REVIEW

MicroRNA-135b as therapeutic target in cancers

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MicroRNAs (miRNAs) are small non-coding RNAs that can negatively regulate gene expression at the post-transcriptional level through the RNA-induced silencing complex (RISC)-mediated inhibition. Because of the imperfect and short seed-binding region of the target sequences, miRNAs hold capacity for multi-targeting and are able to regulate a wide range of cellular functions and signaling. Numerous researches have revealed that dysregulated miRNAs are closely associated with cancer progression. Moreover, genome-wide screening shows that the expression profile of miRNAs can serve as biomarkers for early diagnosis, stratifying patient outcome, and predicting treatment efficiency for cancer patients. Hence, seeking and dissecting the detailed mechanisms of cancer-associated miRNA may provide a new avenue for cancer targeting therapy. This review discussed the current proposed mechanisms of miR-135b involvement in cancer progression and tissue differentiation, both of which are considered as functional equivalents. The regulatory network of miR-135b are also addressed to further clarify the potential oncogenic role of miR-135b.

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The role of miR-135b in lung cancer progression

Several lung cancer-associated miRNAs have recently been identified. Among them, miR-135b antisense stands out for its therapeutic potential or its oncogenic role [1-3]. An up-regulated miR-135b is highly associated with poor overall survival in non-small cell lung cancer (NSCLC). Conversely, LZTS1 and LATS2, the downstream targets of miR-135b, are associated with better overall survival of patients with NSLCL when their proteins show stronger expression [1]. The ectopic expression of miR-135b has also been shown to promote cancer cell invasion, migration, and anchorage-independent growth *in vitro* and *in vivo*. The oncogenic mechanism is via the suppression of LZTS1 and multiple components of the Hippo pathway. Moreover, miR-135b antagonists not only efficiently lessens tumor burden but also reduces lung metastatic capability in a

xenograft model (Fig. 1).

The various oncogenic functions of miR-135b may be attributed to its multi-targeting ability, which involves different mechanisms during tumor progression. However, the oncogenic roles of miR-135b on cancer stemness and drug-resistance are yet to be clarified.

The role of miR-135b in mesenchymal stem cell development

During neuro-ectodermal (NE) development, TGF-β/BMP signaling is important in maintaining the pluripotency and self-renewal ability in human embryonic stem cells. The TGF-β/ BMP signaling can be suppressed by miR-135b [4]. In addition, PAX6 is a critical protein for human NE cell fate determinant that can up-regulate miR-135b during NE development. The miR-135b
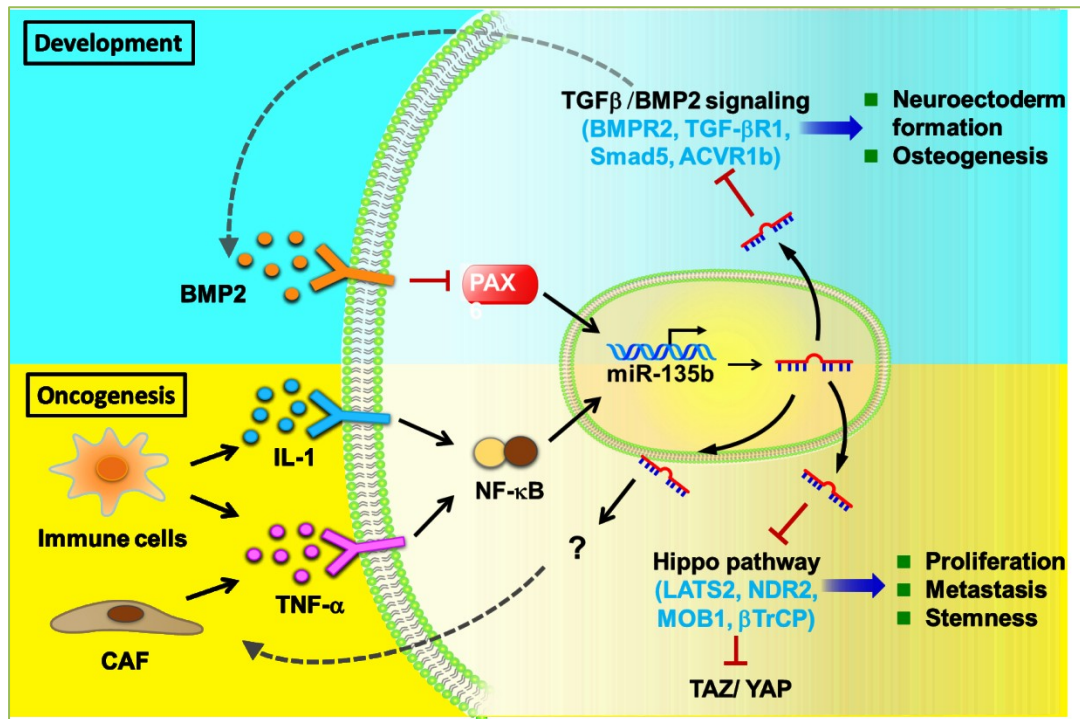
Figure 1. The molecular network miR-135b regulated in tissue development and the progression of cancer.

subsequently suppresses the mRNAs of BMPR2, TGF-βR1, SMAD5, and ACVR1b, by restricting the differentiating direction of human embryonic stem cells (hESC) [4].

The miR-135b is also engaged in the fate-determination of mesenchymal cells by involving the differentiation lineages of muscles and bones [5, 6]. The osteoblastogenesis of pre-myoblast mesenchymal cells is controlled by the potent osteogenic morphogene, bone morphogenetic protein-2 (BMP2) [6]. At the same time, BMP-2 can downregulate miR-135b during the BMP2-mediated C2C12 osteogenic differentiation, while miR-133, a myogenesis-promoting miRNA, is reduced, thereby restricting C2Cl2 to osteogenesis [7]. Evidence suggests that in the presence of miR-135 and miR-133, mesenchymal stem cells undergo myogenesis instead of osteogenesis.

Similarly, miR-135b expressions is the most severely downregulated one among the investigated 157 miRNAs during the osteogenesis of unrestricted somatic stem cells (USSCs) [8]. These results depict the fact that the timing and place of miR-135b are properly controlled by tissue- or organ-specific factors, which in turn may adjust and coordinate the downsteam network and regulate mesenchymal stem cell differentiation.

For the same reason, the inappropriate expression of miR-135b may obstruct cell lineage commitment. For example, multiple myeloma (MM) is composed of uncontrolled and proliferative plasma cells in bone marrow, with a hallmark of osteoblast inhibition and osteoclast activation within its micro-environment niche. Bone marrow-derived mesenchymal stem cells (BM-MSCs) isolated from patients with MM have significantly higher miR-135b expression as compared to those from normal donors [9, 10]. In addition, miR-135b inhibitors can restore the impaired osteogenic phenomenon by relieving the miR-135b/smad5 pathway. Thus, miR-135b may be an alternative therapeutic target for defects in MSC osteogenic differentiation [7, 10].

The expression level of miR-135b is tightly regulated by tissue- specific micro-environment niches during the MSC fate-determination (Fig. 1). The MSCs are capable of differentiating into osteoblasts, adipocytes, chondrocytes, myoblasts, and non-mesenchymal tissues like neuro-ectodermal cells and hepatocytes [11-14]. The miR-135b-mediated circuitry networks are distinct and fluctuated during diverse tissue-differentiation lineages, especially on neuro-ectodermal cells and osteoblasts. The up-regulation of miR-135b by PAX6 in hESC or MSCs helps neural cell fate determination by suppressing several components of the BMP and TGF-β pathways.

On the other hand, miR-135b is suppressed by BMP2 during osteogenic cell commitment. Thus, a reciprocal negative feedback loop is formed between miR-135b and the BMP signaling pathway. This may restrict the divergent gene

expression profile within distinct stem cell lineages (Fig. 1).

Aberrant expression of miR-135b in cancers

Aside from controlling the development of normal embryonic stem cells, aberrant miR-135b expression has been observed in various types of cancers, including colon cancer, prostate cancer, pancreatic ductal adenocarcinoma, anaplastic large-cell lymphomas (ALCLs), mammary carcinomas, and lung cancer [15-20]. A vast number of researches also reveal that miR-135b is up-regulated not only in the tumor part of colorectal cancer but also in fecal samples [21-24]. Furthermore, the expression of stool or tumoral miR-135b is positively correlated with stages of colorectal cancer lesions [15, 21, 22]. These observations support the oncogenic role of miR-135b on cancer progression such that it may serve as a biomarker for cancer.

Role of miR-135b in lung cancer and tumor-microenvironment

The pulmonary epithelial surface is the first line of defense against environmental pollutants and antigens, and contains numerous defensive receptors and secretory cytokines. This immune-pulmonary cell microenvironment is the foundation of the pulmonary innate and/or adaptive immune systems [25]. Recently, a research group has reported a series of results showing the dominant up-regulation of miR-135b in lung tissues upon exposure to cigarette smoke and nano-particles in mice models [26-28]. The activated miR-135b is mediated through the IL-1a/IL-1R pathway, which consequently triggers either the acute or chronic pulmonary inflammation signaling cascade [26-28].

The IL-1/IL-1R super family can recruit several cellular effectors via MyD88, which is connect to the central immuno-hub, the NF-κB signaling [29]. The promoter region of miR-135b as well as its host gene, LEMD1, have been identified as putative NF-κB binding sites. In addition, non-small cell lung cancer (NSCLC) cells treated with TNFκ, a progenitor for provoking NF-κB signaling, reveal signs of NF-κB/ p65 binding on the miR-135b promoter. Moreover, the enhanced NF-κB binding induces miR-135b expression, suggesting an extracellular inflammatory signal trigger for cellular miR-135b via NF-κB cue, leading to the downstream cascade for responding to or resolving extracellular stimulation [18].

Although the role of inflammation in cancer progression is now well accepted, the detailed mechanisms involved remain unclear. NF-κB is the most well-studied pro-inflammatory transcription factor in the recent decade, famous with being a central bridge between inflammation and cancers [30].

Likewise, Matsuyama *et al.* have demonstrated the activation of a proto-oncogene, named Signal Transducer and Activator of Transcription 3 (STAT3), which intrinsically coalesce with NF-κB to form an activating positive feed-forward loop. This signal goes together with IL-6, maintaining the inflammatory-tumor microenvironment in cancers [31, 32]. Together with the aforementioned observations, both of NF-κB and STAT3 transcriptionally promote the miR-135b expression [1, 31, 32]. The expression of miR-135b may functionally provide a clue that may help uncover how inflammatory factors such as NK-κB and STAT3 play decisive roles in multiple aspects of tumor progression. Thus, targeting miR-135b within the dysplastic tissues may be helpful in disrupting the cancer-inflammatory micro-environment.

The miR-135b regulates Hippo pathway and downstream effector TAZ/YAP and promotes cancer stemness

MiR-135b targets multiple Hippo components by directly down-regulating the proteins levels of LATS2, NDR2, MOB1b, and β-TrCP, and subsequently activating nuclear TAZ in NSCLC. Increased nuclear TAZ is associated with poor survival [1]. In some cases, TAZ and YAP are not controlled by the core-Hippo axis, especially after the successive discovery of the novel components of Hippo branches [33, 34]. However, the TAZ proteins eventually go through β-TrCP-mediated degradation [35], suggesting that miR-135b can promote TAZ activity not only through suppressing the Hippo-pathway but also prevent degradation by β-TrCP. Thus, miR-135b potentially leads the limitless growth of tumors by stabilizing the TAZ/YAP protein via regulation of a variety of different targets.

Emerging studies reveal that activated TAZ endow breast cancer stem cells (CSCs) with a self-renewal capacity, which provides metastatic and chemo-resistant activities [36, 37]. Furthermore, the loss of Hippo-related proteins, which belong to YAZ/YAP upstream negative regulators, results in uncontrolled tissue growth and increased population of cancer stem-like cells [38, 39]. These findings suggest that the uncontrolled activation of TAZ and YAP is involved in regulating the population of cancer progenitor cells.

The miR-135b as a therapeutic target for cancer and future prospectives

Gene-based targeting approaches that specifically modulate disease-related gene expression for disease-treatment has robustly been investigated worldwide [40]. Since one miRNA in charge for a specific function or mechanism targets several downstream genes, it will be a remarkable smart way for disease-targeting therapy. For

instance, miR-135b inhibits tumor growth and metastasis by suppressing multiple tumor suppressors and pathways, including the Hippo pathway, LZTS1, APC, FOXO1, DAPK, as well as MTSS-1 (metastasis suppressor-1) [18, 22, 24, 31]. In addition, using miR-135b inhibitors, several researchers have shown that silencing of miR-135b mitigates tumor burden, relieves lung metastatic nodules, and even rescues APC-knockout caused phenotypes [18, 22].

Furthermore, miR-135b displays immuno-modulatory ability by cross-talking with pro-inflammatory cytokines and T cells in anaplastic large-cell lymphoma (ALCL) and in lung cancers [26, 31]. Thus, miR-135 inhibition may further block the communication between tumor cells and its surrounding cancer niche.

The generation of cancer is a complex, multi-step process that includes numerous aberrant gene expressions and dysregulation. Hence, the malformed gene networks are generated. These, in turn, allow cancer cells to gain properties like proliferation, motility, stemness, and drug resistance. The heterogeneity of tumor microenvironment governs an auto-feedback signal that nourishes the malignancy of dysplastic cells. These dysplastic cells further cross-talk with tumor-associated lymphoid cells by reciprocally secreting numerous growth factors and cytokines [41]. Whether the elevated miR-135b serves as a hub in cancer cells and the surroundings, which reconstructs the microenvironment of the neoplastic area by fine-tuning the regulatory network in oncogenic process, warrants further investigation.

Likewise, whether the expression of miR-135b enriches cancer-initiating properties and enhances drug-resistance in cells are important. Thus, targeting miR-135b may be a promising approach for disrupting the communication between tumor cells and its surroundings but also for impairing the oncogenic abilities of cancer cells. For these reasons, dissecting the detailed mechanism of miR-135b involvement in oncogenesis is necessary and targeting miR-135b using antagonists or antisense oligomer may have great potential for lung cancer therapy.

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