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The role of miRNAs in alveolar epithelial cells in emphysema

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Abstract

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease becoming one of the leading causes of mortality and morbidity globally. The significant risk factors for COPD are exposure to harmful particles such as cigarette smoke, biomass smoke, and air pollution. Pulmonary emphysema belongs to COPD and is characterized by a unique alveolar destruction pattern resulting in marked airspace enlargement. Alveolar type II (ATII) cells have stem cell potential; they proliferate and differentiate to alveolar type I cells to restore the epithelium after damage. Oxidative stress causes premature cell senescence that can contribute to emphysema development. MiRNAs regulate gene expression, are essential for maintaining ATII cell homeostasis, and their dysregulation contributes to this disease development. They also serve as biomarkers of lung diseases and potential therapeutics. In this review, we summarize recent findings on miRNAs' role in alveolar epithelial cells in emphysema.

Keywords

Emphysema; Alveolar epithelial cells; MiRNA; Biomarkers

1. Introduction

The mammalian distal lung is a structurally complex and highly dynamic organ [1]. The alveoli are the lung parenchyma's hollow structures, serve as the gas exchange site, and function as an essential barrier to protect tissue from environmental insults. The alveolar compartment is lined with two types of alveolar epithelial cells, the alveolar type II (ATII)

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Conflict of interest statement

None.

and alveolar type I (ATI) cells. ATII cells synthesize and secrete pulmonary surfactants. They also have a stem cell potential and differentiate into ATI cells, forming the blood-air barrier's epithelial component [2]. Chronic exposure to various environmental insults such as cigarette smoke induces pro-inflammatory response and oxidative stress in the lung, leading to alveolar epithelial cells apoptosis, which can contribute to emphysema development [3]. Exposure to cigarette smoke induces senescence in ATII cells isolated from human and murine lungs [4]. This emphasizes their critical role in tissue homeostasis and repair after injury. Emphysema belongs to chronic obstructive pulmonary disease (COPD), and it is defined as an airspace enlargement of the lung following rupture of the alveolar walls. It most frequently develops in people > 45 years old because of smoking [5]. Lung transplantation, lung volume reduction surgery, and endoscopic lung volume reduction are the options for patients with emphysema [6]. Understanding COPD's pathology and molecular mechanisms can lead to identifying novel potential therapeutic targets. This will result in approaches to slow down or reverse the disease development and promote lung regeneration [7,8].

MicroRNAs (miRNAs) are small regulatory RNA genes transcribed from DNA that are not translated. It was estimated that 3–4% of human genes encode miRNA [9]. MiRNAs target mRNA degradation and suppression of protein translation based on sequence complementarity between the miRNA and its targeted mRNA 3'UTR. However, the involvement of miRNAs in gene expression activation was also reported. A cumulating number of reports show a pivotal role of miRNAs in COPD [10]. Also, miRNA-based therapies may prevent and decrease alveolar epithelial cell injury; however, they are still under development for this disease.

2. Alveolar epithelial cells

ATII cells, also called type II pneumocytes, comprise 14% of all lung cells and cover 5% of the lung's internal surface area [11]. They are smaller than ATI cells and harbor a large nucleus. ATII cells are squamous and have a cuboidal shape with an apical surface area of about 250 μm^2 per cell, composed of distinct microvilli. ATII cells provide many components required for the unique microenvironment in the alveolus. They have lamellar bodies (LBs) and secrete pulmonary surfactant, a lipoprotein substance necessary for proper lung function, preventing alveolar collapse by reducing surface tension at the end of expiration and exerting protective, antimicrobial properties [12]. ATII cells synthesize and release surfactant proteins (SP) A, B, C, and D [13]. SP-A plays a crucial role in pathogen clearance and inflammatory responses and stimulates phospholipids' uptake by ATII cells. SP-B is important in LBs biogenesis. SP-C is expressed exclusively by ATII cells, facilitates lipid movement between sheets of membrane and vesicles, and functions along with SP-B to promote surfactant film formation. SP-C also stimulates the reuptake of surfactant phospholipids by ATII cells like SP-A and plays a role in surfactant recycling [14]. SP-D affects the physical structure of phospholipid aggregates in surfactant and their uptake and catabolism by ATII cells. Deletion of *Sp-D* in mice resulted in the pulmonary accumulation of phospholipids and increased alveolar surfactant phospholipid pools. Also, *Sp-D* deletion was associated with increased numbers of activated macrophages, enlarged

distal airspaces, inflammation, and increased lung volumes consistent with emphysema histologic and morphometric features [15].

ATII cells act as progenitor cells in the alveoli, proliferate, and differentiate into ATI cells (called type I pneumocytes), which comprise 8% of the peripheral lung cells [11]. ATI cells cover approximately 95% of the alveolar surface. They are thin and flat with a diameter between 50 and 100 μm , thickness between 0.1 and 0.2 μm , a small nucleus, and an overall cytoplasmic extension around 2300 μm in humans. They reside near the capillary bed and form the air-blood barrier through which gases diffuse and ensure efficient oxygen and carbon dioxide exchange between alveolar spaces and capillaries [16,17]. Also, ATI cells participate in ion and fluid transport, express both the $\alpha 1$ and $\alpha 2$ Na,K-ATPase isoforms, and are capable of active Na^+ transport and fluid reabsorption [18]. We have shown that ATI cells are more susceptible to damage induced by environmental factors than ATII cells [19,20]. Notably, the stemness ability of ATII cells is more activated when ATI cells are injured, which contributes to alveolar renewal and restoration of epithelial integrity [21,22]. Various signaling pathways mediating ATII cell differentiation to ATI cells were identified. WNT/ β -catenin, NOTCH, yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ), bone morphogenetic protein (BMP), and transforming growth factor- β (TGF β) are among these pathways [23]. Therefore, ATII cells play a critical role in the distal lung epithelium and regeneration after injury, and their dysfunction contributes to the pathogenesis of various parenchymal lung diseases, including emphysema [22].

3. Emphysema pathophysiology

Emphysema belongs to chronic obstructive pulmonary disease (COPD) [24]. COPD is a significant contributor to morbidity and mortality worldwide and is the third leading cause of death, with 3.23 million deaths in 2019. About 80% of these deaths occur in low- and middle-income countries [25]. It is often underdiagnosed, with only 10–15% of all cases identified medically, and it rises sharply in people aged > 45 years [5,26]. Without effective prevention, COPD-related deaths would elevate by over 30% in the near few decades. Emphysema consists of a unique alveolar wall damage pattern resulting in marked airspace enlargement. This reduction in the lung surface area traps air in the damaged tissue and prevents oxygen from moving through the bloodstream [27–29]. Emphysema is characterized by tissue destruction, hyperinflation, and ventilation-perfusion mismatch.

Cigarette smoking and secondhand smoking are the key cause of the vast majority of emphysema cases [30,31]. Chronic exposure to cigarette smoke's toxic and oxidant components causes alveolar wall damage due to the generation of reactive oxygen and nitrogen species, free radicals, and unsaturated aldehydes, which alter DNA, lipids, and proteins in alveolar epithelial cells. Genetic predisposition, age, and occupational exposures are important risk factors of emphysema development [32]. This disease can also be caused by the deficiency of an essential anti-protease in the lung, the $\alpha 1$ -antitrypsin (AAT). This protease inhibitor plays a crucial role in modulating key immune cell activities and protecting the lung against damage caused by inflammation [33]. In a recent study, Janosz et al. investigated the pulmonary transplantation of macrophages transgenic for AAT in the

murine lungs [34]. Also, they confirmed the expression and secretion of AAT in human macrophages. Such approaches have promising therapeutic benefits for AAT deficiency.

At the cellular level, the emphysematous lung is characterized by increased numbers of alveolar macrophages, neutrophils, cytotoxic CD8+ T-lymphocytes, and the release of multiple inflammatory mediators such as lipids, chemokines, and cytokines [14,21]. The imbalance of the ongoing inflammation with activated macrophages and neutrophils contributes to reactive oxygen species (ROS) generation leading to oxidative stress. Also, the presence of damaged proteins, peroxidized lipids, and protease/anti-protease imbalance causes alveolar epithelial cell apoptosis and this disease progression [28, 35–37]. We previously showed high oxidative stress, 4-HNE, IL-8, and IL-6 levels and apoptosis in ATII cells obtained from heavy smokers [3,38]. Moreover, ATII cells are abundant in mitochondria, and we have recently reported that dysfunction of these organelles contributes to emphysema pathogenesis [3]. Together, cellular and molecular alterations lead to a matrix and tissue degradation, impeding the ATII cells' ability to serve as progenitor cells to repopulate the injured epithelium and contributing to this disease development [27,29,39].

4. MiRNAs as biomarkers

MicroRNAs (miRNAs) are small non-coding RNAs of 19–25 nucleotides long with no ability to be translated into protein [40]. They bind to the 3'UTR or the target mRNAs' coding region and regulate their expression post-transcriptionally through RNA degradation or translation inhibition. Therefore miRNAs are critical regulators of biological processes such as cell differentiation, proliferation, cell-cell interaction, oxidative stress, and cell death. They are essential for normal pulmonary development and maintaining lung homeostasis. Deregulation of miRNAs expression results in pathological conditions and a broad spectrum of diseases [41].

MiRNAs are mainly in the cell cytoplasm, where they are processed from precursor inactive pre-miRNAs into double-stranded mature miRNAs [42]. Only one strand of the miRNA is integrated into the RNA-induced silencing complex (RISC), which functions as gene expression regulators against its target mRNAs. Mature miRNAs exert their traditional biological function in the cytoplasm. However, recent studies have identified their localization in different cellular compartments, including exosomes, endoplasmic reticulum, nucleolus, and mitochondria. They regulate the expression of multiple target genes and signaling pathways involved in critical processes such as cell growth, proliferation, and apoptosis [43–45].

Exosomes represent a subpopulation of extracellular vesicles (EVs) released into the extracellular spaces [46]. They are present in various fluids such as blood, urine, sputum, or BAL (bronchoalveolar lavage). They have a crucial function in intercellular communication via transferring their content, including miRNAs, to target cells. About 10% of the circulating miRNAs are in exosomes [47]. The rest forms complexes with proteins such as argonaute 2 (AGO2), nucleophosmin 1 (NPM 1), or high-density lipoprotein (HDL). AGO2 is an essential catalytic component of the RISC which mediates canonical miRNA engagement with intracellular target transcripts. NPM 1 was the first protein identified as

crucial for miRNA transport and protects miRNA degradation by RNase. HDL enables the transport of lipids and fat-soluble vitamins through the bloodstream [48]. Studies reported miRNAs' implication as clinical biomarkers for COPD and emphysema [49,50]. Their role in lung inflammation and pathogenesis of idiopathic pulmonary fibrosis (IPF), acute lung injury (ALI), acute respiratory distress syndrome (ARDS), asthma, and lung cancer was also reported.

Exosomal miRNAs can be obtained from body fluids and used as potential non-invasive biomarkers to diagnose respiratory diseases. A recent study showed high exosomal miR-21 levels in sera of smokers and COPD patients [51]. Moreover, smoking altered EVs miRNA profile, including exosomes in the BAL fluid, compared to non-smokers [52,53]. The human bronchial epithelial BEAS-2B cell line exposed to various factors secreted exosomes containing miRNAs into the extracellular space [54,55]. Recently, Chen et al. found that exosomes derived from BEAS-2B cell treated with cigarette smoke extract lead to RAW264.7 macrophages polarization by increasing the percentages of CD86⁺, CD80⁺, CD163⁺, and CD206⁺ cells, but also the secretion of TNF- α , IL-6, iNOS, IL-10, Arg-1, and TGF- β [56]. Exosomes derived from lung epithelium may be used to develop biomarkers for diagnosing and treating cigarette smoke-related lung diseases. Lee et al. isolated a subpopulation of miRNA-enriched EVs' from BAL fluid using ultracentrifugation and sucrose gradient [53]. They were mainly derived from ATI cells, as indicated by caveolin-1 staining. This study provides a novel insight into underlying lung pathology mechanisms, identifying biomarkers, and developing novel therapeutic strategies. MiRNAs deregulation affects molecular pathways such as NOTCH, WNT, hypoxia-inducible factor-1A (HIF-1 α), transforming growth factor, KRAS, and SMAD. They are critical for cell fate determination, inflammation, cellular responses, and survival, suggesting their involvement in COPD pathogenesis. Indeed, miRNAs and exosomes have emerged among various biomarkers as new tools for diagnosis and therapeutic applications in this disease [57].

Microvesicles (MVs) are released from many cell types, including platelets [58], endothelial cells, erythrocytes, and leukocytes. Also, they have been found in blood, urine, synovial fluid, extracellular spaces of solid organs, atherosclerotic plaques, and tumors [59]. Alveolar macrophage-derived MVs can be isolated from murine BAL fluid [60]. Also, MVs obtained from BEAS-2B cells exposed to hyperoxia were studied. These miRNA-enriched epithelial MVs triggered macrophage-mediated lung inflammatory responses [61].

MiRNAs have crucial roles in regulating lung maturation and development, including miR-17, miR-20a, miR-106b, and miR-127 [62]. Increased SP-A, SP-B, and SP-C levels in the miR-26a KO mouse model were observed. MiR-26a overexpression in rat ATII cells inhibited pulmonary surfactant synthesis by reducing SMAD1-associated BMP signaling pathways, which is critical in lung development [63]. Another study demonstrated that SP-C's downregulation in human lung adenocarcinoma is miR-629-3p-mediated by direct binding to *SP-C* mRNA' 3'UTR [64].

5. Dysregulation of miRNAs by exposure to cigarette smoke

Studies have shown the correlation between miRNAs and respiratory dysfunction. The pathogenesis of cigarette smoke-induced lung injury is poorly understood, and this exposure is a risk factor for COPD development [65]. Nouws et al. found that miR-24-3p was decreased in parenchymal lung tissue samples obtained from patients with this disease and inversely correlated with its severity (Table 1) [66]. Its inhibition increased susceptibility of AII cells to apoptosis and emphysema development in cigarette smoke-exposed mice. Also, they detected that in primary human airway epithelial cells (HAECs) exposed to cigarette smoke extract, miR-24-3p decreased apoptosis through the BH3-only protein BIM inhibition, suppressed DNA repair protein BRCA1, and DNA damage response (DDR). Also, miR-24-3p inhibited homologous recombination (HR) but not mutagenic non-homologous end joining (NHEJ) DNA repair pathways. Degreasing cotton smoke reduced Let-7f-1-3p expression in human pulmonary alveolar epithelial cells (HPAEpiC) [67]. Let-7f-1-3p agomir attenuated smoke-induced human small airway epithelial cell (HSAEC) and HPAEpiC apoptosis by repressing the transcription factor Forkhead box-O1 (*FOXO1*) gene expression. FOXO1 plays a pro-apoptotic role because it increases the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), Fas ligand (FasL), and BIM that play a fundamental role in the intrinsic and extrinsic apoptosis pathways. Paschalaki et al. found that miR-126 levels were reduced in lung tissue obtained from mice exposed to cigarette smoke for 28 days [68]. This correlated with increased ataxia-telangiectasia mutated (ATM) protein kinase expression and activation of DDR in the presence of DNA double-strand breaks. Also, decreased miR-126 expression was detected in endothelial and bronchial epithelial cells obtained from COPD patients. Moreover, its inhibition increased *ATM* mRNA and protein levels. These results suggest that miR-126 controls DDR by repressing ATM protein kinase activity and highlighting its importance in this disease pathogenesis. A recent study reported increased miR-155 expression in lung tissue obtained from smokers and COPD patients (Table 2) [69]. Also, its higher levels were detected in alveolar macrophages and lungs in cigarette smoke-exposed mice. Moreover, attenuation of inflammation was observed in BAL fluid and lung tissue in miR-155 deficient mice exposed to cigarette smoke for 4 weeks.

6. Role of miRNAs in emphysema patients

Numerous studies have reported aberrantly expressed miRNAs in various lung diseases [70]. However, little is known about how miRNAs contribute to emphysema development [71]. It was shown that miRNAs levels, e.g., miR-34c, miR-34b, miR-149, miR-133a, and miR-133b, were significantly down-regulated in lung tissue obtained from patients with moderate compared to mild disease [72]. The reduced miR-34c expression was also associated with increased serpin family E member 1 (*SERPINE1*) expression, a protease inhibitor, in BEAS-2B cells, fetal lung fibroblast HFL1 cell line in vitro, and in emphysematous lung tissue *ex vivo*. This indicates a potential mechanism involved in this disease progression [72]. Apoptosis of lung endothelial cells and the implication of miR-199a-5p and miR-34a have also been detected in emphysema development [73,74]. Mizuno et al. found that miR-199a-5p reduced the expression of the hypoxia-inducible factor-1 α (HIF-1 α) in human pulmonary microvascular endothelial cells (HPMVECs) [74].

Increased expression of miR-34a and miR-199a-5p was found in lung tissue obtained from COPD patients, which affected the HIF-1 α -dependent lung structure maintenance program under the control of the p53 signaling pathway. Increased p53 expression caused by oxidative stress in HPMVECs induced miR-34a levels and reduced phosphorylation of AKT, which is known to trigger cell apoptosis. Moreover, *AKT* inactivation caused upregulation of miR-199a-5p, which decreased HIF-1 α levels. This led to impaired VEGF expression and reduced AKT phosphorylation, contributing to HPMVECs apoptosis and emphysema development. Another study showed that increased expression of miR-638 in human lung fibroblasts correlated with this disease severity [71]. It can also regulate the pathways associated with emphysema pathophysiologies, such as oxidative stress and protein calcium regulated heat stable protein 1 (CARHSP1). Increased miR-638 levels in response to oxidative stress in lung tissue contributed to accelerated aging leading to an impaired ability to replace altered extracellular matrix (ECM) components and decreased repair in this disease [71]. Ezzie et al. showed that miR-15b is localized in the bronchial epithelium and alveolar wall at the junction of emphysematous and fibrosis areas [75]. Also, in situ hybridization indicated miR-15b localization in the bronchial epithelium and ATII cells in a smoker with COPD. A recent study on the anti-oncogenic miR-7 showed increased methylation in buccal epithelial cells in COPD. Higher miR-7 methylation was observed in emphysema which may explain the higher incidence of lung cancer in these patients [76]. Recently, Green et al. found increased miR-181b-3p, miR-23c, and miR-429 in human pulmonary endothelial cells (HPECs) in patients with COPD [77]. MiR-429 was also upregulated in lung tumors, suggesting that miRNAs alterations in COPD patients may precede the development of lung cancer.

7. Animal models of emphysema

Animal models complement studies using human samples and allow determining the role of miRNAs in emphysema development. An increased miR-22 expression was detected in lung myeloid dendritic cells (mDCs) in smokers with emphysema [78]. Its levels were high in antigen-presenting cells (APCs) in mice exposed to cigarette smoke or nanoparticulate carbon black, which promoted T_H17 cell-dependent emphysema. Of note, a mouse model with miR-22 deficiency exhibited attenuated T_H17 responses and failed to develop emphysema after this exposure. Moreover, it was shown that miR-22 controls APCs activation and T_H17 responses by activating AP-1 transcription factor complexes and histone deacetylase 4 (HDAC4). The autoreactive T_H17 cells release chemokines that recruit the elastase-secreting neutrophils and macrophages to the lung, causing elastin degradation and developing this disease [79,80]. A recent study showed miR-200b downregulation in the lung tissue in murine elastase-induced emphysema [81]. Its expression also correlated with the inflammatory response in the mouse lung epithelial MLE-12 cell line. Also, zinc finger E-box binding homeobox 2 (ZEB2), a target gene of miR-200b, plays a role in these alterations, as detected by luciferase assay. These results indicate that targeting ZEB2 by miR-200b may attenuate emphysematous changes. Recently, De Smet et al. showed attenuation of cigarette smoke-induced pulmonary inflammation in elastase-induced emphysema in mice with miR-155 deficiency [69]. This study indicates miR-155 as a potential target in COPD therapy.

In summary, downregulated miRNAs and their target signaling pathways in humans and animal models of emphysema are shown Table 1. Upregulated miRNAs are presented in Table 2.

8. Cellular senescence in emphysema

Cellular senescence is one of the major features induced by cigarette smoking and contributes to emphysema pathophysiology [82,83]. Cellular senescence is a state of growth arrest triggered by multiple mechanisms such as telomere shortening, oxidative stress, persistent DDR, or oncogene activation. COPD and IPF are among lung diseases related to aging [84]. Oxidative stress can cause premature senescence [82,85], and emphysema is associated with alveolar epithelial and endothelial cell apoptosis [37,73]. Under normal conditions, the loss of ATII cells is replaced by proliferating cells that undergo cell cycle divisions, maintaining lung homeostasis [37,39]. However, repeated cell cycles eventually cause senescence by telomere shortening leading to inhibition of proliferation and compensation of apoptotic cells [86]. Also, cigarette smoke-induced oxidative stress can cause ATII cell premature senescence without telomere shortening in this disease [86]. Studies showed that smoking increases the levels of pro-senescence markers p16, p21, and p53, and phosphorylation of the DNA damage marker γ H2AX in HSAECs, fibroblasts in vitro, and murine lung [87–89]. A recent study revealed an increased p16 expression, a cell cycle inhibitor, in COPD lungs. Lungs of mice lacking p16 were structurally and functionally resistant to cigarette smoke-induced emphysema due to activation of IGF1/AKT regenerative and protective signaling and increased ATII cell proliferation. Importantly, *Igf1* and *Akt1* mRNAs were diminished in COPD lung [90]. Obtained results indicate that targeting senescence can promote the IGF1 proliferative pathway, facilitate re-epithelialization and lung regeneration.

The mTOR signaling activation is implicated in emphysema pathogenesis, as demonstrated using transgenic mice [91]. mTOR overactivity in lung vascular cells or alveolar epithelial cells contributed to lung senescence with rapid disease development, pulmonary hypertension, and inflammation. This suggests a novel therapeutic approach in COPD by inhibiting cellular senescence via targeting the mTOR pathway. It was also shown that the induction of AMP-activated protein kinase (AMPK) that controls energy balance and metabolism reduces inflammatory responses and expression of genes involved in cellular senescence in BEAS-2B cells, SAECs, and in a mouse model of elastase-induced emphysema [92,93]. AMPK upregulated mitochondrial proteins, antioxidant superoxide dismutase 2 (SOD2), and the histone deacetylase SIRT3 [92], which play an important role in regulating inflammation and cellular senescence [94].

Although studies identified altered signaling pathways contributing to senescence and pulmonary emphysema, miRNAs' role in these processes is not well known. Christenson et al. showed that increased miR-638 expression, caused by emphysema-related oxidative stress in human lung fibroblasts, is implicated in activating multiple pathways such as DDR and telomere attrition [71]. This can lead to cellular senescence, the lung's impaired ability to replace altered ECM components, and contribute to disease progression. In a recent study, Shen et al. found that miR-200b was downregulated in the murine model

of COPD, and its expression was correlated with cellular senescence and inflammatory response [81]. They showed that miR-200b acts as an anti-aging factor in the lungs. Overexpression of miR-200b in MLE-12 cells attenuated cigarette smoke extract-induced senescence-associated beta-galactosidase (SA- β -GAL) activity and deterred the increase in the TNF- α levels. MiR-200b levels were negatively correlated with ZEB2 protein and implicated in cellular senescence. ZEB2 upregulation in MLE-12 cells also induced TNF- α expression contributing to the inflammatory response. The importance of miR-200b was also highlighted in the attenuation of cellular senescence and inflammation by targeting ZEB2 in a murine model of pulmonary emphysema, which suggests a novel therapeutic target for COPD. It was reported that an increased expression of miR-34a caused by hydrogen peroxide-induced oxidative stress in BEA-S2B cells was associated with PI3K α activation and phosphatase and tensin homolog (PTEN) reduction. A reduction of SIRT1 and SIRT6, putative anti-aging enzymes, in the BEAS-2B cell line and peripheral lung samples obtained from COPD patients was also observed. SIRT1, an NAD⁺-dependent protein/histone deacetylase, protected against cigarette smoke- and elastase-induced emphysema in mice through FOXO3-mediated decrease of cellular senescence. This was independent of inflammation since the NF- κ B/IKK2 inhibitor did not have any beneficial effect on emphysema.

Baker et al. reported that miR-34a may serve as a potential biomarker of cellular senescence by acting as an inhibitor of SIRT1 and SIRT6 expression in COPD patients [95]. Moreover, miR-570-3p was involved in reducing SIRT1 levels and activation of cellular senescence in chronic lung diseases such as COPD and interstitial lung disease (ILD) [96]. It was activated by p38 MAP kinase and c-Jun signaling. These results suggest that miR-570-3p can serve as a therapeutic target in lung diseases, and its inhibition may restore cellular growth. Recently, Zeng et al. showed increased miR-494-3p expression in COPD lung tissue, SAECs, and BEAS-2B cells accompanied by an increase in senescence markers like p27, p21, p16, and other pro-inflammatory and profibrotic proteins associated with senescence-like IL-1 β , TNF- α , MMP-2, and MMP-9 [97]. They found that miR-494-3p binds to *Sirt3* and decreases its protein expression in SAECs. The SIRT3 is known to counteract oxidative stress, defend against apoptosis and protect cells from aging. Moreover, miR-494-3p expression was induced by p38 MAPK-c-myc signaling. Therefore targeting senescence through miR-494-3p may be promising approach for targeting COPD and other aging-related lung diseases.

A recent study showed the implication of miRNAs in lung aging by targeting inflammatory and apoptotic pathways [98]. They found an altered expression profile of 25 miRNAs in young and old mice lungs, 8 miRNAs were upregulated, and 17 miRNAs were downregulated. These miRNAs regulate several mRNAs associated with aging-related lung diseases, including emphysema.

9. MiRNAs-based therapeutic strategies

MiRNAs show great potential as treatment targets for numerous diseases [49]. Therapies that effectively slow down the accelerated decline in lung function in patients with COPD are still lacking [99]. In a recent study, Mohamed et al. demonstrated a novel potential

delivery system for miR-146a using poly (glycerol adipate-co- ω -pentadecalactone), (PGA-co-PDL), nanoparticles (NPs) as miR-146a-NP complex to treat COPD. A549 cell transfection with miR-146a-NP reduced target gene *IRAK1* expression. IRAK1 regulates multiple pathways in innate and adaptive immune responses by linking several immune-receptor complexes to TNF receptor-associated factor 6 (TRAF6), leading to activation of immune signaling pathways [100]. MiR-146a was decreased in primary human lung fibroblasts in COPD, and its overexpression had anti-inflammatory effects by downregulating IL-8 and IRAK1 levels and the IL-1 pathway [101]. Further studies in COPD patient-derived lung cells are required to demonstrate miR-146a-NPs potential in clinical settings. To date, the treatment of lung diseases using miRNAs as a new class of drugs lacks details on how they can be designed for effective and specific delivery as potential therapeutics [102]. However, other approaches targeting dysregulated miRNAs can be used in emphysema. For example, miRNAs antagonists, such as anti-miRNAs, can lock nucleic acids and antagomiRs [103]. Indeed, it has been shown that inhibiting miR-638 in human lung fibroblasts led to the modulation of emphysema-related pathways [71]. Also, miR-200b, which is downregulated in mice with elastase-induced emphysema, may serve as a potential novel therapy targeting ZEB2 [81]. Others described treatments in BEAS-2B cells and human primary bronchial epithelial cells obtained from COPD patients using antagomiRs against miR-15b and miR-34a, which are upregulated in lung tissue in individuals with this disease [75,95]. The loss of SIRT1 and SIRT6 expression by miR-34a increased cellular senescence markers in airway epithelial cells obtained from individuals with COPD. They are implicated in several biological processes, including inflammation, cellular senescence, DDR, genomic stability, and autophagy; via the deacetylation of upstream regulatory proteins. AntogomiRs against miR-34a restored SIRT1 and SIRT6 levels. Also, targeting miR-570-3p by antagomiRs in SAECs obtained from COPD patients reversed aging by restoring SIRT1 expression and suppressing p16, p21, and p27 levels. This suggests that restoring cell cycle progression and preventing senescence can lead to cellular growth and attenuating the accelerated aging phenotype in COPD [96]. There are recent advances on inhaled RNA-based therapeutics using a dry powder form as a novel approach for COPD [104]. However, transcending the biological barrier of lungs and successfully reaching deep lung regions is still challenging. Further studies are needed to find appropriate, effective, and safe systems for miRNA-based treatments in COPD.

10. Conclusion

This review described the current knowledge of miRNAs dysregulation in pulmonary emphysema (Fig. 1). We highlighted their contribution to disease severity. Moreover, we focused on pathways implicated in pro-inflammatory responses, DNA damage, cell senescence, and apoptosis. Also, the potential use of miRNAs as biomarkers is the primary approach of research. Furthermore, treating lung diseases using miRNAs as a new class of drugs is limited due to complex challenges. Further studies on miRNAs are urgently needed, which can lead to the development of novel therapeutic approaches.

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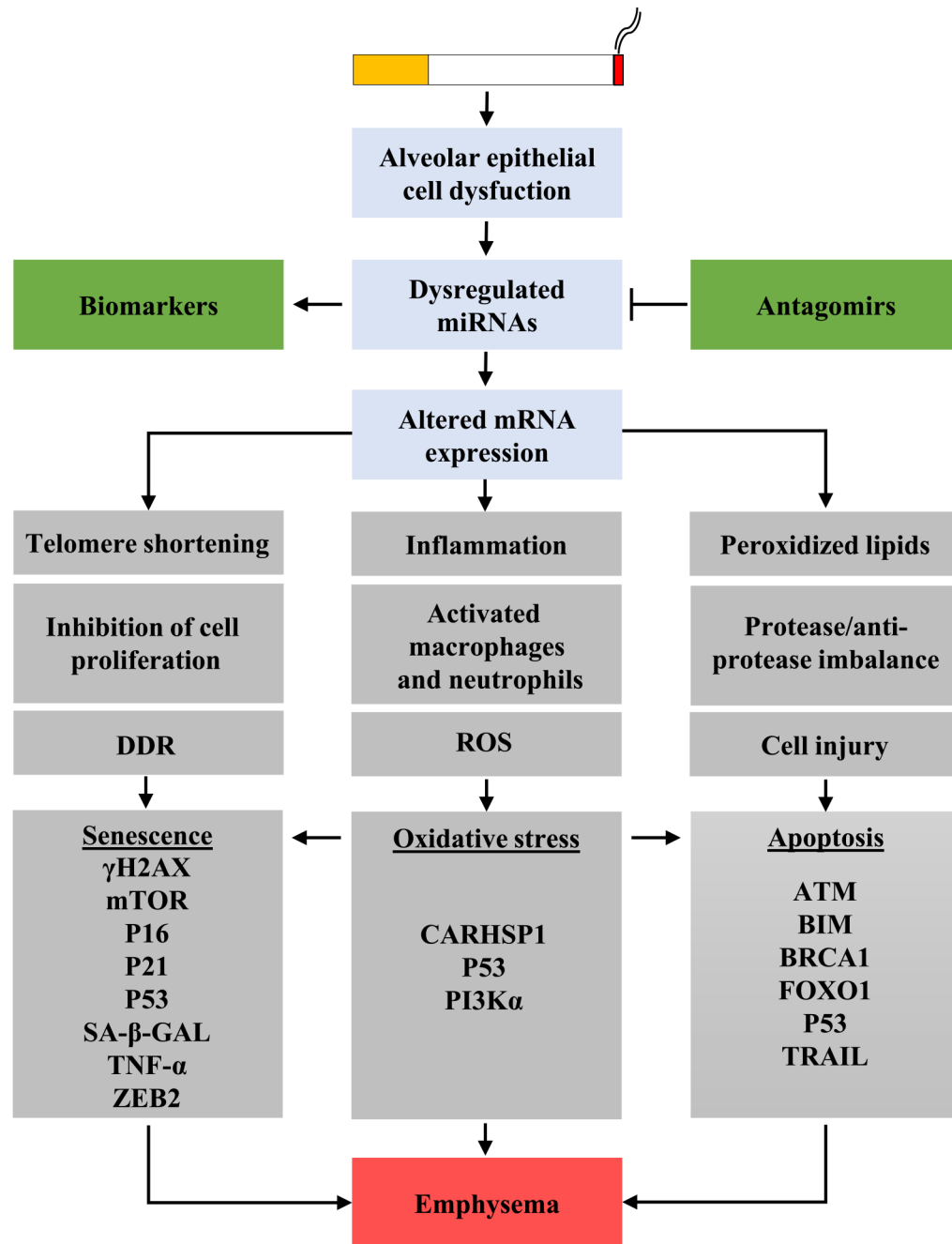


Fig. 1. A conceptual model of miRNAs dysregulation in alveolar epithelial cells in emphysema. Exposure to cigarette smoke causes alveolar epithelial cell dysfunction and miRNAs dysregulation causing mRNAs expression alteration. ROS contributes to alveolar epithelial cell senescence and apoptosis leading to emphysema development.

Table 1

Downregulated miRNA in pulmonary emphysema in human samples and animals.

Downregulated miRNA in emphysema	Upregulated pathways	Human samples and mice models	References
miR-24-3p	BIM and BRCA1, HR, apoptosis	HAECs	[66]
miR-34c	SERPINE1, MAP4K4, ZNF3, ALDOA and HNF4A	BEAS-2B cells	[72]
miR-126	ATM	Murine lung tissue Human endothelial and bronchial epithelial cells	[68]
miR-200b	ZEB2	Murine elastase-induced emphysema MLE-12 cell line	[81]
miR-34b, miR-149, miR-133a and miR-133b	NA	Human lungs tissue	[72]

Abbreviations: HAECs - Human Airway Epithelial Cells, NA - not available

Table 2

Upregulated microRNAs in pulmonary emphysema in human samples and animals.

Upregulated miRNA in emphysema	Affected pathways	Human samples and mice models	References
miR-15b	Decreased SMAD7	Human bronchial epithelium and ATII cells	[75]
miR-22	Decreased HDAC4 expression Increased T helper 17 response	Murine model with miR-22 deficiency	[78]
miR-34a and miR-199a-5p	Decreased AKT phosphorylation HIF-1 α and VEGF expression through p53 signaling	HPMVECs and BEAS-2B cells	[74]
miR-638	Increased DNA damage Decreased DNA repair	Human lung fibroblasts	[71]
miR-520e, miR-302d, miR-92a, miR-211 and miR-150	NA	Human lungs tissue	[71]
let-7c, let-7d, let-7e, let-7f, miR-181c, miR-181d, miR-30a-3p	NA	Human lungs tissue	[71]
miR-7 methylation	NA	Human buccal epithelial samples	[76]
miR-155	NA	Human and murine lung tissue Murine alveolar macrophages	[69]
miR-181b-3p, miR-23c and miR-429	NA	Human pulmonary endothelial cells	[77]
miR-494-3p	Decreased SIRT3 Increased p27, p21, and p16	Human lung tissue, SAECs, and BEAS-2B cells	[97]

Abbreviations: HPMVECs - Human Pulmonary Microvascular Endothelial Cells, SAECs – Small Airway Epithelial Cells, NA – not available