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REVIEW ARTICLE

The role of microRNAs in resistance to targeted treatments of non-small cell lung cancer

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Abstract
Purpose Non-small cell lung cancer (NSCLC), accounting for the most of lung cancers, is usually diagnosed in advanced stage. Targeted treatments boost advanced NSCLC patients with certain mutations, but early drug resistance blocks the advantages of target medicine. MicroRNAs (miRNAs) are regarded as a cluster of small noncoding and posttranscriptionally negative regulating RNAs. We want to explore the role of miRNAs in resistance to targeted treatments of NSCLC to improve the prognosis.
Methods We reviewed recent studies about miRNAs and targeted treatment resistance in NSCLC and classified resistance into two types: EGFR-TKIs resistance and ALK-TKIs resistance.
Results and conclusion Recent studies indicate that miRNAs involve in drug resistance possession in positive and negative manners. Inhibiting expression of certain miRNAs that promote drug resistance and increasing expression of miRNAs that reverse drug resistance may illuminate novel prospect of adjuvant targeted treatments in NSCLC.

Keywords MicroRNAs · Targeted treatment · Drug resistance · NSCLC · EGFR

Introduction

MicroRNAs (miRNAs) are small noncoding single-stranded RNAs (~22 nt), which posttranscriptionally regulate gene expressions via binding to the 3′-UTR (3′-untranslated region) of target messenger RNA (mRNA) [1]. A single 3′-UTR of mRNA may interact with numerous miRNAs; contemporarily, one miRNA is likely to target multiple mRNAs. Thus, miRNAs with their targets constitute an important and complex network in bioinformation [1]. Recent years, miRNAs have been demonstrated momentous effects in tumor progression. Intriguingly, miRNAs involve in occurrence of drug resistance in many cancers, elucidating a new orientation of adjuvant therapy for cancer.

Lung cancer is one of the leading causes of cancer death whether in economically developed or developing countries, both in male and in female [2]. Non-small cell lung cancer (NSCLC) accounts for around about 85 % in lung cancers. Adjuvant therapy plays an important role in the treatment of NSCLC, especially in advanced stage. For patients with anaplastic lymphoma kinase (*ALK*) or epidermal growth factor receptor (*EGFR*) mutations, targeted medicine is first recommended whether surgery or not. Since targeted treatments extend the median survival time of patients, drug resistance tremendously limits its efficacy.

Therefore, this review aims to decipher miRNAs' functions in drug resistance in NSCLC to improve targeted therapeutics.

EGFR-TKIs resistance

EGFR-TKIs (tyrosine kinase inhibitors) are recommended in advanced NSCLC patients with *EGFR* mutations. *EGFR* mutations occur around 10–16 % in NSCLC patients in Spanish population [3]. Deletions of exon 19 (Del19) and the exon 21 L858R point mutation cover 85–90 % in *EGFR* mutations [3]. Gefitinib, erlotinib and afatinib are first-line medicine for patients with *EGFR* mutations. But the

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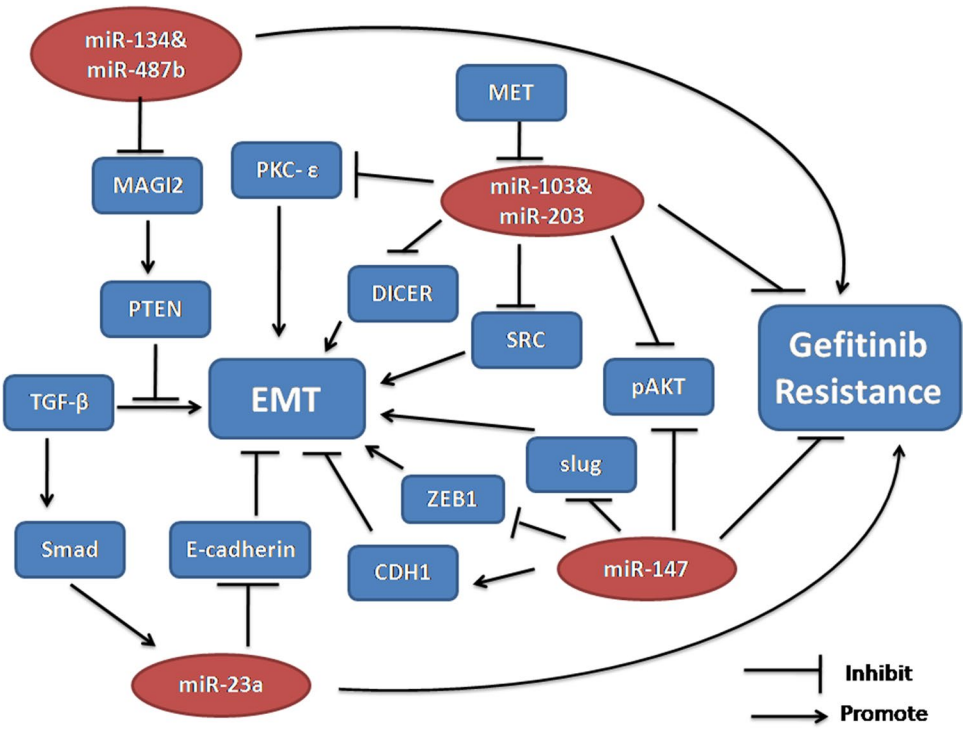

Fig. 1 miRNAs involved in EMT and gefitinib resistance in NSCLC

dilemma of secondary drug resistance tremendously weakens its utility. Interesting, expressions profiles of miRNAs are dissimilar between EGFR-TKIs-sensitive and EGFR-TKIs-resistant cell lines or tissues. And further investigations confirm the significance of discrepant expression.

Gefitinib resistance

Although epithelial-to-mesenchymal transition (EMT) is discovered as a mechanism related to metastasis [4, 5], EMT has recently been found as dispensable for metastasis but contributes to drug resistance in several carcinomas such as pancreatic cancer [6], lung cancer [7]. EMT, as the name suggests, signifies reversion of epithelial phenotype and acquisition of mesenchymal characteristics, accompanied with a biological progression consisting of reversible events [8]. Experiments have shown that transforming growth factor-β1 (TGF-β1) could induce EMT phenotype in vitro and in vivo [9]. In NSCLC, miR-134 and miR-487b, induced by TGF-β1, attribute TGF-β1 to induce EMT through inhibiting *MAGI2* which mediates PTEN instability by phosphorylating PTEN [10]. Similarly, another miRNA induced by TGF-β1 via activating smad, miR-23a, facilitates EMT through inhibiting E-cadherin [11]. In addition, miR-134, miR-487b and miR-23a promote gefitinib resistance [10, 11]. MiR-147, regulating cell invasion and proliferation, could reverse TGF-β1-induced EMT and gefitinib resistance, and repress Akt phosphorylation, while the relationship between biological functions remains unknown [12]. MiR-103 and miR-203, inhibited by MET (the receptor tyrosine kinase for hepatocyte growth factors), block EMT via obstructing *PKC-ε*, *DICER*, *SRC*, inhibit AKT phosphorylation and reverse gefitinib resistance [13]. In conclusion, as shown in Fig. 1, there is a positive correlation between EMT and gefitinib resistance with biological behaviors of miRNAs, which needs further investigations.

The PI3K/AKT/mTOR signal pathway, involving in cell proliferation, survival, differentiation, adhesion, invasion and motility, is an important transduction pathway [14]. Furthermore, activation of PI3K/AKT/mTOR pathway is frequently discovered in NSCLC, related to poor prognosis [15, 16]. As an oncogenic miRNA, miR-21 promotes cell proliferation and suppresses apoptosis in solid tumors like tongue squamous cell carcinomas, pancreatic cancer, breast cancer and lung cancer [17,-22]. In NSCLC, miR-21 could generate gefitinib resistance via activating ALK and ERK and suppressing PTEN [13, 21, 22]. MiR-34a could reverse gefitinib resistance via inhibiting MET phosphorylation which contributes to PI3K/AKT activation [23]. As Fig. 2 shows, miR-21 and miR-34a have opposite roles in gefitinib resistance via the same AKT pathway [13, 21–23].

Around 30 % NSCLC patients with *EGFR* mutations exhibit de novo resistance to EGFR-TKIs treatment. Recent studies have shown that Met activation may participate in this phenomenon [24–26]. MiR-30b and miR-30c, increased by EGFR and MET, promote gefitinib resistance via inhibiting *BIM* [13]. Similar to MiR-30b and MiR-30c, MiR-221 and MiR-222, also increased by EGFR and MET,

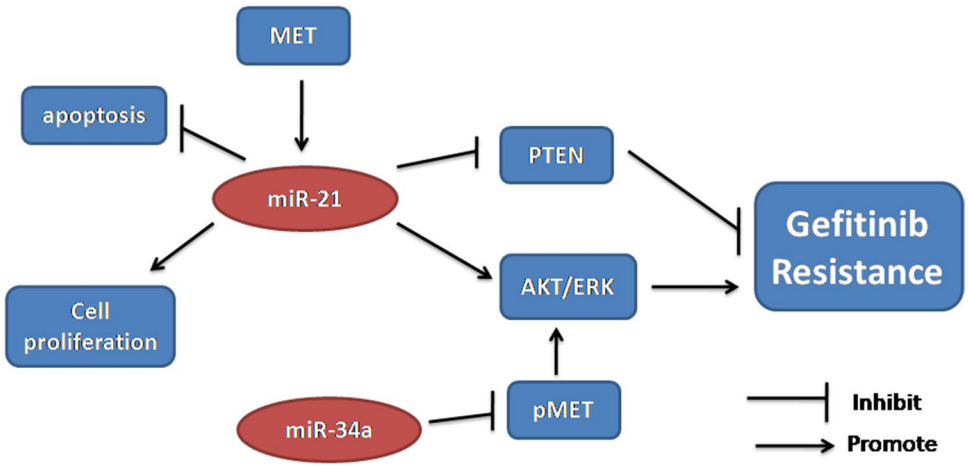

Fig. 2 miRNAs involved in PI3 K/AKT/mTOR signal pathway and gefitinib resistance in NSCLC

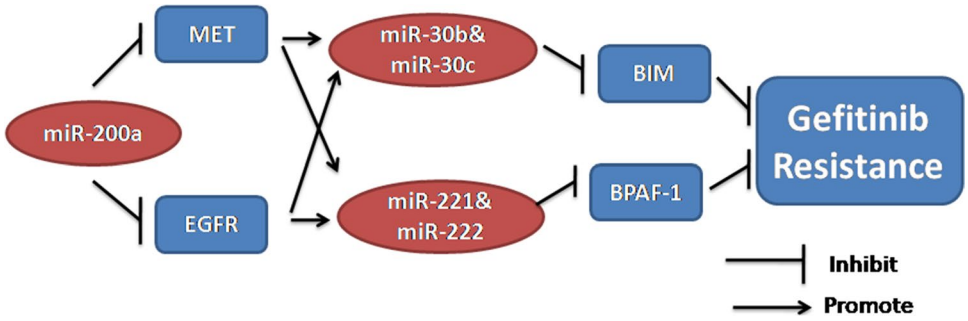

Fig. 3 miRNAs involved in MET, EGFR and gefitinib resistance in NSCLC

promote gefitinib resistance via inhibiting *APAF-1* [13]. On the other hand, miR-200a reverses gefitinib resistance through inactivating *EGFR* and *c-MET* [27]. As shown in Fig. 3, MET and EGFR participate in gefitinib resistance via miR-200a, miR-30b, miR-30c, miR-221 and miR-222 [13, 27].

MiR-7 and miR-138-5p reverse gefitinib resistance in NSCLC in vitro, while the mechanism needs further researches [28, 29].

miRNAs related to EGFR-TKIs resistance mentioned before are summarized in Table 1.

ALK-TKIs resistance

Approximately 4 % of NSCLC patients are discovered anaplastic lymphoma kinase (*ALK*) rearrangements, which is also called *ALK* mutations [30]. Crizotinib, as an oral small-molecule tyrosine kinase inhibitor to *ALK*, is recommend in advanced NSCLC patients with *ALK* mutations [3]. To a certain extent, crizotinib is superior to traditional chemotherapy in *ALK*-positive NSCLC according to researches [31]. Unfortunately, similar to gefitinib, acquired resistance could be later developed in *ALK*-positive patients. Several resistant mechanisms, such as C1156Y, L1196M, G1269A, G1202R, S1206Y and 1152 threonine insertion,

Table 1 miRNAs involved in response/resistance to EGFR targeted therapies of NSCLC

MicroRNAs	−/+[a]	Targets/pathways	References
miR-134 and miR-487b	−	*MAGI2*, TGFβ-EMT	[10]
miR-23a	−	E-cadherin, TGFβ-EMT	[11
miR-147	+	*CDH1*, *ZEB1*, *Slug*	[12]
miR-103 and miR-203	+	*PKC-ε*, *DICER*, *SRC*, pAKT	[13]
miR-21	−	PTEN, PI3K/AKT	[13, 21, 22]
miR-34a	+	PI3K/AKT, pMET	[23]
miR-30b and miR-30c	−	*BIM*	[13]
miR-221 and MiR-222	−	*BPAF-1*	[13]
miR-200a	+	*EGFR*, *c-MET*	[27]
miR-7	+	unknown	[28]
miR-138-5p	+	unknown	[29]

[a] −: Promote gefitinib resistance. +: reverse gefitinib resistance

are reported [32–34]. Several microRNAs are found involving in *ALK*-positive tumor cells progression. MiR-16, miR-29a and miR-135b play a role in promoting *ALK*-positive anaplastic large cell lymphoma (ALCL) tumor cells, while miR-101 decreases the proliferation of *ALK*-positive ALCL cells [35–38]. Nevertheless, the relationship

between crizotinib resistance and microRNAs in NSCLC is not reported before. Though miR-150 enhances antineoplastic effects of crizotinib in crizotinib-resistant osteosarcoma cells [39], does it function in NSCLC needs further investigation.

In conclusion, *ALK* rearrangements are found in many tumors, such as ALCL, NSCLC and inflammatory myofibroblastic tumor (IMT). To date, the investigation of microRNAs and crizotinib resistance in NSCLC seems vacant. There are needs to decipher the roles of microRNAs in crizotinib resistance to improve targeted treatment of *ALK*-positive NSCLC patients.

Discussion

The complex relationships between deregulation of microRNAs and progression of NSCLC illustrate brave potentials of targeting or utilizing miRNAs to perfect the molecular target therapy. But targeted therapy faces the difficulty of secondary resistance which usually occurs early. What is more, microRNAs, as novel biomarkers, are associated with driver mutations, and miRNAs are better reserved in formalin-fixed paraffin-embedded (FFPE) than mRNAs, which is in favor of clinical applications [40].

As previously mentioned, EMT contributes to drug resistance, rather than metastasis [7]. MicroRNAs (miR-23, mir-103, miR-134, miR-147, miR-203 and miR-478b) show positive correlations between functions on gefitinib resistance and capabilities on EMT phenotype [10, 11, 13]. Whether these microRNAs regulate gefitinib resistance via or partially via targeting EMT mechanism remains unknown. For another, the PI3K/AKT/mTOR signal pathway could be the targets of certain microRNAs to impact gefitinib resistance, such as miR-21 and miR-34a [13, 21–23]. Intriguingly, miR-21 plays an oncogenic role in many solid tumors not only in NSCLC, but also in tongue cancer, pancreatic cancer and breast cancer [17–22]. It indicates that miR-21 may be a comprehensive and vital therapy orientation. Not surprising, EGFR and MET involve in gefitinib acquired resistance via miR-30b, miR-30c, miR-103, miR-200a, miR-203, miR-221 and MiR-222 [13, 27].

Disappointing, few studies have addressed ALK-TKIs resistance in *ALK*-positive cancers, especially about correlation between microRNAs and ALK-TKIs resistance. Only one microRNA, miR-150, partly reverses crizotinib resistance in drug-resistant osteosarcoma cells [39]. Further microRNAs investigations may provide a promising future for ALK-TKIs secondary resistant NSCLC patients.

In summary, inhibiting those microRNAs that induce target medicine resistance and indulging those microRNAs that reverse the resistance could restore utilization of target medicine in patients, that is to say, to prolong patients' lives even to cure them. Therefore, besides exploring microRNAs, how to establish a stable and harmless microRNAs expression system is equally important.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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