**EDITORIAL**
Iran J Allergy Asthma Immunol
June 2021; 20(3):259-262.
Doi: 10.18502/ijaai.v20i3.6341

# MicroRNAs in Human Disease: Commentary

**Ian M Adcock, and Sharon Mumby**

*National Heart and Lung Institute, Imperial College London, London, UK*

***Received: 26 April 2021; Accepted: 9 May 2021***

## INTRODUCTION

The Journal has recently published several papers on microRNAs (miRNAs) across a variety of diseases and indicating several important aspects regarding their function in different cell types and inflammatory and immune processes. Here we provide a short introduction to miRNA biology which helps place these studies in the wider context of miRNA biology. We also provide a summary of each of the papers to highlight their strengths and the directions of future research.

MiRNAs are non-coding RNAs of 18-22 nucleotides in length that function to inhibit mRNA translation and/or increase mRNA degradation in most cells in the body. MiRNAs regulate multiple biologic processes involved in inflammation, immunity, and cancer including signal transduction, cell proliferation and differentiation, and programmed cell death/survival. The number of miRNAs increases with progression through the phylogenetic tree ranging from 437 miRNAs in *Caenorhabditis elegans (C. elegans)* to 2-30,00 in man with the number in mice being intermediate at 1,500 miRNAs.[1-3] The expression of miRNAs may be cell- or tissue-specific or common across many cell types. For example, miR-122 represents 70% of the total miRNA content of the liver is highly expressed whilst miR-124 accounts for approximately 50% of the brain miRNAs. The expression of miRNAs in specific cell types alters with the disease.[1-3]

**Corresponding Author:** Ian M Adcock, PhD;
National Heart and Lung Institute Imperial College London, London, SW3 6LY, UK. Tel: (+44 020) 7594 7840, Fax: (+44 020) 7351 8126, E-mail: ian.adcock@imperial.ac.uk

There is a multistep processing pathway for miRNAs starting from nuclear primary miRNAs (pri-miRNAs) which are processed by either the endoribonuclease DROSHA-containing complex or by the splicing machinery to produce 70 nucleotide pre-miRNAs. These pre-miRNAs are exported to the cytoplasm where they are further processed by the endoribonuclease DICER-containing complex resulting in the production of double-stranded miRNA duplexes. These duplexes interact with the RNA-induced silencing complex (RISC) that enables the miRNA guide strand to interact with its target mRNA. Some miRNAs will have a designation -5p or 3p depending on whether the first miRNA of the pre-miRNA is closer to the 5' end of the hairpin structure (5p) or the 3' end (3p).[1-3]

Recent evidence has highlighted the potential importance of miRNAs in mediating cell-cell and tissue-tissue communication. For example, miRNAs can be transported between cells either within extracellular vesicles (EVs) or by interactions with proteins. A large number of mRNAs and miRNAs are found within EVs which can donate their cargoes to recipient cells. There is much debate about the classification and definitions of EVs but in general, exosomes are small EVs (<200 nm) generated by the fusion of the plasma membrane with multivesicular bodies and subsequent exocytosis. In contrast, larger EVs (>200 nm), known as microvesicles, are resulted from outward budding and fission of the plasma membrane. Active loading of EVs likely occurs as the EV miRNA profile generally differs from that of the parent cell.[1-3] These actively loaded miRNA-containing EVs can be transported around the body to be taken up by different target cells and/or tissues to produce

functional responses. In some cases, however, miRNAs are found in the blood associated with protein complexes e.g. with high (HDL) and low-density (LDL) lipoproteins, and are taken up by cells via specific scavenger receptors. MicroRNAs are protected from ribonucleases and are therefore stable and may be detected even after storage for many years. Thus, the presence of miRNAs in blood or EVs may serve as biomarkers of disease or cancer activity and or cellular drug response.[1-3]

**MicroRNAs in Disease: Commentary**

Asthma is a heterogeneous chronic inflammatory disease of the airways associated with distinct clinical phenotypes and pathophysiological mechanisms. A recent review in the Journal has summarised the role of epigenetics in the pathogenesis of asthma highlighting the importance of miRNAs such as miR-146a, miR-221 in regulating airway smooth muscle (ASM) cell function and how an expression changes with disease severity.[4] In addition, miR-140-3p, miR-708, and miR-142-3p are implicated in ASM cell hyperplasia and hypertrophy.[5] The review also indicated miRNAs involved in Th2 responses and IgE production.[4] The dysregulation of some of these miRNAs such as up-regulation of miR-21, miR-223, miR-146a, miR-146b, miR-155, and downregulation of the let-7 family is also observed in asthma-related diseases such as atopic dermatitis and allergic rhinitis suggesting a key role in the Atopic March from childhood to adulthood.[5] This review also indicated how miRNA expression is markedly affected by environmental factors including pollution, allergens, and diet as well as prenatal events.[4]

The importance of large studies in disease-associated cells was indicated in a small study of 7 moderate asthmatics and 8 healthy controls investigated the relative expression of 9 miRNAs (miR-21, miR-106a, miR-126, miR-146a, miR-150, miR-155, miR-181a, miR-221, and miR-223) previously identified as being dysregulated in asthmatic airway epithelial cells and peripheral blood CD4+ and CD8+ cells.[6] This study found no significant differences in the expression of these 9 miRNAs in peripheral blood CD8+ T cells from asthmatic patients compared with healthy controls. However, treatment of cells with an inhaled corticosteroid (fluticasone furoate) and/or a long-acting $\beta_2$-agonist (Vilanterol) induced that fluticasone alone enhanced miR-221 expression and together with vilanterol suppressed miR-223 expression.[6]

The expression of miR-23a-3p and miR-181b-5p have been implicated in the immune, inflammatory and apoptotic processes associated with the neurodevelopmental disorder autism.[7] A small study of 37 autistic patients and 40 healthy controls examined the expression of these miRNAs and IL-6 in peripheral blood mononuclear cells (PBMCs) using RT-qPCR. PBMC expression of IL-6 mRNA was significantly different between health and disease whilst that of miR-181-5p and miR-23 did not reach significance.[7] This may reflect differences between where patients were located (Tehran or Amol) or other environmental factors. In contrast, the expression of miR-1, miR-133, miR-208a, miR-206, miR-17, miR-29, miR-223, miR-326, and miR-155 were significantly increased in PBMCs from 45 patients with coronary artery disorder slow coronary flow (SCF) compared to 45 age-and sex-matched healthy controls. This was linked to enhanced serum expression of IL-1β, IL-8, and TNF-α proteins.[8] These data indicate the need for larger studies and the need to ideally conduct studies in purified cell populations that are associated as much as possible with the disease pathology.[8] Further studies are required to confirm the mechanisms that link circulating miRNA levels in patients with heart failure and SCF.

Tuberculosis (TB) is a major health risk globally and defining how mycobacteria overcome host immunity is critical in controlling the infection.[9] The expression of 3 miRNAs implicated in infection and the regulation of metabolic pathways important for intracellular TB survival (miR-1224, -484, and -425) was significantly up-regulated following culture of PBMC-derived macrophages (MDM) with Bacillus Calmette-Guérin (BCG).[9] The effect of BCG on miR-1224 expression at 72hrs was 10-15-fold greater than with the other 2 miRNAs highlighting the possible key role of this miRNA in re-patterning the host metabolism in response to mycobacterium infection.

Cell-specific expression of miRNAs is important and can modulate cell phenotypes. Thus, miR-21 transfection of naive human CD4+ T cells increased the expression of forkhead box P3 (Foxp3), transforming growth factor-beta (TGF-β), and interleukin-10 (IL-10), and this was associated with their transformation into induced regulatory T-cells (iTregs).[10] The miR-21

transformed iTregs were highly similar to cytokine-differentiated iTreg.[10]

Several papers in the Journal have examined the role of miRNAs in the etiology of the autoimmune disease multiple sclerosis (MS).[11] For example, genetic studies identified a single nucleotide polymorphism in miR-23a (rs3745453), but not in miR-155 (rs767649) or miR-196a2 (rs11614913), was associated with a 2.3-fold increased risk of MS in 80 MS compared with 80 healthy subjects from Isfahan.[11] miR-326 induces T helper (Th) 17 differentiation and maturation and therefore has been implicated in the immunopathogenesis of MS. The impact of interferon (IFN)-β therapy on the expression of miR-29b-3p and miR-326 in Th1 and Th17 cells, previously identified as being critical in the differentiation of these cell types, isolated from responder and non-responder populations of MS patients (n=40) has also been investigated.[12] However, in vivo analysis demonstrated no difference in expression of these two miRNAs between MS and healthy subjects or in response to treatment in Th1 and Th17 cells purified from PBMCs. These data matched the effect of disease and therapy on TH1 and Th17 cell numbers and of plasma IFN-γ and IL-17A in MS patients across drug responsiveness.[12]However, there was a statistically significant difference in the levels of miR-326 in exosomes released from anti-CD3/CD28-stimulated conventional Treg cells purified from the blood of 10 relapsing-remitting (RR) MS patients and controls.[13] In contrast, the exosomal expression of miR-146a, miR-29a, and miR-155did not differ.[13] The differences in the results of these papers regarding miR-326 expression may relate to the site of cell isolation (PBMCs), the potential pathogenic role of exosomal miR-326, or the fact that other miRNAs are also important in Th17 expression and activation.[14]

Chronic allograft dysfunction (CAD) is due to interstitial fibrosis and tubular atrophy (IFTA) leading to renal transplant failure.[15] The expression of miR-142-5p and miR-142-3p was significantly up-regulated in biopsies of CAD patients with IFTA (n=16) compared to subjects with normal allografts (n=17). In contrast, the expression of miR-211 was significantly down-regulated in renal allograft biopsies.[15] Similar results were observed for miR-142-3p and miR-211, but not miR-142-5p, in PBMCs. The paper indicates that PBMC miRNA levels reflect disease tissue only in some cases but where they do they may have potential as non-invasive biomarkers of disease.

Changes in miRNA expression may persist for many years after initial lung insult and reflect key pathogenic pathways.[16] For example, the expression of miR-21-5p, a regulator of TGF-β signaling and SMAD7 expression, was significantly down-regulated 2.7-fold in the lung tissue of subjects exposed to mustard gas.[16] The expression of miR-21-5p correlated inversely with that of SMAD7 in patients exposed to mustard gas and suggest a potential opportunity for therapeutic intervention.

Advanced bioinformatic analysis of mRNA and miRNA expression levels in the skin may be used to identify gene module hubs involved in the autoimmune disease vitiligo that is a common cause of depigmentation of skin and hair.[17] Weighted gene co-expression network analysis (WGCNA) of previously published data identified a module (turquoise) associated with vitiligo and pathways involved in important disease processes including G protein-coupled receptor signaling pathway, lymphocyte chemotaxis, chemokine activity, neutrophil migration, and granulocyte chemotaxis. The miRNA prediction tool (miRWalk) identified several miRNAs that regulated the top up-regulated genes within the turquoise module including miR-939-3p, miR-612 and miR-939.[17]

Analysis of the WGCNA turquoise module also produced a drug-target network that identified therapeutic drugs.[17] Another recent study has demonstrated how miRNAs may not only be markers of drug responses but may also act as novel drug interventions particularly in oncology.[18] Prostate cancer (PC), the second leading cause of cancer death in males, has been characterized by enhanced expression of miR-93 and low expression of miR-34a.[18] Treatment of human lymph node carcinoma of the prostate (LNCaP) cells with a miR-34a mimic or/and a miR-93 inhibitor significantly reduced the expression of the androgen receptor (AR) and prostate-specific antigen (PSA) which both are important in the initiation and progression of PC. Additionally, co-treatment of cells with the green tea extract epigallocatechin-3-gallate (EGCG) further reduced AR and PSA levels.[18] Future studies need to examine the impact of these therapies on primary PC cells to set the scene for a Phase II clinical study.

In summary, the recognition of the importance of miRNAs in human disease and treatment has been recognized by researchers in Iran and by the Journal. Increasing awareness of the need to have large studies; utilizing samples as closely linked to the diseased tissue as possible will make additional studies more disease-relevant. Analysis of miRNAs will form an increasingly important part of our understanding of human disease pathogenesis.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## REFERENCES


1. Mori MA, Ludwig RG, Garcia-Martin R, Brandão BB, Kahn CR. Extracellular miRNAs: From Biomarkers to Mediators of Physiology and Disease. Cell Metab. 2019;30(4):656-73.
2. Coll-Bonfill N, de la Cruz-Thea B, Pisano MV, Musri MM. Noncoding RNAs in smooth muscle cell homeostasis: implications in phenotypic switch and vascular disorders. Pflugers Arch. 2016;468(6):1071-87.
3. Zhang L, Xu X, Su X. Noncoding RNAs in cancer immunity: functions, regulatory mechanisms, and clinical application. Mol Cancer. 2020; 19(1):48-56.
4. Alizadeh Z, Mortaz E, Adcock I, Moin M. Role of Epigenetics in the Pathogenesis of Asthma. Iran J Allergy Asthma Immunol. 2017;16(2):82-91.
5. Specjalski K, Jassem E. MicroRNAs: Potential Biomarkers and Targets of Therapy in Allergic Diseases? Arch Immunol Ther Exp. 2019;67(4):213-23.
6. Badalzadeh M, Mazinani M, Pourpak Z, et al. In Vitro Analysis of Nine MicroRNAs in CD8+ T Cells of Asthmatic Patients and the Effects of Two FDA-approved Drugs. Iran J Allergy Asthma Immunol. 2019;18(4):358-68.
7. Atwan H, Assarehzadegan MA, Shekarabi M, et al. Assessment of miR-181b-5p, miR-23a-3p, BCL-2, and IL-6 in Peripheral Blood Mononuclear Cells of Autistic Patients; Likelihood of Reliable Biomarkers. Iran J Allergy Asthma Immunol. 2020;19(1):74-83.
8. Danaii S, Shiri S, Dolati S, et al. The Association between Inflammatory Cytokines and miRNAs with Slow Coronary Flow Phenomenon. Iran J Allergy Asthma Immunol. 2020;19(1):56-64.
9. Alipoor SD, Mortaz E, Tabarsi P, et al. miR-1224 Expression Is Increased in Human Macrophages after Infection with Bacillus Calmette-Guerin (BCG). Iran J Allergy Asthma Immunol. 2018;17(3):250-7.
10. Namdari H, Ghayedi M, Hadjati J, et al. Effect of MicroRNA-21 Transfection on In-vitro Differentiation of Human Naive CD4+ T Cells to Regulatory T Cells. Iran J Allergy Asthma Immunol. 2017;16(3):235-44.
11. Golshani Z, Hojati Z, Sharifzadeh A, Shaygannejad V, Jafarinia M. Genetic Variation in Intergenic and Exonic miRNA Sequence and Risk of Multiple Sclerosis in the Isfahan Patients. Iran J Allergy Asthma Immunol. 2018;17(5):477-84.
12. Karimi L, Eskandari N, Shaygannejad V, et al. Comparison of Expression Levels of miR-29b-3p and miR-326 in T Helper-1 and T Helper-17 Cells Isolated from Responsive and Non-responsive Relapsing-remitting Multiple Sclerosis Patients Treated with Interferon-beta. Iran J Allergy Asthma Immunol. 2020;19(4):416-25.
13. Azimi M, Ghabaee M, Naser Moghadasi A, Izad M. Altered Expression of miR-326 in T Cell-derived Exosomes of Patients with Relapsing-remitting Multiple Sclerosis. Iran J Allergy Asthma Immunol. 2019;18(1):108-13.
14. Atabaki M, Shariati-Sarabi Z, Barati M, Tavakkol-Afshari J, Mohammadi M. MicroRNAs as the Important Regulators of T Helper 17 Cells: A Narrative Review. Iran J Allergy Asthma Immunol. 2020;19(6):589-601.
15. Soltaninejad E, Nicknam MH, Nafar M, et al. Altered Expression of MicroRNAs Following Chronic Allograft Dysfunction with Interstitial Fibrosis and Tubular Atrophy. Iran J Allergy Asthma Immunol. 2015;14(6):615-23.
16. Salimi S, Noorbakhsh F, Faghihzadeh S, Ghaffarpour S, Ghazanfari T. Expression of miR-15b-5p, miR-21-5p, and SMAD7 in Lung Tissue of Sulfur Mustard-exposed Individuals with Long-term Pulmonary Complications. Iran J Allergy Asthma Immunol. 2019;18(3):332-9.
17. Derakhshani A, Mollaei H, Parsamanesh N, et al. Gene Co-expression Network Analysis for Identifying Modules and Functionally Enriched Pathways in Vitiligo Disease: A Systems Biology Study. Iran J Allergy Asthma Immunol. 2020;19(5): 517-28.
18. Mokhtari H, Yaghmaei B, Sirati-Sabet M, et al. Epigallocatechin-3-gallate Enhances the Efficacy of MicroRNA-34a Mimic and MicroRNA-93 Inhibitor Co-transfection in Prostate Cancer Cell Line. Iran J Allergy Asthma Immunol. 2020;19(6): 612-23.