MicroRNAs are Potential Biomarkers and Therapeutic Targets of Atopic Dermatitis

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Abstract

microRNAs (miRNAs) are a class of small, non-coding RNAs that regulate the expression of a diverse array of genes and pathways, with important roles in disease pathogenesis. Many of these miRNAs are currently under investigation as biomarkers or therapeutic options in a range of diseases. Here, we discuss the role of miRNAs in atopic dermatitis, a relapsing chronic pruritic inflammatory skin disease of unknown etiology, as they have been strongly implicated in the pathogenesis of skin inflammation. We outline the history and application of miRNAs for the detection and treatment of atopic dermatitis in comparison with other diseases, such as cancer and cardiovascular disease, which may assist the development of diagnostic and therapeutic strategies.

Keywords: MiRNA; Atopic Dermatitis; Biomarker; Therapeutic Application

Abbreviations

miRNA	: microRNA;
AD	: Atopic Dermatitis;
CVD	: Cardiovascular Diseases;
Th cells	: T Helper Cells;
MDC	: Macrophage-Derived Chemokine;
ST-miRCCL22	: *Salmonella typhimurium* Expressing CCL22 miRNA

Introduction

microRNAs (miRNA) are a class of small, non-coding RNAs ~21-25 nucleotides in length. Over 1000 human miRNAs have been identified to date, primarily located in the intronic regions of other genes. These structures represent an important form of post-transcriptional gene regulation by directly inhibiting gene translation through their binding of the 3′ untranslated regions of their target messenger RNA (mRNA), resulting in lower mRNA stability, or translation inhibition [1,2]. The effects of these miRNAs are far reaching, with more than 30% of the entire genome affected, including critical processes, such as development, differentiation, cell growth, and apoptosis. This central role of miRNAs in overall cell function has drawn considerable interest from cancer researchers, as cancers ultimately arise as a result of aberrant gene expression. As miRNAs are readily detected in accessible body fluids, such as saliva, blood, urine, and even hair follicles, this suggests the possibility of miRNAs as potential biomarkers of human diseases. This review summarizes the molecular *mec*hanisms of miRNA activity, highlights recent studies demonstrating their application as both biomarkers and therapy targets, and explores their therapeutic potential for the treatment of atopic dermatitis (AD).

miRNAs as biomarkers and therapeutic targets in cancer and cardiovascular disease

miRNAs were first identified in the mid-1990s with the discovery of Let-7 and Lin-4 in the model organism *Caenorhabditis elegans* [3,4]. Homologs have since been identified in nearly all eukaryotic organisms, with significant conservation among species. Given the strong conservation of these structures, and their central role in processes such as development, differentiation, cell growth, and apoptosis, it was not surprising reports of aberrant miRNAs expressed in cancers and other diseases emerged soon thereafter.

Ongoing clinical trials are currently assessing the correlation between miRNA expression and disease prognosis in cancer. As *in vitro* expression profiles of many tumor-derived miRNAs have shown promise for the diagnosis of patients, miRNA expression profiles might be used to precisely classify various cancer types, and might be superior to gene expression profiles in classification of tumors. More widespread screening before the onset of disease is also possible; stable miRNAs detected in easily accessible fluids, such as serum, plasma, and urine, as well as hair, have shown distinctive patterns of miRNA expression among patients and controls highlighting their possible use as diagnostic markers [5-7].

Interestingly, a single miRNA can simultaneously regulate both tumor suppressive and oncogenic target genes within a single cancer [8-11]. For example, miR-196b can target not only the

HOXA9/MEIS1 oncogenes, but also FAS tumor suppressor gene in mixed lineage leukemia-rearranged leukemia [8,9]. This implies that tumor initiation and development, as influenced by miRNAs, might be more complex than previously thought, which has important implications for using miRNAs as therapeutic agents. To date, several drugs have been shown to alter miRNA expression, including the bioactive agent docosahexaenoic acid, which inhibits the expression of miR-21, a protumorigenic miRNA [12]. Researchers are currently designing inhibitors for oncogenic miRNAs, along with mimics for tumor-suppressor miRNAs, which can act alone or synergistically with currently approved treatments [12,13].

In addition to cancer, many miRNAs have been identified as novel biomarkers and potential therapeutic targets for cardiovascular diseases (CVD). miRNAs play a crucial role in the biogenesis and function of the cardiovascular gene regulatory system, and have been implicated as dynamic regulators of cardiac and vascular signaling and arterial remodeling [14-16]. For example, in atherosclerosis, miRNA expression is directly regulated by blood flow, with endothelial cell miRNA expression increased in response to laminar or high flow, and other decreased in cases of low or disturbed flow [15,16]. Together, this regulatory dynamic suggests a possible therapeutic use of miRNAs as inhibitors of atherosclerosis development. The endothelial cell-derived *mec*hano-sensitive miR-143/145 can be delivered to smooth muscle cells via endothelial extracellular vesicles, exerting atheroprotective effects in smooth muscle cells [16]. Recent research has also suggested the use of stable miRNAs circulating in body fluids as potential biomarkers for cardiovascular diseases. The muscle-enriched miR-1, miR-133 and miR-499-5p, as well as the cardiomyocyte-specific miR-208, have been extensively investigated for their diagnostic ability in plasma of patients with coronary artery disease [17,18]. These observations strongly suggest a role for circulating miRNAs as a blood-based biomarker for cardiovascular diseases.

Several studies have demonstrated the potential therapeutic use of miRNAs to modulate disease processes by antagonizing miRNA expression or increasing their inhibitor functions. For example, targeted expression of miR-590 and miR-199a in heart cells using an adeno-associated viral vector demonstrated a re-entry of cardiomyocytes into the cell cycle, resulting in reduced infarct size and improved cardiac function [18].

miRNAs and allergic diseases

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease that results from a combination of genetic susceptibility and heightened immunologic responses to environmental allergens. Recently, the role of miRNAs in disease pathogenesis has begun expanding outside of the realms of cancer and CVD to allergic diseases, such as AD, with signif-

icant attention paid to both genetic and environmental factors. AD is an inflammatory skin disease that results from a combination of genetic predisposition, imbalanced immune responses, epidermal barrier abnormalities, and severe pruritus. A Th1/Th2 imbalance is a key factor in the pathogenesis of allergic diseases such as AD, with some reports implicating miRNA regulation of innate and adaptive immune responses in Th2 polarization [19-23]. Immune cells, including monocytes/macrophages, and dendritic cells, as well as T and B lymphocytes, play a key role in AD [23]. miRNAs have been shown to regulate an array of immune cell functions, including macrophage-derived cytokines (MDC) and the expression of inflammatory mediators in the context of AD [23,24]. miRNA expression profiling of human skin with psoriasis and atopic eczema revealed differential miRNA expression compared to healthy subjects, with multiple miRNAs differentially expressed in lesions skin relative to that of healthy controls [25]. Depending on the miRNA involved, these structures can function as suppressors or activators of various skin diseases (Table 1).

The Th1/Th2 imbalance plays a central role in the clinical expression of allergy and asthma, with Th2 cytokines acting as a driving factor in the pathophysiology of allergic diseases [19,22,23]. This balance of immune responses is heavily influenced by miRNAs, with serum levels providing insights into the pathology of allergic diseases [26]. The let-7 family of miRNAs has been shown to regulate IL-13 production by human T cells, resulting in reduced IL-13 production in the lungs, and alleviation of airway inflammation in a murine model of asthma [27]. Similarly, the miR-200 family of miRNAs regulates expression of E-cadherin, with suppression of E-cadherin is associated with increases in CCL17, a Th2 cell chemoattractant [28]. Other miRNAs, such as miR-1 and miR-155, also play a role in allergic inflammation, as suppression of these miRNAs by VEGFA contributes to Th2 inflammation in the endothelium and promotes recruitment of activated T cells and subsequent eosinophilic inflammation, together with Th2 cytokine production [29,30].

Recently, our group demonstrated that miRNA targeting CCL22 suppressed inflammatory responses in macrophages and in an animal model of AD [31,32]. This MDC/CCL22 axis was directly implicated in Th2-associated skin inflammatory reactions with significant increases in serum concentrations strongly correlated with disease severity in AD.

In those studies, a recombinant strain of *Salmonella typhimurium* expressing CCL22 miRNA (ST-miRCCL22) was used for the *in vivo* knockdown of CCL22 as a treatment for AD. ST-miRCCL22 was shown to significantly downregulate CCL22 expression in activated lymphocytes *in vitro*. Subsequent *in vivo* analyses in a mouse model of AD revealed decreases in both IL-4 and IgE expression, alongside increases in IFNγ; Th17 cells were also suppressed in AD mice treated with ST-miRCCL22. Together, these data suggest that targeted miRNA delivery may be an effective method for the treatment of AD (Figure 1).

Table 1. Roles of miRNAs in Skin Diseases

| Diseases	MiRNA	Up-regulated	Down-regulated	Function of MiRNA
Atopic dermatitis	miR-21	↑		Inhibition of apoptosis in human T cells
	miR-125b	↑		Suppression of keratinocyte proliferation by targeting FGFR2.
	miR-146a	↑		Suppression of chronic skin inflammation
	miR-155	↑		Promotion of T helper (Th) cell proliferation through the direct targeting of CTLA4 Suppression of BCL6 in inflammation
Psoriasis	miR-21	↑	↓	Induction of proliferation by targeting PTEN in human keloid fibroblasts
	miR-31	↑	↓	Induction of inflammatory cytokine and chemokine production by directly targeting serine/threonine kinase 40 (STK40), a negative regulator of NF-κB signaling
	miR-99		↓	Wound healing by downregulating the AKT/mTOR signaling
	miR-125b	↑		Suppression of hyper-proliferation and aberrant differentiation of keratinocytes.
	miR-146a	↑		Suppression of innate immune responses in keratinocytes
	miR-203	↑		Suppression of highly proliferating keratinocytes
	miR-221	↑		Tissue inhibitor of metalloprotease-3
	miR-424		↓	Inhibition of keratinocyte proliferation through downregulation of MEK1 or cyclin E1

Figure 1. Yoon et. al.
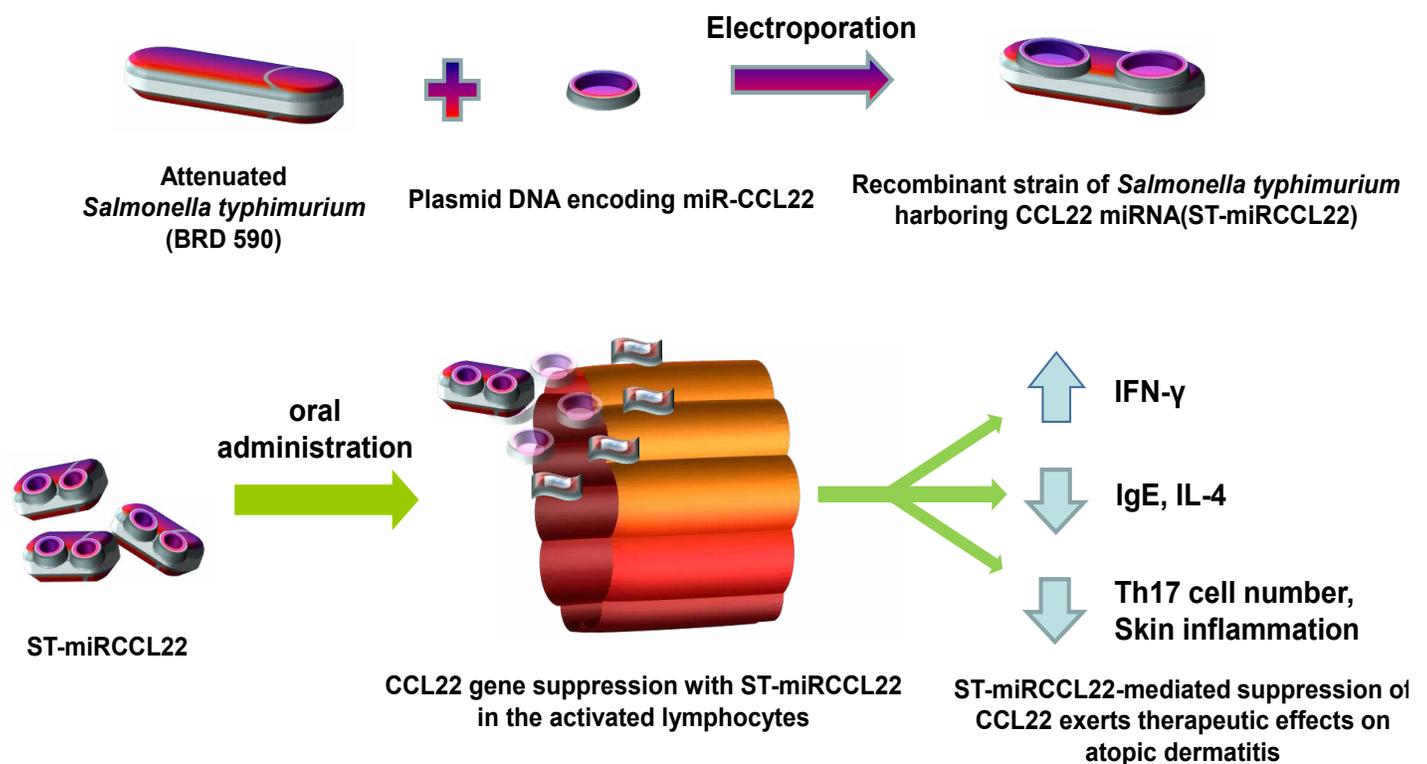
Figure 1. Oral administration of miRNA-CCL22 using *Salmonella typhimurium*. *Upper panel*, The recombinant plasmid pcDNATM6.2-GW/EmGFP-miR were electroporated into attenuated *S. typhimurium*. *Lower panel*, After oral administration of ST-miRCCL22, the therapeutic effects of ST-miRCCL22 on atopic dermatitis-like skin in mice were assessed. Changes in IL-4, IFN-γ, and IgE levels in serum were measured by ELISA; Th17 cells were enumerated by FACS analysis. Samples were collected 7 days after oral inoculation.

Conclusions

miRNAs have been suggested as potential biomarkers and therapeutic agents for the treatment of human diseases, such as cancer and cardiovascular disease; similar strategies may also be applicable for the treatment of allergic diseases, such as AD, with multiple products currently in development. Although the current state of knowledge regarding the expression, regulation, pharmacokinetics, and safety of miRNAs as a therapeutic strategy remain limited, these compounds hold tremendous promise for the treatment of all stages of AD.

Acknowledgement

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