BASIC SCIENCE FOR THE CHEST PHYSICIAN

MicroRNAs in lung diseases

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Received 26 May 2011
Accepted 14 July 2011
Published Online First
11 August 2011

ABSTRACT

The advent of RNA sequencing technology has stimulated rapid advances in our understanding of the transcriptome, including discovery of the vast RNA complement generated by transcript splice variation and the expansion of our knowledge of non-coding RNAs. One non-coding RNA subtype, microRNAs (miRNAs), are particularly well studied, primarily because of their important roles as post-transcriptional gene regulators. The first miRNA was identified in the early 1990s and there are now thought to be around 1000 distinct miRNAs in man, with each cell type expressing a distinct repertoire. Increasing evidence has implicated miRNAs as having causative roles in a variety of lung diseases and has driven investigations into their potential as therapeutic targets.

DERIVATION AND FUNCTION

miRNAs are generally derived from precursor transcripts termed pri-miRNAs. These pri-miRNAs can either contain a single short miRNA hairpin of only about 100 nucleotides (nt) in length, or they can contain multiple miRNA hairpins. A number of miRNAs, known as miRtrons, are excised from the introns of protein-coding genes by the cellular splicing machinery.

The product of miRNA processing is a 19–25 nt RNA molecule that can be incorporated into a cytoplasmic protein complex called RISC, the RNA-Induced Silencing Complex. Once in the context of RISC, miRNAs target specific mRNAs through Watson–Crick base pairing which predominately involves only bases 2–8 of the miRNA, known as the 'seed'. A single miRNA may bind to a number of target transcripts and a single transcript may contain multiple interaction motifs for a single miRNA or for different miRNAs. The interaction of a miRNA and target message culminates in degradation of the target mRNA, translational repression, or a combination of both (figure 1). It is thought that around 60% of all mRNAs within the cell are targeted by miRNAs, but should just one of those RNAs encode a critical component of a signal transduction pathway, then profound downstream influences may be triggered by its diminished expression.

TARGET IDENTIFICATION

Despite progress in our understanding of miRNA biology, the list of validated miRNA–mRNA interactions is not comprehensive. Target identification is a major hurdle in miRNA research and is complicated by the fact that the interaction of miRNAs with target mRNAs is tolerant of some degree of mismatch. Where are the regions of mRNAs that miRNAs target?

Recent unbiased approaches suggest that miRNA interaction sites may be located throughout the mRNA transcript. However, historic studies have shown that interaction motifs tend to be enriched in the 3′ untranslated region (3′UTR) of the target mRNA, a region often involved in the regulation of mRNA stability. Algorithms commonly used to predict miRNA targets exploit this bias towards the 3′ UTR, possibly leading to the under-investigation of other regions.

A growing body of literature suggests that there is disrupted expression of specific miRNAs either in lung pathologies themselves or experimental models thereof. Examples of this are cited for lung cancer, asthma, fibrosis and chronic obstructive pulmonary disease (COPD).

Lung cancer

The let-7 miRNA family and the miR-17-92 cluster, a single transcript which can give rise to seven distinct miRNAs, have well-defined roles in lung cancer as tumour suppressors and oncogenes, respectively. The let-7 family is frequently deleted in lung cancer and has been shown to regulate RNA stability of transcripts encoding multiple oncogenes including Ras[1] and cycle regulators such as cyclinD2. By contrast, miRNAs from the miR-17-92 cluster are overexpressed in small cell lung cancer and this overexpression is associated with inactivation of function of the retinoblastoma tumour suppressor.[2]

Asthma

Mouse models and in vitro studies of human cells have implicated miRNAs as having causative roles in asthma. Rodriguez *et al* have demonstrated that a total knockout of miR-155 causes the spontaneous development of an asthma-like phenotype, including inflammatory infiltration into the lung and airway remodelling.[3] TLR4-induced Th2 inflammation induces increased expression of miR-126 and administration of cholesterol-linked single-stranded inhibitors of miR-126 decreases the levels of TLR4-induced inflammatory infiltrate and airway hyper-responsiveness.[4] In addition, treatment of human bronchial smooth muscle cells with interleukin 13 causes a decrease in the expression of miR-133a. Artificial inhibition of miR-133a function in smooth muscle cells through the use of antagomirs was shown to increase the expression of RhoA, a known procontractile protein.[5]

Fibrosis

Liu *et al* have described increased miR-21 expression in both a murine bleomycin-induced fibrosis model and the lungs of patients with idiopathic pulmonary fibrosis. The authors speculate that miR-21

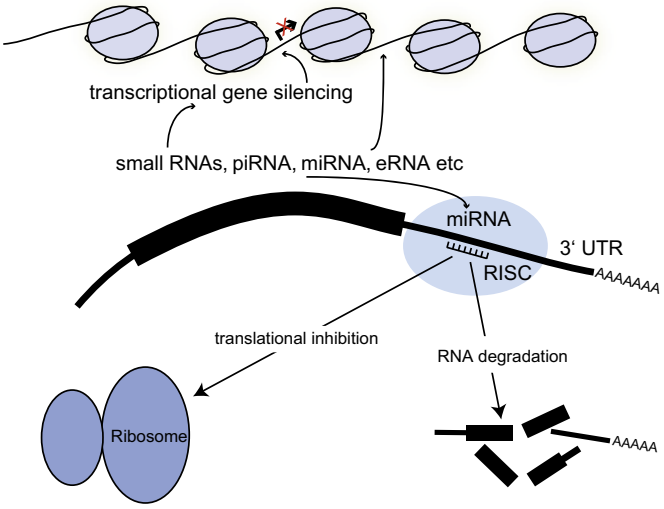

Figure 1 Advances in identification of RNA species through deep sequencing have revealed a number of novel RNA species such as eRNAs and identified more members of known families such as miRNAs. miRNAs have been the subject of intensive study and these have now been shown to anneal to target mRNAs through complementary base pairing. In most cases this interaction leads to cleavage of the target mRNA thereby preventing translation. A second mechanism of action involves blockade of mRNA translation.

may have a role in fibrosis through regulation of an inhibitory Smad, Smad7.[6] miRNA expression profiling of human fibrotic lung biopsies revealed differential expression of 46 miRNAs compared with cells from controls, including downregulation of miR-126 and let-7d. Reduction of let-7d expression resulted in increased collagen deposition and alveolar septal thickening in vivo and epithelial-mesenchymal transition in vitro.[7]

COPD

It is well established that smoking is a major risk factor for the development of COPD. A comparison of the miRNA expression profile of bronchial epithelial cells from smokers and never-smokers identified 28 miRNAs with differential expression and suggested that miR-218 in particular may play an important role in modulating epithelial gene expression following exposure to cigarette smoke.[8]

CLINICAL RELEVANCE

The increasing evidence for disrupted miRNA expression and function in disease processes makes them interesting targets for therapeutic intervention. Applications of miRNA-based therapy for lung disease are less advanced than for some other diseases.[9] However, promising data are emerging from model systems. In a recent report it has been shown that the systemic administration of miRNA mimics to the known tumour suppressor miRNAs miR-34a and let-7 decreases the in vivo tumour burden in a mouse model of non-small cell lung cancer (NSCLC).[10]

Perhaps a more immediate way of exploiting miRNAs in the pathogenesis of pulmonary disease is through molecular diagnostics, particularly risk stratification and outcome prediction. miR-155 and miR-let7a-2 expression correlate with poor overall survival in patients with lung adenocarcinoma,[11] while a five-miRNA signature (miR-137, mir-372, miR-182*, miR-221 and let-7a) has been shown to correlate with disease-free survival in patients with NSCLC.[12] A further feature of miRNAs is that they are stable and detectable in blood plasma and serum by qPCR or array hybridisation. Indeed, Chen *et al* have recently shown that the expression profile of serum miRNAs can serve as an NSCLC fingerprint.[13]

We are now reaching an exciting juncture in the miRNA field as continuing research into the mechanistic role of miRNAs in a wide range of diseases is occurring alongside demonstrations that miRNA are viable targets for both diagnostic screens and therapeutic intervention.

Competing interests None.

Contributors TP and PL wrote this document.

Provenance and peer review Commissioned; internally peer reviewed.

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