miR-206/133b Cluster: A Weapon against Lung Cancer?

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Lung cancer is a deadly disease that ends numerous lives around the world. MicroRNAs (miRNAs) are a group of non-coding RNAs involved in a variety of biological processes, such as cell growth, organ development, and tumorigenesis. The miR-206/133b cluster is located on the human chromosome 6p12.2, which is essential for growth and rebuilding of skeletal muscle. The miR-206/133b cluster has been verified to be dysregulated and plays a crucial role in lung cancer. miR-206 and miR-133b participate in lung tumor cell apoptosis, proliferation, migration, invasion, angiogenesis, drug resistance, and cancer treatment. The mechanisms are sophisticated, involving various target genes and molecular pathways, such as MET, EGFR, and the STAT3/HIF-1α/VEGF signal pathway. Hence, in this review, we summarize the role and potential mechanisms of the miR-206/133b cluster in lung cancer.

Lung cancer is a severe disease with a poor prognosis, giving rise to a growing body of patient deaths. In China, many people lose their life as a result of cancers, and lung cancer is the primary cause of them. About 610,000 individuals were estimated to die of lung cancer in 2015, contributing to a grave disease burden.1 Lung cancer can be divided into four leading types according to histologic pathology: large cell carcinoma, small cell carcinoma, squamous cell carcinoma, and adenocarcinoma.2 Nonetheless, lung cancer is also historically classified into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) in terms of the diverse clinical presentation, metastasis, and sensitivity to therapy.2,21 Approximately 80% patients with lung cancer could be diagnosed as having NSCLC, and only 10%–15% of patients can survive for 5 years or longer.1 Nowadays, patients with lung cancer are predominantly given chemotherapy to relieve or control their conditions. The effect is unsatisfactory; hence, it is imperative to investigate novel and helpful approaches against lung cancer.

MicroRNAs (miRNAs) 20–25 nucleotides in length belong to small non-coding RNAs and make sense in regulation for the targeted gene by targeting a specific 3′ UTR of miRNAs.6–8 Accumulating evidence indicates that miRNAs participate in diverse cancers, including lung cancer. miR-187-3p functioned as a tumor suppressor in lung cancer, where it can repress proliferation, migration, and invasion, as well as enhance cell apoptosis by targeting oncogene BCL6.9 miR-326 and miR-329 are both downregulated in patients’ tissue and cell lines and act in an anticancer role in NSCLC.10,11 However, some cancer-associated miRNAs might act as cancer promoters, and miR-17-92 cluster is an example. miR-17-92 regulated lung tumorigenesis by directly targeting hypoxia-inducible factor 1α (HIF-1α), and upregulation of c-myc resulted in induction of miR-17-92 and downregulation of HIF-1α.12,13 Furthermore, miR-346 has been revealed as a tumor enhancer by modulating the XPC/ERK/Snail/E-cadherin pathway in NSCLC, as well as being associated with poor prognosis.14 Therefore, various miRNAs may have different functions in human cancer for reasons that remain unknown.

The miR-206/133b cluster is located on the human chromosome 6p12.2, which is essential for growth and rebuilding of skeletal muscle; it is not expressed in the heart.15 This miRNA cluster includes not only so-called the myoRNAs miR-206 and miR-133b but also the long non-coding RNA linc-MD1, which plays a pivotal part in muscle differentiation.16 Nonetheless, linc-MD1 has not been deeply investigated, and there is no evidence showing that this long non-coding RNA participates in human diseases, including human cancer. Accordingly, the miR-206/133b cluster specifically refers to miR-206 and miR-133b in this paper. It has been reported that miR-206/133b can be discovered in slow myofibers in adult muscle, and its expression is regulated by a network of myogenic genes, such as MyoD, a kind of muscle regulatory factor (MRF).17,18 Moreover, it was disclosed as being involved in immune response and may be a biomarker for a Th17-type immune reaction in T cells.19 In addition, these miRNAs appeared to dysregulate a multitude of human cancers, including breast cancer,20 cervical carcinoma,21 colon cancer,22 and lung cancer.23 Both miR-206 and miR-133b are significantly downregulated in lung cancer (Table 1), suggesting they may play parts in lung tumorigenesis.

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In the present review, we highlighted the function, target genes, and mechanisms of the miR-206/133b cluster in lung cancer cell apoptosis, proliferation, migration, invasion, angiogenesis, and cancer treatment to provide evidence for further investigations and the clinic in the future.

### Promotion of Cell Apoptosis

Tumor cell apoptosis is regulated by a network of factors, and dysregulation of cell apoptosis is linked to a body of diseases, referring to numerous molecular pathways and proteins. Eukaryotic cells motivate caspase-7 and caspase-3 to promote apoptosis via death receptor- and mitochondria-induced signal pathways. The B cell lymphoma (Bcl) 2 family includes three subsets: anti-apoptosis proteins such as Bcl-xL and Bcl-2, pro-apoptosis proteins such as Bak and Bax, and BH3-only proteins, which play a vital role in cell apoptosis.26,27

MET is the tyrosine kinase receptor of the hepatocyte growth factor (HGF) and greatly relates to tumorigenesis. Evidence shows that the MET plays a key role in p53-mediated regulation of migration and invasion through the signal pathway of phosphatidylinositol 3-kinase (PI3K)-AKT and mTOR.28

#### Table 1. Dysregulation of the miR-206/133b Cluster in Lung Cancer

| Lung Cancer Type | miRNA | Expression | Target Genes | Tissue/Cells | Reference |
|------------------|-------|------------|--------------|--------------|-----------|
| LAC              | miR-206 | down       | Smad3        | cells        | 37        |
| NSCLC            | miR-206 | down       | 14-3-3z      | cells        | 46        |
| Lung cancera     | miR-206 | down       | CCL2, VEGFA  | cells        | 48        |
| NSCLC            | miR-206 | down       | MET          | both         | 31,37,48  |
| NSCLC            | miR-206 | down       | c-Met        | both         | 57        |
| NSCLC            | miR-206 | down       | c-Met, Bcl2  | both         | 58        |
| NSCLC            | miR-206 | down       | SOX9         | both         | 58        |
| LSCC             | miR-206 | down       | MET, EGFR    | cells        | 30        |
| LAC              | miR-206 | down       | G6PD, PGD, TKT, GPD2 | cells | 50,58,78 |
| NSCLC            | miR-206 | down       | –            | both         | 50        |
| LAC              | miR-206 | down       | –            | –            | 80        |
| NSCLC            | miR-133b | down      | FSCN1        | cells        | 60        |
| NSCLC            | miR-133b | down       | PKM2         | cells        | 72        |
| NSCLC            | miR-133b | down       | EGFR         | both         | 72        |
| LAC              | miR-133b | down       | MCL-1, BCL2L2 | both | 50        |
| Lung cancera     | bothb   | down       | –            | –            | 80        |
| LAC              | miR-133b | down       | –            | both         | 40        |
| NSCLC            | miR-133b | down       | –            | tissue       | 42        |
| Lung cancera     | miR-133b | down       | –            | tissue       | 72        |

**LAC, lung adenocarcinoma; LSCC, lung squamous cell carcinoma; PKM2, pyruvate kinase isoform M2.**

*aLung cancer types were not mentioned.

*bIncluding miR-206 and miR-133b.*
In another investigation, c-Met, Bcl2, and cyclin D1 were overexpressed in lung cancer tissues, and downregulation of them enhanced NSCLC cell (SK-MES-1 cell and A549 cell) apoptosis. Further assays verified that both Bcl-2 and c-Met were the appropriate targets of miR-206, and miR-206 prominently reduced their levels in NSCLC. 

Taking all these findings into consideration, we suppose the miR-206/133b cluster might be a promoter in lung cancer cell apoptosis. The relationship between these lung cancer-related miRNAs and corresponding targets or signaling pathway are briefly summarized in Figure 1.

Inhibition of Cell Proliferation

It is generally known that cell proliferation plays a critical role in tumorigenesis and tumor progression. Cells lack some factors of control and regulation, giving rise to the development of neoplasms. There is a growing body of evidence showing that the miR-206/133b cluster participates in a great deal of tumor cell proliferation. Ren et al. revealed that miR-206 could suppress colorectal cancer cell proliferation by targeting formin-like 2 (FMNL2), which was validated as an emerging member of diaphanous-related formins (DRFs). miR-133b was found to be decreased in renal cell carcinoma cells, and restoration of its expression significantly repressed cell proliferation by regulating matrix metallopeptidase 9 (MMP-9).

Smad3, a transcription factor, is characterized as a key regulator in the TGF-β1 (transforming growth factor β1)/Smad signal pathway, which involves in tumorigenesis. Zhang et al. reported that the TGF-β1 could lessen the expression of miR-140 and miR-206, and further study demonstrated these miRNAs acted as tumor inhibitors in the LAC cell by targeting Smad3 and affecting the TGF-β1-related pathway, contributing to repression of cell proliferation. They also revealed that these miRNAs associated with tribbles homolog 2 (TRIB2) in inhibition of cell proliferation in vivo, but no further investigations were performed to confirm whether the TRIB2 was a target of miR-206. In addition, miR-206 tightly correlated with NRF2 (nuclear factor erythroid-2-related factor 2) and the pentose phosphate pathway, and all of them (NRF2 and pentose phosphate pathway) were dramatically dysregulated in lung tumor. NRF2 was able to downregulate the expression of miR-206 and modulate the metabolic flux of lung cancer cells by affecting fatty acid synthesis, the tricarboxylic acid cycle, and the pentose phosphate pathway. miR-206 was uncovered to target the pentose phosphate pathway genes (G6PD, PGD, TKT, and GPD2), reducing pentose phosphate pathway-related NADPH production and ribose synthesis and suppression of the growth of the H1437 cell and A549 cell. The analogous role of miR-206 was observed in vivo. Our research group disclosed that restoration of miR-206 does not merely boost lung cancer cell apoptosis but also represses proliferation by binding to Bcl-2 and c-Met mRNA. In LAC, Zhang et al. discovered four miRNAs were dramatically dysregulated via miRNA microarray and qRT-PCR assays, including miR-133b. Upregulation of miR-133b significantly suppressed SPC-A1 cell and A549 cell proliferation, whereas there was no function to migration and invasion of these cancer cells.

In this section, evidence shows that the miR-206/133b cluster acts as a suppressor in lung cancer cell proliferation. The link between these lung cancer-related miRNAs and corresponding targets or signaling pathway was briefly generalized in Figure 2.

Blocking Tumor Angiogenesis

Angiogenesis is a crucial process in tumorigenesis and tumor development. If there are effective and credible approaches to treatment that blocks angiogenesis, a great deal of patients will live longer. The mechanisms of angiogenesis are sophisticated and complicated, involving anti-angiogenic factors such as Notch molecular pathway,
matrix metalloproteinases, and pro-angiogenic factors such as FGF (fibroblast growth factor) and VEGF (vascular endothelial growth factor). Increasingly, investigations have shown that miRNAs associate with tumor angiogenesis. For instance, Tu et al. uncovered that upregulation of miR-497 markedly repressed tumor angiogenesis by targeting VEGFR2, a key receptor of VEGF. Furthermore, miR-206 was disclosed to tightly correlate with angiogenesis in lung cancer. Previous findings suggested that the 14-3-3 family dysregulated and played a critical role in NSCLC. Xue et al. found the expression of 14-3-3z was increased in lung cancer cell lines, which could induce tumor angiogenesis in vivo and in vitro. Nevertheless, miR-206 was discovered to play an inverse role, suppressing lung tumor angiogenesis. The underlying mechanism of miR-206-regulated angiogenesis was suppression of the 14-3-3z/STAT3/HIF-1α/VEGF signaling pathway. Similarly, the relationship among miR-206 and HGF-induced angiogenesis was investigated. HGF was able to induce tubules formation and metastasis of human umbilical vein endothelial cells (HUVECs), whereas miR-206 dramatically repressed it and the mechanism correlated with blocking PI3k/Akt/mTOR molecular pathways. In addition, Shen et al. investigated the underlying mechanism by which normal fibroblasts (NFs) transform into cancer-associated fibroblasts (CAFs) in lung cancer. They found CAFs promoted tumor growth and angiogenesis, and miR-206 inhibited the conversion of NFs to CAFs by targeting VEGFA/CCL2.

It has been reported that HUVECs qualified as a model of angiogenesis in vitro study. In addition, miR-133 could enhance HUVEC apoptosis and inhibit proliferation and migration by modulating by complicated factors. The epithelial-to-mesenchymal transition (EMT) is a well-established element in the transformation of early-stage tumors into advanced cancer. Decreased expression of E-cadherin, a calcium-dependent transmembrane glycoprotein, is a key hallmark in EMT. E-selectin targeting PEGylated-thioaptamer is able to block breast cancer metastasis. Accumulating evidence suggests that dysregulation of E-cadherin results in tumor metastasis and an unsatisfactory prognosis in a variety of human tumors. In addition, it has been reported that miRNAs tightly correlate with EMT in cancer metastasis. HGF was able to induce EMT and contribute to migration and invasion of lung cancer A549 and 95D cells, while miR-206 markedly blocked HGF-induced EMT, as well as attenuating lung cancer cell migration and invasion by modulating c-Met and its downstream PI3k/Akt/mTOR molecular pathway. Wang et al. also discovered miR-206 was downregulated and that overexpression of it could diminish the metastasis of the 95D cell. However, the potential targets and mechanisms of it were not further investigated. miR-206 and miR-140 have been previously described to suppress lung cancer cell proliferation. They also have been confirmed to inhibit LAC cell metastasis, which could upregulate the level of E-cadherin by targeting Smad-3 and triggering TRIB2-related activity. Furthermore, miR-206 was found to inhibit lung cancer cell migration and invasion by regulating MET. Mataki et al. pointed out miR-206 attenuated metastasis of lung squamous cell carcinoma EBC-1 cells via wound healing and Matrigel invasion assays through regulation of MET and the EGFR downstream signaling pathway. These findings indicates that miRNA-mediated MET plays a critical role in lung cancer.
cancer cell migration and invasion. In addition, miR-206-related SOX9 (sex-determining region Y [SRY] box 9) activity participated in lung cancer cell migration and invasion.68

Fascin1 (FSCN1), a member of FSCN family, has been validated to enhance NSCLC cell migration and invasion and have no effect on tumor cell proliferation.69 Yang et al.60 uncovered that FSCN1 was a direct target of miR-133b and increased in NSCLC cells. As in previous investigations, FSCN1 significantly promoted NSCLC cell migration and invasion, whereas miR-133b played an inverse role when compared with the FSCN1.60 In addition, low expression of miR-133b dramatically associated with lymph nodes metastasis and an advanced stage in NSCLC.61 However, the mechanisms and other functions of miR-133b were not further investigated. The relationship between these lung cancer-related miRNAs and corresponding targets or signaling pathway were briefly summarized in Figure 1.

Treatment and Prognosis
It is widely acknowledged that chemotherapy is a pivotal approach to antagonizing a tumor; however, it seems to generate drug resistance and has undesirable prognosis and complications. With the relationship between miRNAs and human cancer gradually uncovered, miRNA-based therapy may be an underlying approach in the future. For instance, miR-34 has been the first miRNA mimic for cancer treatment in a phase I clinical,62 which was also considered a cancer repressor by regulating multitudes of genes and molecular pathways, such as the Notch molecular pathway, c-MYC, and CDK6.63 Sorafenib, a well-recognized medicine for patients with advanced hepatocarcinoma, has been verified to give rise to elevate the expression of miR-423-5p in hepatocarcinoma patients’ serum, and miR-423-5p was detected that promoted hepatocarcinoma cell autophagy.64 It has been reported that the microtubule-associated protein (MAP) kinase and interacting kinase aptamers inhibit tumor cell proliferation.65,66 The miR-29b-p53-mediated pathway is regard as one of the most critical modulated pathways in carcinomas therapeutic. LK-L1C/K6W/L8C, a novel synthesized amphiphilic peptide, has been demonstrated as being able to induce cancer cell apoptosis by the miR-29b-p53-mediated pathway.66

Cisplatin, a well-established chemotherapeutic drug, is commonly applied for lung cancer treatment. Nonetheless, it has been reported that lung cancer patients always trigger cisplatin resistance in clinical application.67 Cisplatin-resistant H1299/DDP and A549/DDP cells were inclined to appear EMT, invasion, and migration.68 Low expression of miR-206 was observed in cisplatin-resistant LAC cells, whereas re-expressed miR-206 facilitated the cells to be sensitive to cisplatin treatment and antagonized EMT, invasion, and migration.68 Further assays revealed miR-206 blocked EMT, cell metastasis, and cisplatin resistance by targeting MET and repressing its downstream PI3K/Akt/mTOR molecular pathway.69 PF-04691502, a dual PI3K/mTOR suppressor, in combination with VEGF small interfering RNA (siRNA), blocked NSCLC, which may be useful for lung cancer patient therapy.70 Moreover, PCTAIRE1 siRNA-lipid nanoparticles reduced tumor growth significantly compared with the scramble control group.70 In addition, Liu et al.71 found miR-133b was able to promote sensitivity of NSCLC cells toward gefitinib chemotherapy, of which mechanisms may include the EGFR-related pathway. Gemcitabine, another chemotherapeutic agent, combined with platinum treatment, has been widely applied in advanced NSCLC.72 Evidence suggested gemcitabine combined with miR-133b mimic exhibited a remarkable effect in terms of lung cancer cell chemotherapy.73 miR-133b was also verified to correlate with radiation therapy in lung cancer cells, and it was low expressed in radioresistant lung cancer cells.74 Elevating expression of miR-133b contributed to radioresistant lung cancer cells being resensitive, and the relevant pathway was involved in pyruvate kinase isofrom M2-mediated glycolysis.75 In addition, Wu et al.73 demonstrated that cationic lipids combined with pre-miR-133b significantly elevated the level of mature miR-133b in the lung cancer A549 cell and in the mouse model. This study may greatly contribute to how miRNA-based treatment can be more efficient in the future.

Furthermore, the miR-206/133b cluster correlates with lung cancer patients’ prognosis and survival. Zhang et al.76 discovered TRIB2 was higher in lung cancer samples and linked to a poorer prognosis, whereas miR-206 could inhibited TRIB2-related activity in lung cancer cells. This investigation implied miR-206 may be beneficial for lung cancer patients’ survival. Xue et al.77 investigated a cohort of 116 NSCLC patients, studying the relationship between expression of miR-206/14-3-3z and prognosis. They disclosed that the miR-206 high/14-3-3z low group had the longest survival compared with the miR-206 low/14-3-3z high group through Kaplan-Meier survival analysis.66 In addition, LAC patients were divided into solid subtype positive and negative groups, and the solid subtype positive group had poorer prognosis than the negative one, while miR-133b was dramatically decreased in the solid subtype positive group.78 However, miR-133b was also found to positively associate with lung cancer patients’ overall survival.79 These findings suggested miR-133b may be beneficial for lung cancer patients’ prognosis.

This section mainly generalizes the role of the miR-206/133b cluster in lung cancer treatment and prognosis. We assume that these miRNAs may facilitate drug-resistant lung cancer cells in becoming resensitive, as well as contribute to better prognosis and survival in lung cancer.

Others
Long non-coding RNAs (lncRNAs) have been reported to play pivotal roles in lung cancer progression.74,75 Furthermore, IncRNAs are able to function as competing endogenous RNAs. For instance, the long non-coding RNA NEAT1 promoted lung cancer development by blocking miR-377-3p, resulting in the de-repression of its endogenous target E2F3.76 RMRP, a member of the IncRNAs, was found to inhibit expression of miR-206 and elevate the level of SOX9, FMNL2, and Kirsten rat sarcoma viral oncogene (KRAS) in lung cancer.77 Further experiments confirmed this IncRNA played an oncogenic function by targeting miR-206 and promoting expression of
SOX9, FMNL2, and KRAS. The crosstalk between IncRNAs and miRNAs may be further explored and contribute to uncover the unrecognized fields of cancer.

Cui et al. discovered eight so-called hub genes (HSPD1, POLA1, SMARCA4, ENO1, HSPA5, CDC42, CTSD, and CALR) were underlying functional targets of miR-206 in the A549 cell line via quantitative proteomics and protein network analysis. Nevertheless, the definitive function and link between these genes and miR-206 has not been investigated in lung cancer. Until now, it has not been reported that the miR-206/133b cluster plays a part in circulating miRNAs in lung cancer patients. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a critical component of tobacco, could be a high risk for lung carcinogenesis. In NNK-induced rat lung tumor experiments, both miR-206 and miR-133b were found to be upregulated in the rats’ serum in the early phase of NNK-induced lung tumorigenesis. However, serum miR-206 and miR-133b were remarkably decreased in a late-stage study, which corresponded with the result in NNK-induced rat lung tumors. If these findings are convincing and scientific, we hypothesize that rats antagonize NNK-induced microenvironmental alteration and strike a balance, giving rise to lung cancer suppressor-associated miRNAs that elevated early. Nonetheless, after tumor formation, some mechanisms and factors have been repressed or impaired, and then these miRNAs appear to be downregulated. Further investigations are required to determine whether miR-133b and miR-206 are diagnosed biomarkers in lung cancer patients.

Conclusions
In this review, we summarized the roles and mechanisms of the miR-206/133b cluster in lung cancer. We discovered miR-206 and miR-133b could promote cell apoptosis, repress cell proliferation, block tumor angiogenesis, and inhibit cell migration and invasion in lung cancer. Moreover, we uncovered that these miRNAs boosted drug-resistant and radioresistant lung cancer cells to be resensitive, and upregulation of these miRNAs might be beneficial for lung cancer patients’ prognosis and survival. The mechanisms were sophisticated, including MET, Smad3, EGFR, and 14-3-3z-associated signaling pathways. Altogether, these findings indicated the miR-206/133b cluster acted as a suppressor in lung cancer by targeting diverse genes and related molecular pathways, which might provide evidence for clinical applications and further study.

AUTHOR CONTRIBUTIONS
J.-Y.P. was responsible for conceiving, designing, and writing part of the project, while C.-C.S., Z.-Y.B., and Z.-L.C. conceived, designed, and wrote the remainder of the project, in addition to checking and modifying the manuscript. S.-J.L., Q.-Q.L., Y.-X.W., and Y.-Y.B. proposed some independent ideas to improve the manuscript. D.-J.L. and C.-C.S. are the principals of our research group and the corresponding authors.

CONFLICTS OF INTEREST
The authors disclose no potential conflicts of interest.

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