regulate gene transcription, which is associated with cell differentiation, proliferation and apoptosis (55). Src-homology domain 2-containing protein tyrosine phosphatase 1 (SHP-1), SHP-2 and suppressor of cytokine signal transduction (SOCS) family proteins are negative regulators of the JAK/STAT signalling pathway. In cancer, miRNA-21 can activate the JAK/STAT pathway by downregulating the expression of SOCS1 (56). Additionally, miRNA-21 targets PDCD4, downregulates SHP-1 expression and activates STAT3 expression, which in turn helps arsenic induce bronchial epithelial cells to undergo malignant transformation and acquire cancer stem cell stemness (57).

4. Role of miRNA-21 in lung cancer

Numerous studies have shown that miRNA-21 is an oncogene that frequently has higher expression in lung cancer. However, only few studies have reported low expression of miRNA-21 in lung cancer cells (58). Notably, miRNA-21 can regulate proliferation, invasion, metastasis, apoptosis and angiogenesis in lung cancer cells by targeting different downstream target genes, whereas knocking down or inhibiting miRNA-21 expression impairs the progression of lung cancer (59) (Fig. 3).

Clinical trials of miRNA-21 in lung cancer have not been conducted, to the best of our knowledge. However, numerous preclinical animal and cellular studies have shown that inhibiting miRNA-21 expression can suppress lung cancer progression (60-69). Several preclinical animal and cellular studies involving miRNA-21 in lung cancer are presented in Table I.

Regulation of the cell cycle and inhibition of lung cancer cell proliferation by miRNA-21. The cell cycle leads to cell proliferation, allowing cells to grow, replicate genetic material and divide. The cell cycle has four main phases: Gap 1 (G1), DNA

replication (S), G2 and mitosis (M). Alterations in the mechanisms of the cell cycle constitute one of the main pathways by which normal cell proliferation is transformed into uncontrolled cell division and, ultimately, carcinogenesis and cancer progression (70). Cell cycle proteins and cyclin-dependent kinases (CDK) are key regulators that influence the progression of the cell cycle (71). Targeting key regulators of the cell cycle in cancer is an effective approach to treat tumors. Overexpression of miRNA-21 increases the expression of the cell cycle protein D1 and the cell cycle protein E1, which in turn promotes the proliferation of lung cancer cells (72). Xia *et al* (73) reported that the expression of anti-miRNA-21 significantly decreased the expression of cell division cycle 2 and cell cycle protein B1 in lung cancer, which in turn increased the number of cells in G2/M phase and decreased the number of cells in S phase. Zhong *et al* (74) reported that downregulation of miRNA-21 expression resulted in arrest of lung cancer cells in G2/M phase, which inhibited cell proliferation. By contrast, overexpression of miRNA-21 increased the number of tumor cells in S-phase, which promoted cell proliferation. The underlying mechanism was related to the fact that miRNA-21 can target human mutS homolog 2 (74). Similarly, Li *et al* (61) found that the expression of miRNA-21 increased considerably after plasmid transfection, leading to a decrease in the G2-phase population of cells and an increase in the S-phase population. These changes imply that the lung cancer cells were still undergoing DNA replication and cell proliferation (61). Jin and Yu (60) reported that Hypoxic lung cancer cell-derived exosomal miRNA-21 can mediate macrophage M2 polarization and promotes lung cancer cell proliferation by target interferon-regulatory factors 1. Circ_0001287 can upregulate PTEN expression by sponging miRNA-21, which in turn inhibits the proliferation of lung cancer cells, whereas overexpression of miRNA-21 reversed this process (64). These studies indicate that increasing the expression of miRNA-21 promotes the proliferation of lung cancer cells.

Lung cancer cell invasion and metastasis are promoted by miRNA-21. Lung cancer is a highly malignant and lethal cancer associated with cell invasion and metastasis. Metastasis of tumour cells to distant sites leads to severe organ failure at secondary sites, where epithelial-mesenchymal transition (EMT) is an important route to promote the invasion and metastasis of lung cancer (75). The occurrence of EMT causes the cells to change from a non-motile epithelial state to a motile mesenchymal state, which increases the malignant characteristics of the tumour cells. Among them, the expression of epithelial marker E-cadherin decreases, whereas the expression of mesenchymal markers such as N-cadherin and waveform protein increases (76). Additionally, tumor cell invasion and metastasis are also associated with an increase in the expression of matrix metalloproteinase (MMP), which promotes tumor cell invasion and metastasis by facilitating the degradation of the extracellular matrix (77). Sinomenine downregulates miRNA-21 expression, which in turn increases E-cadherin expression, reversing the induction of cysteine-rich protein with kazal motifs (RECK), tissue inhibitor of metalloproteinase 1 (TIMP1) and TIMP2 expression, inhibits the expression of MMP2, MMP9, waveform protein and CD147 (which can activate MMP proteins), and thus reduces the

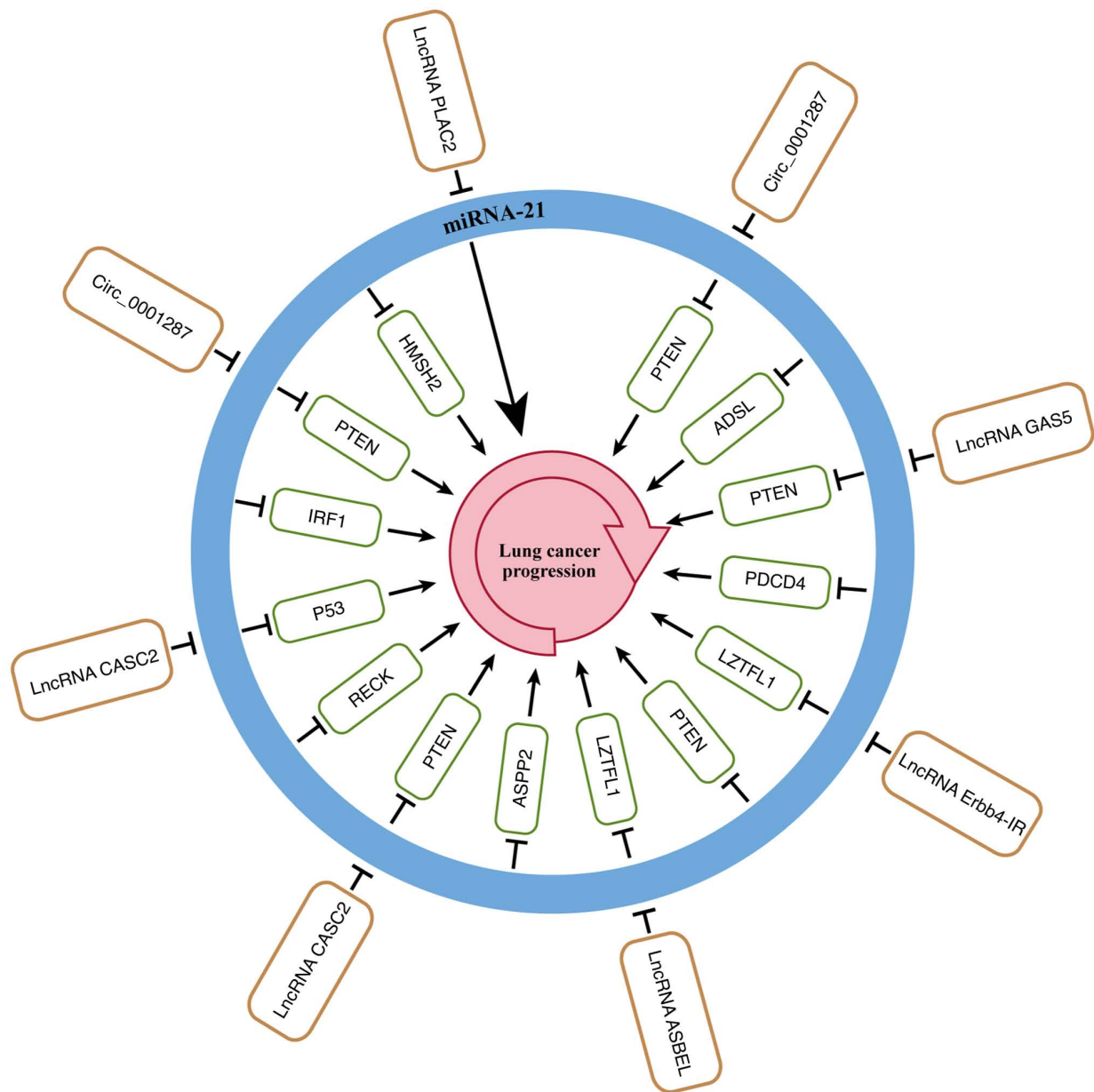


Figure 3. MiRNA-21 can promote lung cancer progression by directly targeting downstream target genes. However, miRNA-21 is regulated by a variety of lncRNAs and circRNAs that affect the expression of miRNA-21 or downstream target genes, which in turn regulates lung cancer progression. MiRNA-21, microRNA-21; circRNA, circular RNA; lncRNA, long non-coding RNA. LncRNA CASC2, lncRNA cancer susceptibility candidate 2; LncRNA PLAC2, lncRNA cancer susceptibility candidate 2; LncRNA GAS5, lncRNA growth arrest-specific transcript 5; LncRNA Erbb4-IR, lncRNA Erb-B2 receptor tyrosine kinase 4 IR; LncRNA ASBEL, lncRNA anti-sense transcript of BTG3 (B-cell translocation gene 3); PTEN, phosphatase and tensin homolog; RECK, reversion inducing cysteine rich protein with kazal motifs; IRF1, interferon-regulatory factors 1; HMSH2, human mutS homolog 2; ADSL, adenylosuccinate lyase; PDCD4, programmed cell death factor 4; LZTFL1, leucine zipper transcription factor-like 1; ASPP2, apoptosis stimulating protein 2 of p53.

invasive ability of lung cancer cells. The underlying mechanism is related to the targeting of the downstream target gene RECK by miRNA-21 (78). RECK can negatively regulate EMT and the MMP and reduce the invasion and metastasis of cancer (79). Lung cancer tumor spheroids are enriched with miRNA-21, which can promote macrophage polarisation and EMT, accelerate ERK/STAT3 signalling and thus promote lung cancer cell metastasis to the brain (80). Xia *et al* (66) found that the lncRNA PLAC2 can act as a sponge for miRNA-21 and downregulate the expression of miRNA-21 to promote PTEN expression, which in turn inhibits the migration and invasion of lung cancer cells, whereas overexpression of miRNA-21 can reverse the inhibitory effect of the lncRNA PLAC2 on

lung cancer cells. The combined effect of downregulation of miRNA-21 expression and upregulation of let-7 (one of the two earliest discovered miRNA) was superior to single regulation in inhibiting the invasion and migration of lung cancer cells. The combined effect of the two is also a direction of research on cancer treatment in the future (81). These studies suggest that high expression of miRNA-21 promotes metastasis and invasion of lung cancer.

Inhibiting apoptosis. Apoptosis is the main form of programmed cell death. In cancer, cell-autonomous apoptosis constitutes a common tumor suppressor mechanism, and the induction of apoptosis in tumor cells is the primary

Table I. Preclinical animal and cellular studies of miRNA-21 in lung cancer.

Model	Experimental application	Mechanism of action of miRNA-21	Result	(Refs.)
Nude mice, H1299 cells	Transfection of macrophages with miRNA-21 mimics- H1299 cells	MiRNA-21/IRF1	MiRNA-21 mimics promote proliferation in lung cancer nude mice and cells	(60)
BALB/c nude mouse, A549 cells	miRNA-21 inhibitor- A549 cells	MiRNA-21/PTEN/ RECK	MiRNA-21 inhibitor suppresses lung cancer progression	(61)
C57BL/6 mice	Intraperitoneal injection of miRNA-21 inhibitor	MiRNA-21/RUNX1	MiRNA-21 inhibitor suppresses tumour growth	(62)
BALB/c nude mouse,	miRNA-21 mimics- A549 and H460 cells	Circ-SLC7A6/ miRNA-21/QKI	MiRNA-21 mimics promote cell proliferation and invasion	(30)
PC-9 and A549 cells	miRNA-21 mimics- PC-9 and A549 cells	LncRNA CASC2/ miRNA-21/p53	MiRNA-21 mimics reverse the inhibitory effect of lncRNA CASC2 on lung cancer	(29)
A549 cells	miRNA-21 inhibitor- A549 cells	MiRNA-21/ASPP2	MiRNA-21 inhibitor promotes apoptosis	(36)
H226 and H2170 cells	miRNA-21 mimics- H226 and H2170 cells	LncRNA ASBEL, lncRNA Erbb4-IR/ miRNA-21/LZTFL1	MiRNA-21 mimics promote chemoresistance to gemcitabine and cisplatin in lung cancer cells	(63)
A549 cells	miRNA-21 mimics- A549 cells	Circ_0001287/ miRNA-21/PTEN	MiRNA-21 mimics promote cell proliferation, metastasis and radioresistance	(64)
KLN205 and HCC827 cells	miRNA-21 mimics- KLN 205 and HCC827 cells	LncRNA SNHG10/ miRNA-21	MiRNA-21 mimics promote cell proliferation	(65)
H650 and H1581 cells	miRNA-21 mimics- H650 and H1581 cells	LncRNA PLAC2/ miRNA-21	MiRNA-21 mimics promote cell migration and invasion	(66)
A549/PTX and A549/DDP cells	miRNA-21 mimics- A549/PTX and A549/DDP cells	MiRNA-21/HBP1	MiRNA-21 mimics promote cell migration, invasion and EMT	(67)
A549 cells	miRNA-21 inhibitor- A549 cells	MiRNA-21/FBP1	MiRNA-21 inhibitors inhibit cellular glycolysis and growth	(68)
A549 cells	Liposomal nanoparticle delivery of miRNA-21 to A549 cells	MiRNA-21/PTEN/ EGFR	MiRNA-21 improves sensitivity of lung cancer cells to erlotinib and inhibits cell growth <i>in vitro</i>	(69)

miRNA-21, microRNA-21; IRF1, interferon-regulatory factor 1; PTEN, phosphatase and tensin homolog; RECK, reversion-inducing cysteine-rich protein with kazal motifs; RUNX, runt-related transcription factor 1; QKI, KH domain containing RNA binding; lncRNA CASC2, lncRNA cancer susceptibility candidate 2; ASPP2, apoptosis stimulating protein 2 of p53; lncRNA ASBEL, anti-sense transcript of BTG3 (B cell translocation gene 3); lncRNA SNHG10, lncRNA small nucleolar RNA host gene 10; lncRNA PLAC2, lncRNA cancer susceptibility candidate 2; HBP1, HMG box transcription factor 1; EMT, epithelial-mesenchymal transition; FBP1, fructose-1,6-biphosphatase; A549/PTX cells, paclitaxel-resistant A549 cells; A549/DDP cells, cisplatin-resistant A549 cells; EGFR, epidermal growth factor receptor.

method of cancer treatment (82). Apoptosis is mainly mediated by the death receptor-mediated extrinsic pathway, the mitochondria-mediated intrinsic pathway and the endoplasmic reticulum stress-mediated apoptotic pathway, which are mainly mediated by the B-cell lymphoma 2 (Bcl-2) family, the cysteinyl aspartate specific proteinase (Caspase) family, the Fas ligand and Fas receptor, and the tumor necrosis factor (TNF) receptor and TNF ligand binding, which are involved in the apoptotic process of the above pathway (83). Wu *et al* (29)

reported that the lncRNA CASC2 promoted p53 protein and Bcl-2 associated X protein (Bax) expression and inhibited Bcl-2 expression by binding to miRNA-21, which in turn promoted apoptosis in lung cancer cells, and overexpression of miRNA-21 reversed this process. A similar study showed that direct inhibition of miRNA-21 expression in lung cancer cells promotes Bax expression and inhibits Bcl-2 expression, which in turn induces apoptosis (81). Ge *et al* (84) developed an antisense oligonucleotide drug that targets and inhibits

the expression of miRNA-21 and subsequently promotes the expression of caspase-3 and caspase-8, which in turn promotes apoptosis in lung cancer cells. This, in turn, promoted apoptosis in lung cancer cells. Zhou *et al* (36) reported that miRNA-21 inhibitors can inhibit the PI3K/Akt/NF- κ B signalling pathway, which promoted the expression of caspase-3, caspase-8 and caspase-9 and induced apoptosis in lung cancer cells, and the mechanism was related to the targeting of miRNA-21 to ASPP2. To summarize, downregulating miRNA-21 expression can promote apoptosis in lung cancer cells.

Promoting angiogenesis in lung cancer cells. Malignant cells require nutrients to survive and proliferate. Rapidly growing tumors are heavily vascularised, whereas dormant tumours are not. Hence, tumor angiogenesis needs to be initiated for tumor progression (85). Vascular endothelial-derived growth factor (VEGF) is a key angiogenic factor in tumors and is involved in the initial stages of tumor development, progression and metastasis. Therefore, VEGF and its receptor-mediated signaling pathways have become important therapeutic targets for the treatment of various cancers (85). Liu *et al* (86) found that knocking down STAT3 in human bronchial epithelial cells inhibited the expression of miRNA-21, and inhibiting miRNA-21 expression reduced VEGF levels, which in turn inhibited angiogenesis and the malignant transformation of human bronchial epithelial cells. Zhao *et al* (87) reported that during the transformation of human bronchial epithelial cells into tumor cells induced by arsenite (a carcinogen), miRNA-21 and VEGF expression are elevated; however, downregulation of miRNA-21 expression suppressed the expression of VEGF, suggesting that arsenite exerts a protumor angiogenic effect through miRNA-21 expression. Dong *et al* (88) noted that the lung cancer cells in the miRNA-21-inhibited group rarely formed tubes and had shorter tube lengths and fewer junctions; however, the lung cancer cells in the negative control and mock groups had dense and uniform lumens, suggesting that miRNA-21 is closely associated with promoting angiogenesis in lung cancer.

Promotion of resistance to chemotherapy, radiotherapy and targeted therapy in lung cancer cells by miRNA-21. Certain patients with lung cancer are diagnosed in the middle-to-late stage or after metastasis has occurred, as the disease remains inconspicuous for a long time. After such a late diagnosis, the opportunity for surgery is lost and conservative treatment becomes the main treatment method, involving chemotherapy, radiotherapy and targeted therapy (89). However, the five-year survival rate of patients receiving conservative treatment is <20% (5). The underlying reasons for these poor prognostic outcomes are related to drug resistance, increased adverse effects and poor response to radiotherapy. Certain studies found that miRNA-21 was associated with resistance to chemotherapy, radiotherapy and targeted therapy in lung cancer cells (38,90-93). The roles of miRNA-21 in the chemotherapy, radiotherapy and targeted therapy of lung cancer are presented in Table II.

Chemotherapy. The IC₅₀ values of carboplatin and paclitaxel were lower in A549 cells with miRNA-21 knockdown than in lung cancer A549 cells without miRNA-21 knockdown. This suggested that knockdown of miRNA-21 expression promotes the sensitivity of lung cancer cells to carboplatin and

paclitaxel (59). Extracellular vesicles are nanoscale vesicles that can be used to deliver miRNA. Extracellular vesicles in lung cancer metastatic soft brain membranes can deliver miRNA-21 to lung cancer cells and are highly expressed, with suppressed expression of PTEN and PDCD4 but increased phosphorylated Akt protein levels. This, in turn, promotes methotrexate resistance in leptomeningeal metastasis lung cancer cells (90). Additionally, the lncRNA CASC2 binds to miRNA-21 and miRNA-18, promotes PTEN expression and inhibits the PTEN/PI3K/Akt pathway, which in turn increases the sensitivity of NSCLC to cisplatin (38). Liang *et al* (63) reported that the lncRNA ASBEL and the lncRNA Erbb4-IR can promote the expression of leucine zipper transcription factor-like 1, a downstream target gene of miRNA-21, and increase the sensitivity of lung cancer cells to gemcitabine and cisplatin, whereas the chemosensitivity-promoting effects of the lncRNA ASBEL and the lncRNA Erbb4-IR were attenuated when cells were transfected with miRNA-21 mimics. Perftoran (perfluorodecalin), an artificial oxygen carrier, can attenuate the hypoxia resistance of lung cancer cells to carboplatin by inhibiting miRNA-21 expression (94). Thus, miRNA-21 can reduce the sensitivity of lung cancer cells to chemotherapeutic drugs through multiple pathways. The combination of miRNA-21 with chemotherapeutic drugs may be a way to improve the efficacy of chemotherapy for treating lung cancer in the future.

Radiotherapy. Overexpression of miRNA-21 in lung cancer cells inhibits sensitivity of radiotherapy. For instance, the lncRNA growth arrest-specific transcript 5 can act as a sponge for miRNA-21 and inhibit its expression, promoting PTEN expression and inhibiting Akt phosphorylation, which in turn promotes the radiosensitivity of lung cancer cells (91). Zhang *et al* (92) designed ribonuclease-targeting chimeras that could degrade miRNA-21, and the degradation of miRNA-21 increased the expression of PDCD4, which in turn improved the sensitivity of lung cancer cells to radiation therapy. It has also been reported that circ_0001287 sponges miRNA-21, increases PTEN expression and promotes the radiosensitivity of lung cancer cells (64). In addition, miRNA-21 can promote glycolysis in NSCLC by upregulating the expression of hypoxia-inducible factor 1 α , which in turn promotes radioresistance in NSCLC cells (95). These studies illustrate that overexpression of miRNA-21 promotes radioresistance in lung cancer cells. Inhibiting miRNA-21 expression in lung cancer cells may be an effective method to reverse resistance to radioresistance.

Targeted therapy. Targeted therapy is an important treatment option for patients with advanced lung cancer. EGFR-tyrosine kinase inhibitors (TKIs) are the most widely used targeted drugs for treating lung cancer and can improve patient survival, but resistance to these drugs decreases efficacy and may lead to cancer progression (96). It has been shown that miRNA-21 increased purine metabolism by targeting adenylosuccinate lyase, leading to persistent resistance to oxitinib in lung cancer cells (93). Gefitinib is an EGFR-TKI and miRNA-21 promotes phosphorylation and activation of Akt, facilitating resistance to gefitinib in lung cancer cells (97).

Potential of miRNA-21 as a biomarker for lung cancer. With the advancement in technology, the determination of miRNA-21 has become more precise and sensitive (98,99).

Table II. Role of miRNA-21 in chemotherapy, radiotherapy and targeted therapy in lung cancer.

Expression	Upstream regulator	Target	Major functions	(Refs.)
Up	LncRNA CASC2	NA	Inhibits the sensitivity of lung cancer cells to cisplatin	(38)
NA	NA	NA	Inhibits sensitivity of lung cancer cells to carboplatin and paclitaxel	(59)
Up	LncRNA ASBEL and LncRNA Erbb4-IR	<i>LZTFL1</i>	Promotes resistance to gemcitabine and cisplatin in lung cancer cells	(63)
Up	Circ_0001287	PTEN	Promotes radioresistance	(64)
NA	NA	PTEN and PDCD4	Promotes methotrexate resistance in leptomenin-geal metastasis lung cancer cells	(90)
NA	NA	PTEN	Inhibits the radiosensitivity of lung cancer cells	(91)
NA	NA	PDCD4	Inhibits the radiosensitivity of lung cancer cells	(92)
Up	NA	ADSL	Promotes purine metabolism, leading to persistent resistance to oxitinib in lung cancer cells	(93)

LncRNA CASC2, lncRNA cancer susceptibility candidate 2; lncRNA ASBEL, lncRNA anti-sense transcript of B cell translocation gene 3; lncRNA Erbb4-IR, lncRNA Erb-B2 receptor tyrosine kinase 4 IR; LZTFL1, leucine zipper transcription factor-like 1; PTEN, PTEN, phosphatase and tensin homolog; PDCD4, programmed cell death factor 4; ADSL, adenylosuccinate lyase; Up, upregulated; NA, no information available.

Studies have found that miRNA-21 can also be used as a biomarker for lung cancer diagnosis, prognosis and response to treatment. Pang *et al* (100) revealed that miRNA-21 was highly expressed in the serum of patients with lung cancer and that miRNA-21 can be used as a diagnostic marker for lung cancer, with an area under the curve (AUC) value of 0.901. Based on TNM staging, miRNA-21 expression in patients with stage III and IV lung cancer was significantly higher than that in patients with stage I and II disease. The expression of miRNA-21 in patients with metastasis was significantly higher than that in patients without metastasis. These findings suggest that miRNA-21 can be used as a diagnostic biomarker for lung cancer diagnosis and pathological staging (100). The expression of peripheral blood miRNA-21 and miRNA-486 was highly accurate for early cancer in lung nodules, with an AUC value of 0.855 (101). This can help in the early diagnosis of lung cancer. Liu *et al* (102) reported that the expression level of exosomal miRNA-21 can distinguish between patients with lung cancer and healthy individuals.

One study reported that plasma miRNA-21 expression was significantly elevated in patients with NSCLC and that high miRNA-21 expression was positively correlated with lung cancer tumour size (103). Another study revealed that extracellular vesicular miRNA-21 expression in lung cancer pleural lavage fluid was correlated with lung cancer pleural infiltration and that miRNA-21 induced peritoneal cavity mesothelial mesenchymal transition, leading to cancer spread (104). High expression of miRNA-21 was associated with poor prognosis in patients with soft meningeal metastases of lung cancer, and miRNA-21 expression was negatively associated with OS in patients with soft meningeal metastases of lung cancer (90). Xu and Shi (105) found an increase in the expression of miRNA-21 in the serum of patients with NSCLC by conducting PCR. Another study revealed that miRNA-21 expression was significantly upregulated in 112 patients with NSCLC recurrence

or metastasis (experimental group) compared to 68 control patients with newly diagnosed NSCLC. After a follow-up time of three years, the mortality rate of the experimental group was found to be 91.96%, while the mortality rate of the control group was only 57.35% (105). These findings suggested that miRNA-21 is associated with poor prognosis in patients with NSCLC.

Zhu *et al* (106) reported that the overall effective rate of treating patients with lung cancer brain metastases via whole-brain radiotherapy combined with intensity-modulated radiotherapy was 86% and the miRNA-21 expression level of the treated patients with lung cancer brain metastases was significantly lower. A low level of miRNA-21 was associated with prolonged OS in treated patients with lung cancer brain metastases and low miRNA-21 expression levels in treated patients were associated with carcinoembryonic antigen, neuron-specific enolase, squamous cell carcinoma antigen and other tumor marker expression levels (106). These findings suggest that the expression level of miRNA-21 can predict the efficacy of whole-brain radiotherapy combined with intensity-modulated radiotherapy (106).

Therapeutic strategies for lung cancer based on miRNA-21.

As mentioned earlier, miRNA can be classified into two types: Tumor suppressors and oncogenes. The use of miRNA mimics or inhibitors and techniques to knock down miRNA expression may be an effective therapeutic strategy for treating cancer. In addition, the combination of miRNA mimics or inhibitors with chemotherapy, radiotherapy and targeted therapy can improve therapeutic sensitivity and benefit patients. Furthermore, miRNA-21 is an oncogene and inhibiting the expression of miRNA-21 has therapeutic potential for cancer treatment. CRISPR/Cas9 gene editing technology can identify activated oncogenes, inhibit proliferation and suppress growth in cancer cells by knocking

out oncogenes (107). These effects subsequently reduced the proliferation, migration and colony formation of lung cancer cells and improve their sensitivity to carboplatin and paclitaxel (59). Certain cancer therapeutic agents, such as Tanshinone IIA and cryptotanshinone, inhibit the expression of miRNA-21, which in turn inhibits the proliferation and invasion of lung cancer cells via a mechanism related to the downregulation of miRNA-21, which promotes the expression of tight junction proteins (108). Downregulation of miRNA-21 combined with upregulation of let-7 had a stronger inhibitory effect on lung cancer than downregulation of miRNA-21 expression alone (81). These findings provide a new idea for future regulation of the expression of both miRNAs for lung cancer treatment. In addition, miRNA-21 plays a role in promoting therapeutic resistance to various chemotherapeutic agents, targeted drugs and radiotherapy, and the use of miRNA-21 inhibitors in combination with these approaches can increase treatment efficacy.

Strategies to inhibit miRNA-21 in lung cancer also include anti-miRNA-21 oligonucleotides (95), small molecules (109), miRNA sponges (110) and anti-miRNA-21 nanoparticle delivery strategies. Zhang *et al* (92) used an acidic pH/H₂O₂-activated ribonuclease-targeted chimera that could precisely degrade miRNA-21 expression in lung cancer cells, whereas other control miRNAs were not degraded; the degradation of miRNA-21 promoted PDCD4 expression, thereby increasing the sensitivity of lung cancer cells to radiotherapy and inhibiting tumour growth. Small-molecule inhibitors can induce specific changes in the structure and kinetics of pre-miR-21 by binding to pre-miR-21 and affecting the Dicer enzyme cleavage site, which in turn reduces miRNA-21 expression (109). Numerous lncRNAs, such as the lncRNA ASBEL and the lncRNA Erbb4-IR, can act as sponges for miRNA-21 and inhibit its expression, thereby preventing the progression of lung cancer (63).

A study reported that when QTsome lipid nanoparticles encapsulated with anti-miRNA-21 (QTplus-AM21) were delivered to lung cancer cells, miRNA-21 expression was strongly inhibited, followed by significant inhibition of lung cancer cell growth *in vitro* (69). This study also revealed that QTplus-AM21 promoted the sensitivity of lung cancer cells to erlotinib via a mechanism in which QTplus-AM21 promoted PTEN expression and inhibited EGFR expression (69). Of note, the delivery of miRNA-21 inhibitors and adriamycin via a calcium phosphate-polymer nanoparticle system caused greater cytotoxicity and promoted the expression of the tumor suppressor PTEN in lung cancer cells compared to that recorded after using adriamycin alone (111). Additionally, co-delivery of anti-miRNA-21 and anti-miRNA-95 by calcium phosphate nanoparticles significantly inhibited lung cancer cell growth and promoted radiation sensitivity (112).

5. Advances in clinical studies of miRNA mimics or inhibitors

Problems with the *in vivo* specificity, safety and instability of miRNA have limited most of these studies to preclinical research. Clinical trials of miRNA-21 inhibitors or mimics in lung cancer have also not been conducted, to the best of our knowledge. However, a few miRNA mimics and inhibitors have

entered the clinical stage (113-135) (Table III). These studies provide insights for the entry of miRNA-21 into clinical trials.

The earliest miRNA mimic was MRX34, a miRNA-34 liposomal mimic that was initiated in 2013 and was included in multiple tumor studies but was ultimately terminated early owing to severe immunotoxic reactions (113,114) (NCT02862145, NCT01829971). These results highlight the need to critically assess the potential off-target effects and immunotoxicity of miRNA. However, these two trials evaluated maximum tolerated doses of 93 and 110 mg/m² in patients with hepatocellular carcinoma (HCC) and non-HCC patients, respectively, which helped determine the dosage of miRNA to be used in subsequent clinical trials.

The miRNA-16 mimic MesomiR-1 was administered to five patients with malignant pleural mesothelioma and advanced NSCLC. The findings revealed that patients experienced a transient increase in inflammatory factors and liver enzymes shortly after MesomiR-1 was injected, the quality of life improved in three patients, and the initial phase I trial showed good tolerability; however, the researchers did not continue with clinical trials or the publication of relevant experimental results (NCT02369198) (115,116).

The oligonucleotide RG-101 of miRNA-122 was used in a clinical trial of chronic hepatitis C, which demonstrated tolerability and good results in terms of massive viral decline after four weeks (117-120). However, the clinical trial was not followed up and the results were not published.

Miravirsen (SPC3649f) is a miRNA-122 antagonist that has been used in clinical trials for chronic hepatitis C. SPC3649f is effective in the treatment of chronic hepatitis C. Although three phase II clinical studies have been conducted, no further studies or results have been subsequently published (121-124).

AMT-130 is an artificial miRNA delivered to brain tissue via adeno-associated virus type 5 to silence the HTT gene and thus treat Huntington's disease. This clinical trial is currently undergoing concurrent phase I and phase II studies (125,126).

MGR-201 (an miRNA-29 mimic), MRG-110 (an miRNA-92a antagonist) (130,131), CDR132L (an miRNA-132-3p antagonist) (132) and RGLS4326 (an miRNA-17 antagonist) (133) have all completed phase II clinical trials. Further studies and the publication of related results are anticipated.

RG-012 is an miRNA-21 antagonist for Alport syndrome. The FDA designated RG-012 as an orphan drug and a phase II clinical trial was completed; however, for unknown reasons, the trial was terminated early (134,135). Other miRNA clinical studies currently being terminated include those on INT-1B3 (an miRNA-193a-3p mimic), RG-125/AZD4076 (an miRNA-103/107 antagonist) (127) and MRG-106 (an miRNA-155 antagonist) (128,129).

6. Conclusion and prospects

In this review, the multiple roles of miRNA-21 in lung cancer were summarized. Although miRNA-21 regulates biological processes by targeting downstream genes, it is also controlled by upstream regulators, such as transcription factors, DNA methylation and ceRNA. Most studies have shown that miRNA-21 acts as an oncogene in lung cancer and that high expression of miRNA-21 is associated with poor prognosis for patients with lung cancer. It also affects multiple pathways that

Table III. Clinical trials with miRNA therapeutics.

Drug names for miRNA	Target miRNA	Mode of action	Disease/condition	Treatment modalities	Clinical trial nos. ^a	(Refs.)
MRX34 ^b	miRNA-34a	miRNA mimics	Hepatocellular carcinoma, pancreatic cancer, bile duct cancer, neuroendocrine tumour, colorectal cancer, non-small cell lung cancer, breast cancer, cervical cancer, bladder cancer, oesophageal cancer, leiomyosarcoma, ovarian cancer, pheochromocytoma, adenocarcinoma of the appendix, gastrointestinal stromal tumors and renal cell carcinoma	Intravenous/liposomal delivery	NCT02862145, NCT01829971	(113,114)
MesomiR-1 ^c	miRNA-16	miRNA mimics	Malignant pleural mesothelioma and non-small cell lung cancer	Intravenous/vehicle transfer of nonliving bacterial nanocells (EDVs or TargomiRs) Subcutaneous injection/ biomolecule conjugation (GalNAc)	NCT02369198	(115,116)
RG-101 ^c	miRNA-122	Anti-miRNA	Chronic hepatitis C virus		EudraCT no. 2013-002978-49, 2015-001535-21, 2015-004702-42, 2016-002069-77	(117-120)
Miravirsen/ SPC3649 ^d	miRNA-122	Anti-miRNA	Chronic hepatitis C virus	Subcutaneous injection/ chemical modification (LNA)	NCT01200420, NCT02508090, NCT02452814, NCT01872936, NCT01727934, NCT01646489, NCT04675996	(121-124)
INT-1B3 ^b	miRNA-193a-3p	miRNA mimics	Advanced solid tumours	NA		-
AMT-130 ^{e,f}	Artificial miRNA	A miRNA expression	Huntington's disease	Stereotaxic infusion/ viral transfer (adenoviral associated vector)	NCT04120493	(125,126)
MGR-201 ^d	miRNA-29	miRNA mimics	Normal individuals and healthy individuals with scars	Intradermal injection	NCT02603224, NCT03601052	-
RG-125/ AZD4076 ^b	miRNA-103/107	Anti-miRNA	Non-alcoholic steatohepatitis and type 2 diabetes mellitus	NA	NCT02612662, NCT02826525	(127)

Table III. Continued.

Drug names for miRNA	Target miRNA	Mode of action	Disease/condition	Treatment modalities	Clinical trial nos. ^a	(Refs.)
MRG-106 ^b	miRNA-155	Anti-miRNA	Cutaneous T-cell lymphoma /mycosis fungoides	Subcutaneous injection, intravenous infusion or intra-lesional injection	NCT03713320, NCT03837457	(128,129)
MRG-110 ^c	miRNA-92a	Anti-miRNA	Wounds	Skin injection/chemical modification (LNA)	NCT03603431	(130,131)
CDR132L ^c	miRNA-132-3p	Anti-miRNA	Heart failure	Intravenous/chemical modification (LNA)	NCT04045405	(132)
RGLS4326 ^c	miRNA-17	Anti-miRNA	Autosomal dominant polycystic kidney disease	Subcutaneous injection	NCT04536688	(133)
RG-012/lademirsen/SAR339375 ^b	miRNA-21	Anti-miRNA	Alport syndrome	Subcutaneous injection/chemical modification (phosphorothioate)	NCT03373786 NCT02855268	(134,135)

^aNCT-numbered trials are registered at ClinicalTrials.gov; EudraCT-numbered trials are registered in the EU Clinical Trials Register (clinicaltrialsregister.eu); ^bStopped/terminated; ^cPhase I completed; ^dPhase II completed; ^ePhase I ongoing; ^fPhase II ongoing; LNA, locked nucleic acid; miRNA, microRNA; EDVs, EnGeneC Dream Vectors; GalNac, N-acetyl-galactosamine.

contribute to lung cancer progression, including the PI3K/AKT, MEK/ERK, TGF- β /SMAD, Hippo, NF- κ B, and STAT3 pathways. Mechanistically, miRNA-21 can contribute to lung cancer progression by regulating the cell cycle, promoting proliferation, metastasis and invasion, and angiogenesis, and inhibiting apoptosis in lung cancer cells.

In addition, elevated expression of miRNA-21 has been linked to drug resistance in lung cancer. The subsequent application of miRNA-21 inhibitors in combination with chemotherapy, radiotherapy and targeted therapy may represent a novel strategy to enhance clinical efficacy. However, this approach requires further validation through extensive clinical trial data. Furthermore, miRNA-21 holds promise as a biomarker for diagnosis, prognosis and therapeutic response in patients with lung cancer. It is possible to conduct miRNA-21 urine and sputum miRNA-21 detection experiments to further analyze whether the use of miRNA-21 as a lung cancer biomarker in other fluids is suitable. These non-invasive operations have the advantages of easy sampling and negligible harm to patients.

Considering the cancer-promoting role of miRNA-21, strategies for its inhibition in lung cancer may be summarized, and these approaches include the use of CRISPR/Cas9 technology, natural anticancer drugs, oligonucleotides, small molecules and miRNA sponges to knock down or inhibit miRNA-21 expression. There are few studies related to the delivery of miRNA-21 inhibitors using viral vectors. However, the use of nanoparticle technology to deliver miRNA-21 inhibitors is an effective and promising method for precise and safe delivery to cancer cells, with fewer immune responses and other adverse effects (69).

The anti-lung cancer strategy involving miRNA-21 has not entered clinical trials. However, its great clinical translational potential has received considerable attention from researchers. Research on miRNA-21 therapy needs to address several challenges before it can become a routine clinical therapy for lung cancer. First, the stability and targeted delivery of miRNA-21 need to be addressed. Since miRNA-21 is easily broken down in the bloodstream, which makes it difficult to reach therapeutic concentrations, a reliable delivery system needs to be developed to accurately deliver the therapeutic concentration of miRNA-21 to lung cancer cells. The available viral delivery systems (such as lentiviruses and adeno-associated viruses) are highly efficient, but their adverse effects on the immune response and insertion mutagenesis need to be further addressed. However, nonviral delivery systems (such as exosomes and nanoparticles) are safer delivery methods, but their low delivery efficiency and potential toxicity require further optimization. Additionally, the clinical safety of miRNA-21 therapy needs to be confirmed. For instance, MRX34 was the first miRNA-34 mimic to enter the clinic; however, the trial had to be terminated early due to the occurrence of severe immune reactions (114). Therefore, future experiments need to critically assess the potential off-target effects and immunotoxic reactions of miRNA-21. Finally, although the expression and therapeutic targets of miRNA-21 in lung cancer are now preliminarily understood, whether the multiple downstream target genes of miRNA-21 interact with each other needs to be determined to gain a more comprehensive understanding of the regulatory network of miRNA-21.

Such information can provide a more comprehensive theoretical basis for the entry of miRNA-21 into clinical studies of lung cancer.

To summarize, miRNA-21 plays the role of an oncogene in lung cancer and miRNA-21 may serve as a suitable therapeutic target for lung cancer. However, current studies are mostly limited to animal experiments and relatively few clinical experiments. In the future, researchers can perform more multicentre and large-sample experiments to achieve clinical validity. Once these problems are solved, these treatment strategies can benefit patients with lung cancer or other cancers. Therefore, further in-depth research holds significant promise.

Acknowledgements

Not applicable.

Funding

This work was funded by the Guangxi University of Traditional Chinese Medicine (grant no. 2024LZ029) and the Guangxi Zhuang Autonomous Region Administration of Traditional Chinese Medicine (grant no. GXZYB20230493), a self-funded project.

Availability of data and materials

Not applicable.

Authors' contributions

ZL wrote the major parts of the manuscript and prepared the figures and tables. HZ and GW analysed the literature. WG and YL made changes to the article. ZC conceptualized the study and oversaw the process. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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