*Inflammation, Vol. 40, No. 2, April 2017 (© 2016)*
DOI: 10.1007/s10753-016-0488-y

REVIEW

# MicroRNA-155: a Novel Armamentarium Against Inflammatory Diseases

**Wu Xiaoyan,[1,2,3] Eva Maria Arriero Pais,[1,2,3] Li Lan,[1,2,3] Chen Jingrui,[1,2,3] Miao Lin,[1,2,3] Patrick Asare Fordjour,[1,2,3] and Fan Guanwei[1,2,3,4,5]**

*Abstract*—An increasing number of diseases are being newly closely associated with inflammation, where microRNAs seem to play a critical role in the whole disease process from initiation to development. MicroRNAs are small non-coding RNAs that govern gene expression and modulation by means of mRNA degradation or translational repression. After several profound research studies, new correlations between microRNA-155 and inflammation-related diseases are strongly emerging. Hence, we review in this paper the possible molecular mechanisms of microRNA-155 in inflammatory disorders. Furthermore, we also consider the feasibility of targeting it as a bright alternative to improve the early diagnose statistics and treatments in those diseases. MicroRNA-155 features a novel breakthrough in fine-tuning inflammatory responses and, thereby, in treating a wide spectrum of diseases with inflammation as a common denominator.

**KEY WORDS:** microRNA-155; inflammation; mechanism of action; diagnosis and treatment of inflammatory diseases.

## INTRODUCTION

MicroRNAs or miRNAs are a group of small non-coding 18–22-bp-long RNAs that regulate post-transcriptionally gene expression by means of messenger RNA (mRNA) degradation or translational repression. Approximately 30% of the human genes are modulated by miRNAs, where metabolism, growth, and other processes are as well involved [1].

[1] State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, 300193, China
[2] Ministry of Education Key Laboratory of Pharmacology of Traditional Chinese Medical Formulae, Tianjin University of Traditional Chinese Medicine, Tianjin, 300193, China
[3] Institute of Traditional Chinese Medicine Research, Tianjin University of Traditional Chinese Medicine, Tianjin, 300193, China
[4] Tianjin State Key laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, # 312 Anshanxi Road, Nankai District, Tianjin, 300193, China
[5] To whom correspondence should be addressed at Tianjin State Key laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, # 312 Anshanxi Road, Nankai District, Tianjin, 300193, China. E-mail: fgw1005@hotmail.com

One of the miRNAs particularly well studied is the conservative miRNA-155 (miR-155). It is encoded by the MIR155 host gene (MIR155HG), which was originally considered transcriptionally activated by a promoter insertion at a general retroviral integration site in B cell lymphomas. Hence, MIR155HG was initially named B cell integration cluster (BIC) and contained three exons along 13.024 kb, the human chromosome 21 [2, 3]. MIR155HG displays a strong sequence homology among chicken, mouse, and human species and can be found highly expressed in lymphoid organs, hinting at a conserved evolutionary function. Its mature form arises from the sequential processing of an initial precursor MIR155HG transcript in either the 3′ or 5′ arms through the Drosha and Dicer enzymes. Once matured, miR-155-5p or miR-155-3p can control gene expression by binding to the 3′ untranslated (UTRs) coding regions in the targeted mRNAs.

Recent data reveal that miR-155 can be localized in immune responses, including B and T cell differentiation and development. MiR-155 overexpression results in human chronic inflammatory condition [4]. Inflammation is a protective response requiring the formation of blood vessels and the mobilization of immune cells and molecular



0360-3997/17/0200-0708/0 © 2016 Springer Science+Business Media New York

mediators. However, excessive inflammation is detrimental to the host and can lead to tissue damage or even cell death. Multiple disorders are associated with inflammation including cancer and atherosclerosis, among others. These diseases account to the ones most affecting our high life quality standards in an enormous proportion.

In this paper, we review the relationship between miR-155 and inflammation to achieve a better molecular understanding and, thereby, provide with theoretical basic foundations in miR-155-based treatments for inflammatory diseases.

## THE MOLECULAR MECHANISM OF MIR-155 IN INFLAMMATION

### An Overview

Bioinformatic analysis using the Target Scan 7.0 (release date August, 2015) revealed that human mRNA targets of miR-155-5p contained in the total 50 conserved sites and 127 poorly conserved sites "http://www.targetscan.org/cgibin/targetscan/vert_70/targetscan.cgi?species=Human&mir_sc=miR-155-5p." An integrated list of miR-155-5p targets was gathered through a reporter assay—a total of 140 genes and modulatory proteins for leukemogenesis, myelopoiesis, and inflammation were reported. Interestingly, target genes such as the inhibitor of nuclear factor κ-B kinase ε (IKK ε), myeloid differentiation primary response 88 (MyD88), receptor interacting protein kinase 1 (RIPK1), transcription factor PU.1 (SPI1), and cytokine signaling 1 (SOCS1) were experimentally identified by high-throughput genome-based seed sequencing of miR-155-5p and recognition of the endogenous transcript modulation [5] "https://en.wikipedia.org/wiki/MiR-155."

### The Strong Interrelationship of MiR-155 and Inflammation

*The Primary Process of Inflammatory Responses*

The primary origin of inflammatory responses comes from the innate immune system which acts as the first defensive barrier against invading pathogens [6]. This complex system is primarily composed by dendritic cells (DCs), granulocytes, and monocytes/macrophages. Once stimulated, the activated macrophages release several pro-inflammatory mediators, including chemokines and cytokines, to trigger the inflammatory cascade response. Pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) govern the macrophage activation upon sensitization by the pathogen-associated molecular patterns (PAMPs) from viruses or other pathogens [7].

*The Relationship of MiR-155 with Inflammatory Signaling Pathways*

With the development of microarray technology, Taganov and co-researchers concluded in 2006 that some lipopolysaccharides (LPS), concretely the TLR4 ligand, influenced the miRNA expression in human THP-1 monocytes; one out of the three upregulated miRNAs was miR-155 [8]. Even though that their main further research has been focusing ever since more on miR-146 rather than on miR-155, they investigated whether relevant viral stimuli on TLRs could trigger any miR-155 expression. They as well revealed that inflammatory mediators like polyriboinosinic:polyribocytidylic acid or poly(I:C) and tumor necrosis factor α (TNF-α) can strongly boost miR-55 expression *via* either the TRIF or MyD88 signaling pathway in macrophages and monocytes. In turn, inflammatory factors activate NF-κB through the intermediary TRIF or MyD88 signaling pathways. The direct association of NF-κB with the miR-155 gene promoter enhances miR-155 expression, thus inducing cytokine's release such as TNF-α. On the contrary, in cells transfected with pNF-κB-luc, miR-155 decreased the activity of NF-κB in a concentration-dependent way [9].

DCs have a prominent pattern of differentiation (maturation) to control immunity in response to inflammatory stimulation. In 2009, it was shown that several miRNAs display abnormal expression in human monocyte-derived DCs when stimulated with LPS, where the miR-155 level was as high as expected. To successfully demonstrate that miR-155 directly targets the relevant downstream TNF receptor-associated factor 6 and TAK1-binding protein 2 (TRAF6–TAB2) signal pathways, a selective miR-155 targeting through the TLR/interleukin–1 (IL-1) inflammatory pathway was conducted using a microarray technology in combination with RNA silencing. Consequently, it could be stated that miR-155 constitutes a part of the negative feedback loop in mature human DCs responding to microbial challenge [10].

The Src homology 2 domain-containing inositol-5-phosphatase 1 (SHIP1) was recognized as a firsthand miR-155 target, since it was repressed by miR-155 *via* direct 3′ UTR interactions, as shown through gain and loss of function experimental techniques. Both *in vivo* and *in vitro* experiments in LPS-stimulated hematopoietic cells showed that miR-155 overexpression repressed endogenous SHIP1 levels, thereby hyperactivating the kinase

Akt. Moreover, a myeloproliferative disorder (MPD) phenotype was fully recovered after specific SHIP1 knockdown through a retroviral vector possessing a miR-155 formatted SHIP1 siRNA cassette in the hematopoietic mice system; noteworthy similarities were present in miR-155$^{+/+}$ and SHIP1$^{-/-}$ mice. Conclusively, a molecular connection between SHIP1 and miR-155 was unveiled, providing proof of concept that SHIP1 repression is a hallmark in miR-155 biological regulation [11].

Activated miR-155-5p not only represses negative inflammatory regulators such as SHIP1 and inositol polyphosphate-5-phosphatase D (INPP5D) but also SOCS1 [12, 13]. Negative feedback mechanisms are essential to regulate the intensity as well as the duration of the inflammatory reaction. On the one hand, the inflammation-inhibitor SOCS1 can be upregulated by inflammatory cytokines, in turn preventing the activation of the cytokine signaling by targeting the JAK/STAT pathway [14]. On the other hand, SOCS1 can restrain LPS-induced inflammatory response by blocking TLR4 signaling through targeting nterleukin-1 receptor-associated kinase 1 and 4 (IRAK1/4) [15, 16]. Hence, miR-155 is thought to promote the TLR signaling and inflammatory response by suppressing the SOCS1 feedback loop effects [17]. Besides in 2010, contributions of Tang and colleagues promptly discovered in humans the miR-155-mediated suppression of Myd88, an adaptor protein of the TLR4 signaling pathway. Nevertheless, the miR-155 binding site did not underlie to any homology among different species. Consequently, it is to be expected that the miR-155 effect on the TLR4 signaling pathway may differ among multiple species [18].

TLR3 is a tightly regulated pivotal modulator of both tumor suppression and antiviral immunity which functions as a natural buffer system against excessive immune response and inflammation. Initially, it was hypothesized that its post-transcriptional mRNA regulation was due to miR-155, since it exhibited binding sites to TLR3 coding regions in manifold species. Nonetheless, this could not be validated until the year 2015, and it was additionally established that the TLR3 coding district also modulated IFN-β production [19]. Interestingly, the miR-155 role in the TLR3 response is extremely complicated. Following the rational standpoint of O'Neill and colleagues in 2011, miR-155 might act as a fine tuner or a "brake"' that suppresses partially the hyperactivation of the pro-inflammatory response [17]. Besides, miR-155 upregulation was shown in poly (I:C)-stimulated chicken embryo fibroblasts.

Inhibition of the c-Jun N-terminal kinase (JNK) blocked the abovementioned miR-155 level boosting which resulted from the poly (I:C) and TNF-α stimulation, suggesting a regulative role of mitogen-activated protein kinase (MAPK) signaling pathway in miR-155 expression. Moreover, their findings discovered that miR-155 is not only a constitutive part of the primary macrophage response but also one of the oncogenic miRNAs that may bridge uncontrolled inflammation with cancer [9].

LPS-stimulated transgenic mice B cells, possessing an ectopic miR-155 expression (E μ-miR-155 transgenic mice), released more TNF-α in an autocrine or paracrine manner, showing off a remarkable hypersensitivity to LPS compared with their wild-type (WT) counterparts [20]. The fact that the miR-155 expression is dependent on LPS is concordant with the discovery that miR-155 targets the protein transcription step of the LPS/TNF-α signaling pathway. By conferring stability to protein-encoding transcripts like fas-associated death domain protein (FADD) and IKK ε, its protein expression resulted as well influenced positively [17, 21]. LPS can promote as well the NF-κB nuclear import from the cell cytoplasm through activation of a signaling cascade, as correlates with the observation that MIR155HG expression was activated by a NF-κB-mediated mechanism in LPS-sensitized RAW264.7 cells [20]. Moreover, primary murine bone marrow-derived macrophages infected by H. pylori displayed a NF-κB-dependent MIR155HG upregulation, concretely miR-155-5p [22]. Nevertheless, MIR155HG may also restrain the intensity of the NF-κB-dependent inflammatory response by supporting the activation of defense pathways [23], indicating that miR-155 shifts different functions depending on the inflammation stages. Given that both NF-κB and the transcription factor activator protein 1 (AP-1) can regulate in parallel the MIR155HG activation, it can be hypothesized that modulation of this gene shall occur in a cell context-dependent manner "https://en.wikipedia.org/wiki/MiR-155."

As aforementioned, miR-155 has been proven to be an element of the primary macrophage response by orchestrating the release of different kinds of inflammatory mediators. Additionally, miR-155 modulates one of the critical mediators of the interferon network (IFN), namely the STAT1 protein. Findings of Kohanbash and co-workers in 2012 supported the existence of a positive feedback loop between miR-155 and STAT1 in response to inflammatory mediators or infection [24].

### *Endogenous MiR-155 Participating in Inflammatory Process by Targeting Inflammatory Gene Expression*

Recent evidence in 2015 reflected that miRNAs target gene expression not only post-transcriptionally—affecting

cells in which they are transcribed—but also functionally through their transfer between immune cells. Margaret and colleagues found out that the two key co-regulated miRNA feedback regulated the inflammation process, namely endogenous miR-146a and miR-155, and that DCs discharged them as exosomes targeting other DCs. Upon assimilation, exogenous microRNAs can reprogram the cellular response to endotoxin shock and regulate gene expression. Besides, the assembly of both miR-146a and miR-155 in exosomes and posterior distribution to immune cells was confirmed in an *in vivo* model. Exosomal miR-155 promotes, while miR-146a inhibits, endotoxin-induced inflammation in mice [25].

In conclusion, these findings provide with robust proof of concept that endogenous miRNAs lead the regulative mechanism of the inflammatory response [25].

## THE DIAGNOSIS AND TREATMENT OF INFLAMMATION-RELATED DISEASES *VIA* MIRNA-155

Evidence showed that nowadays, even more diseases display a close association with inflammation, *i.e.*, atherosclerosis, cancer, and diabetes. Therefore, we hypothesize that inflammation modulation *via* miR-155 should have an impact on inflammation-related diseases. For example, in atherogenic programming of macrophages, which sustains and amplifies vascular inflammation, miR-155 plays a central role. Macrophages, which escalate the vascular inflammation into a lipid overloading response, are the primary effective cells in atherosclerosis. miRNAs influence their activity by modulating key signaling transcription factors. So, it should be of significance to research the functional role that miRNAs play in the immune response during atherogenesis, since there have been already reports of miR-155 upregulation in macrophages suffering of atherosclerotic lesions and inflammation [26]. These results may indicate that miR-155 facilitated the inflammatory response of macrophages during atherosclerosis. In lesional and mox-LDL or IFN-γ-stimulated macrophages, reduction of miR-155 levels downregulated the chemokine (C–C motif) ligand 2 (CCL2) expression, which in turn boosted the recruitment of monocytes to atherosclerotic plaques. In addition, miR-155 mobilized macrophages to inflammatory activation site by blocking the B cell lymphoma 6 (BCL6)-mediated inhibition of NF-κB signaling.

### The Feasibility of Regarding miRNAs as a Method in Diagnosis and Treatment

Lately, it has been confirmed that specific miRNAs, which are produced in human organs or tissues, have smaller molecular mass and rather strong stability. Besides, they are more likely to enter the blood circulation system, facilitating their detection in the blood plasma while incurring in minimal damage. All in all, it is plausible to consider miRNAs as a novel disease and prognosis biomarker in inflammation-related diseases.

Ideal biomarkers should meet these criteria: (1) be acquired through non-invasive methods and have high specificity and sensitivity to the pathology or disease; (2) reliable handling in early disease diagnosis and detection, even before clinical symptoms appear; (2.1) have the ability to assign different pathologic stages to specific expression variations as a consequence of therapeutic response or disease progression; (3) be able to easily extrapolate its effects from generated model systems to the human model; (3.1) fluid samples should be inexpensive and accessible; and (4) have a long half-life to promote a high accuracy in detection.

Secreted miRNAs possess the necessary features as suitable biomarkers: (1) found in different body fluids in a stable form; (2) mostly their sequences are conserved among a wide range of species, easing the results' extrapolation step from the experimental animal models to the human one; (3) their expression pinpoints different biological disease stages or tissues; and (4) easy and affordable detection by quantitative polymerase chain reaction (PCR) or microarrays [27].

A coherent approach to use miRNAs to combat any related disorder would be mainly the rectification of abnormal miRNA expression. For this purpose, we could take advantage of the promising tools in gene-knockout approaches in regard to high miRNA expression profiles. Biochemical agents such as the anti-miRNA oligonucleotide (AMOs), the miRNA sponges, antagomirs, morpholinos, and locked nucleic acids (LNAs) are now available. By those means, mature miRNA can be suppressed by either complementary sequence pairing or mimicking. Aside from these, exogenetic miRNAs can be injected into lower expression cells. Among the dominating miRNA-delivery therapeutics, the following examples can be found: lipid encapsulated miRNA mimics, cationic polymers, and adenoassociated virus (AAV) packaged systems [28].

## Pharmacological Compounds Targeting MiR-155 to Mitigate Inflammation

### *Glucocorticoids Inhibit Inflammation via miR-155 Blockage*

Glucocorticoids (GCs) are one of the most effective therapies for a wide range of chronic inflammatory diseases. In the study of Zheng *et al*., a systematic microarray screening revealed that dexamethasone (Dex) repressed miR-155 expression in LPS-induced RAW264.7 cells. Though, transfection of miR-155 hindered the accomplished Dex inhibition and re-established miR-155-related influence. Treatment with other miR-155 inhibitors resembled the same behavior as macrophages treated with LPS and Dex, revealing that miR-155 plays a functional role in the anti-inflammatory GC effects. In addition, the inhibitory effect of Dex in miR-155 expression follows an NF-κB dependency. Furthermore, bioinformatics analysis of luciferase assays showed that the NF-κB binding site, which binds to the promoter district of the BIC as an allosteric inhibitor, was necessary for regulating the GC-driven inhibition of miR-155. Furthermore, combined treatment of Dex with suppression of miR-155 strengthened the anti-inflammatory Dex function in LPS-stimulated RAW264.7 cells [29].

### *1, 25-Dihydroxyvitamin D Diminish Inflammation via Repressing miR-155*

Negative feedback loops stay in foreground as a duration and intensity regulator of the inflammatory reaction. Studies of Chen and colleagues regarding the immunomodulatory mechanisms of vitamin D provided first-hand evidence that innate immune response modulation through 1, 25-dihydroxyvitamin D (1, $25(OH)_2D_3$) is related with miR-155-SOCS1 pathway in macrophages, which should result in an enhanced negative feedback inhibition of TLR4 signaling. Exactly, this was observed when he additionally sensitized cells with LPS; TLR-modulated inflammation was weakened by the abovementioned negative loop *via* the vitamin D receptor (VDR) signaling. On the contrary, VDR inactivation generates a miR-155 overproduction and so, SOCS1 repression, finally leading to a hyper-inflammatory response in mice macrophages. MiR-155 knock down ameliorates vitamin D repression of LPS-induced inflammation, ascertaining that 1, $25(OH)_2D_3$ provokes SOCS1 expression by downregulating miR-155.

In conclusion, taking the extensive functions of miR-155 into consideration, it is likely that vitamin D may regulate immune activities and the interaction of other immune cells through miR-155 [30].

### *Punica Granatum Inhibits the Inflammation in Breast Cancer Cells Through the MiR-155 Axis*

Previous studies have unveiled that polyphenolics extracts from pomegranates (Punica Granatum, PG) has the potency to suppress inflammation in lung cancer cell lines. What is more, an elementary screening revealed that PG extract could decrease inflammation *via* miR-155, leading narrowly to SHIP1 expression [20, 31–34]. Hence, further investigations about the role of miR-155-SHIP-1-PI3K in the cytotoxicity and the anti-inflammatory activity of PG extract were conducted in breast cancer cell lines. Here, PG extract induced SHIP-1 expression, accompanied by the suppression of PI3K-dependent phosphorylation of AKT and downregulation of miRNA-155 in BT474 cells. In analogy, nude mice tumors bearing BT474 cell xenografts, which were pre-treated with PG extracts, had similar results. This confirmed the cytotoxic and anti-inflammatory effects of PG extract, which were attributed to the simultaneously blockade of the axis miR-27a and the zinc finger and BTB domain containing 10-specificity protein (ZBTB10-sp) [35].

In summary, there is evidence that anti-inflammatory activity of PG extract is significantly mediated through the influential interrelationship between miR-27a-ZBTB10-Sp and miR-155-SHIP1-PIP3-AKT-NF-κB [35].

## MiR-155: Gene Therapy-Based Clinical Approaches

### *Diagnosis of Cytogenetically Normal Acute Myeloid Leukemia*

Guido and co-workers evaluated the leukemogenic role of miR-155 in a clinical trial comprising 363 patients with primary cytogenetically normal acute myeloid leukemia (CN-AML). Interestingly, quantification of MIR155HG and miR-155 expression profiles in blood samples by means of Nano String Counter assays and microarrays allowed correlating the miR-155 level with clinical outcomes in CN-AML. Furthermore, this correlation was proven to be independent of other typical molecular and clinical biomarker predictors, but whether miR-155 may provide with a better identification of molecular risk is still an open question. As well, higher levels of miR-155 expression were related to a higher risk for disease relapse (DR) or death and lower probabilities of achieving complete remission (CR). Additionally, old participants with the same aforementioned expression profile showed

a lower overall survival (OS); analogously, the younger ones presented lower disease-free survival (DFS) and OS as well.

Extrapolating from these results, we can take to knowledge that letting novel miR-155 antagonist compounds access to the extensive clinic landscape in the near future will surely constitute an important milestone in curing AML disease [36].

*Gene Therapy of Wound Healing*

Wound healing is a highly controlled process which underlies a wide range of cell process such as cell proliferation and maduration, hemostasis, and inflammation [37, 38]. As previously discussed, miR-155 is induced by inflammatory mediators and plays a crucial modulatory role in the immune response. Taken this together, miR-155 must play a key role in wound healing. In fact, miR-155 is upregulated in wounded mice tissue compared to healthy skin, involving the influx of macrophages into the wound and leading generally to an amplification of the inflammatory response and slower repair, as suggested by Diegelmann and colleagues [39]. Subsequently, heterozygous miR-155-deficient (miR-155$^{-/-}$) mice experienced a more salutary wound healing process [38]. Surprisingly, an association between an increased wound closing rate and mobilized macrophages resulted proven in wounded tissue. miR-155 deficiency has, thereby, explicit rewarding effects in wound repair. This leads to the hypothesis that patients suffering from type 1 and type 2 diabetes, who present commonly a complex impaired wound healing, could benefit from miR-155 modulation. Reducing miR-155 expression during wound healing could represent an urgently needed innovative breakthrough in diabetes treatment which would herald new venues in the targeting-based therapeutic industry [40].

An insurmountable clinical problem in the wound healing process is the development of fibrosis and an uncontrolled inflammatory response. One of the highlights in fetal wound repair is the lack of an inflammatory response, required for a scarless wound healing [40, 41]. Besides, miR-155 is narrowly related to inflammatory cell differentiation and development. Apart from that, Yang and colleagues confirmed the previously foreseen beneficial effect of the miR-155 inhibition by injecting antagomir locally in wound edges. As expected, wounded tissues experienced multiple drawbacks characterized by not only a decrease in immune system response but also a down-regulation of pro-inflammatory factors such as TNF-$\alpha$ and IL-$\beta$; on the contrary, the anti-inflammatory factor IL-10$\beta$ increased. Meanwhile, expression of collagens 1 and 3 (Col1/3) and $\alpha$-smooth muscle actin ($\alpha$-SMA) at locally antagomir pre-treated wound sites decreased in protein expression as well as in mRNA levels. In the wound healing mice model, treatment with antagomir resulted consequently in a more regularly arranged dermal structure and thinner collagen fibers. Controversially, the healing rate was not significantly affected [42].

Briefly summarized, these results yield obvious evidences that antagomir not only can improve the wound healing process by decreasing the inflammatory response, but also represents a potent therapeutic approach in skin fibrosis remission [42].

*Prognostic Marker in Chordoma Bone Cancer*

Chordoma is a rare, low-grade bone cancer deriving from benign notochordal rests [43]. After the original diagnosis, chordomas can take several years to spread and develop metastases—as 10–50% of patients do experience. The 5-year and 10-year relative OS rates are about 45–77 and 28–50%, respectively [44]. Besides, the absence of prognostic markers and therapeutic treatments for this cancer type worsens the above-mentioned OS dilemma and implies an imperative need in their discovery; it must be put forward as a race against time.

In the study of Eiji Osaka *et al*., the relationship between clinic–pathological features such as the miR-155 expression level and a poor outcome was analyzed in chordoma patients. Significant differences in metastasis-free survival (MFS) and OS were observed between low and high miR-155 expression groups, which indicate miR-155 levels independently affect prognosis in chordoma. Moreover, further analysis showed a direct significant correlation between high miR-155 levels and disease recurrence with lower OS, independent of other prognostic biomarkers. They also reported markedly favorable outcomes if low miR-155 and high miR-1 were available, suggesting the greater clinical utility of a dual miRNA panel. MiR-155 inhibition arrested cell cycle proliferation and diminished malignant cell invasion and migration potential. Hence, their data implies that miR-155 expression is not only a potential prognostic marker, but also a possible therapeutic target for chordoma patients [45].

We could not list all the inflammation-related diseases that have association with miR-155 in this paper, so we choose the typical diseases to represent. The diseases mainly include common diseases, whose mechanisms have close and explicit relation with inflammation. What is more, when reading articles, we prefer diseases that

multi-papers all have similar conclusions that these diseases have connection with inflammation and miR-155. In our opinion, the diseases that have connection with inflammation and miR-155 will be more and more extensive with further research.

## MiR-155 Deficiency as Diagnostic and Treating Approaches

Mirna Therapeutics developed the first miRNA-based anti-cancer drug to treat a kind of hepatocellular carcinoma named MRX-34. This liposome-based miR-34 mimic was first ever applied to clinic in the year 2013 (see mirnarx.com; NCT01829971). On the one hand, although current achievements have delineated a great promising future in miRNA-based therapeutics, improvements in stability, intake efficiency *via* endocytosis, and off-target effects still need to be made [28]. On the other hand, detection of circulating miR-155 has still not been mastered due to the limiting amount of total RNA in blood, which makes practically impossible to determine the quality and concentration of the isolated RNA. A more serious problem is miR-155 show abnormal expressions during various diseases, so that the broad impact of miR-155 functioning makes it probably difficult to use miR-155 as a specific disease biomarker. Apart from this, it is crucial to normalize accurately the measured concentration values after the miRNA extraction and the values of a reliable circulating housekeeping RNA, which is still a dilemma. Nevertheless, miRNAs like U6 or miR-16 are being currently used as a housekeeping RNA. However, levels of these RNAs are often unstable under pathological conditions. Alternatively, Mitchell and colleagues successfully reported a spiked-in normalization method based on the combination of three miRNAs of the *Caenorhabditis elegans* nematode in the RNA purification step to produce a synthetic miRNA as a normalizing value—with nonhuman homology, though [46]. Regarding the seemingly greater instability of synthetic miRNA compared to the endogenous ones [47], we strongly suggest the further research of a stable transfectable miRNA encoding vector in order to generate temporary or permanently an *in situ* constitutively expressed miRNA normalizer with no dependence on pathological cell stages, following analogously the rationale of the dual-reporter luciferase assay (plasmid with Renilla and Firefly luciferases).

Even that miR-155 has become a main modulator of a manifold of inflammation-related diseases, yet much needs to be learned about the exact mechanisms involving miR-155, focusing on their regulation following a tissue-specific pattern and in the context of chronic diseases. To fully clarify miR-155 activity under conditions of disease and homeostasis, we need an in-depth comprehension of its mechanism of action as well as novel biologic models. Meanwhile, the tendency of oversimplifying the molecular mechanism of miR-155 action regarding merely one single setting of downstream targets should be eradicated [48], as diseases generally are a linear combination of more than one deregulated pathway in which each condition and disease type should be weighted to achieve a selective effective treatment.

Development of clinical applications of miR-155 is progressing quickly, but there are still many boundaries left to surpass respecting the improvements in our fundamental experimental research field, which hinder enormously the application of miR-155 modulation in patients. In the first instance, clinical trials should consider and try to improve the following aspects: (1) the divergence in homology between experimental *in vivo* models and the human species; (1.2) the resulting disparity between *in vivo* and *in vitro* models; (2) limited trials size; (3) problematic development of effective stable carriers to target specifically molecules or tissues, *i.e.*, miRNA mimics and inhibitors are relatively unstable and chemical modification alters their biological properties; (4) off-target effects, which translate into unexpected side effects; (5) the essential necessity of miRNA delivery systems; and (6) the carrier selectivity targeting different organs and conversely, a wide spectrum of carriers targeting one single organ [49].

## DISCUSSION

Inflammatory diseases commonly are a complex slow evolving process leading to uncontrolled health damage and correspond to a field which still indispensably seeks for novel treatments. Due to a variety of inflammation-related diseases sharing miR-155 as one main disorder modulator, the idea of taking advantage of it in an early disease diagnosis and treatment materialized spontaneously.

Especially because the traditional diagnostic methods lack of enough sensitivity, the clinical application of miR-155 is still ongoing. Besides, prospective studies should emphasize on the systematic characterization of any stable constitutive miRNAs in order to facilitate the normalization of miR-155 values in the blood plasma [47].

Since miR-155-associated inflammatory diseases continue to increase, a new interest in targeting it had arisen. Nonetheless, this promising novel therapeutic has turned out to be a burden problem not only in selecting practically its target genes or a specific effective carrier but also in targeting

selectively a certain gene region. With respect to the effective miR-155 delivery, we need to exploit high efficient harmless suitable vector vehicles to import miR-155 into the wished organelle or gene region. Besides, future research studies should have an experimental focal point leading to illustrate accurately the mechanisms by which miR-155 can initiate mRNA degradation and restrain translation. The exact sources, location, and role of miR-155 need to be as well better discerned. In regard to the clinical landscape, clinical trial design should be reasonable enough and enable larger trials to ensure reliably additional benefits of miR-155 in comparison with other discovered biomarkers. Lastly, a fast, reproducible trustworthy quantification of circulating miR-155 entirely relies on current technological development. Once achieved, all of this clinical application of miR-155 will be enormously eased [50].

Despite heralding new venues in scientific research is tortuous, we have a bright future ahead if we successfully surpass miR-155 boundaries.

## ACKNOWLEDGMENTS

This work was support by grant from the National Key Basic Research Program of China (973 Program) (No.2012CB518404), the National Natural Science Foundation of China (81273891), and the National Science and Technology Support Program Projects (2014BAI05B01).

## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest.** The authors declare that they have no conflict of interest.

## REFERENCES


1. Bartel, D.P. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function [J]. *Cell* 116(2): 281–297.
2. Tam, W., D. Ben-Yehuda, and W.S. Hayward. 1997. bic, a novel gene activated by proviral insertions in avian leukosis virus-induced lymphomas, is likely to function through its noncoding RNA [J]. *Molecular and Cellular Biochemistry* 17(3): 1490–1502.
3. Tam, W. 2001. Identification and characterization of human BIC, a gene on chromosome 21 that encodes a noncoding RNA [J]. *Gene* 274(1-2): 157–167.
4. O'Connell, R., D. Rao, and D. Baltimore. 2012. microRNA regulation of inflammatory responses [J]. *Annual Review of Immunology* 30: 295–312.
5. Neilsen, P.M., J.E. Noll, S. Mattiske, C.P. Bracken, P.A. Gregory, R.B. Schulz, S.P. Lim, R. Kumar, R.J. Suetani, G.J. Goodall, and D.F. Callen. 2012. Mutant p53 drives invasion in breast tumors through up-regulation of miR-155 [J]. *Oncogene* 32: 2992–3000.
6. Medzhitov, R. 2010. Inflammation 2010: new adventures of an old flame [J]. *Cell* 140(6): 771–776.
7. Takeda, K., and S. Akira. 2005. Toll-like receptors in innate immunity [J]. *International Immunology* 17(1): 1–14.
8. Taganov, K.D., M.P. Boldin, K.J. Chang, and D. Baltimore. 2006. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses [J]. *Proceedings of the National Academy of Sciences of the United States of America* 103(33): 12481–12486.
9. O'Connell, R.M., K.D. Taganov, M.P. Boldin, G. Cheng, and D. Baltimore. 2007. MicroRNA-155 is induced during the macrophage inflammatory response [J]. *Proceedings of the National Academy of Sciences of the United States of America* 104(5): 1604–1609.
10. Ceppi, M., P.M. Pereira, I. Dunand-Sauthier, E. Barras, W. Reith, M.A. Santos, and P. Pierre. 2009. MicroRNA-155 modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells [J]. *Proceedings of the National Academy of Sciences of the United States of America* 106(8): 2735–2740.
11. O'Connell, R.M., A.A. Chaudhuri, D.S. Rao, and D. Baltimore. 2009. Inositol phosphatase SHIP1 is a primary target of miR-155 [J]. *Proceedings of the National Academy of Sciences of the United States of America* 106(17): 7113–7118.
12. Wang, P., J. Hou, L. Lin, C. Wang, X. Liu, D. Li, F. Ma, and Z. Wang. 2010. Cao X Inducible microRNA-155 feedback promotes type I IFN signaling in antiviral innate immunity by targeting suppressor of cytokine signaling 1 [J]. *Journal of Immunology* 185(10): 6226–6233.
13. Androulidaki, A., D. Iliopoulos, A. Arranz, C. Doxaki, S. Schworer, V. Zacharioudaki, A.N. Margioris, P.N. Tsichlis, and C. Tsatsanis. 2009. The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs [J]. *Immunity* 31: 220–231.
14. Alexander, W.S. 2002. Suppressors of cytokine signalling SOCS in the immune system[J]. *Nature Reviews Immunology* 2: 410–416.
15. Kinjyo, I., T. Hanada, K. Inagaki-Ohara, H. Mori, D. Aki, M. Ohishi, H. Yoshida, M. Kubo, and A. Yoshimura. 2002. SOCS1/JAB is a negative regulator of LPS-induced macrophage activation [J]. *Immunity* 17: 583–591.
16. Akira, S., and K. Takeda. 2004. Toll-like receptor signalling [J]. *Nature Reviews Immunology* 4: 499–511.
17. O'Neill, L.A., F.J. Sheedy, and C.E. McCoy. 2011. MicroRNAs: the fine-tuners of Toll-like receptor signaling [J]. *Nature Reviews Immunology* 11(3): 163–175.
18. Tang, B., B. Xiao, Z. Liu, N. Li, E.D. Zhu, B.S. Li, Q.H. Xie, Y. Zhuang, Q.M. Zou, and X.H. Mao. 2010. Identification of MyD88 as a novel target of miR-155, involved in negative regulation of helicobacter pylori-induced inflammation [J]. *FEBS Letters* 584: 1481–1486.
19. Hu, X., J. Ye, A. Qin, H. Zou, H. Shao, and K. Qian. 2015. Both microRNA-155 and virus-encoded MiR-155 ortholog regulate TLR3 expression [J]. *PLoS One* 10(5): e01260.
20. Tili, E., J.J. Michaille, A. Cimino, S. Costinean, C.D. Dumitru, B. Adair, M. Fabbri, H. Alder, C.G. Liu, G.A. Calin, and C.M. Croce. 2007. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock [J]. *The Journal of Immunology* 179: 5082–5089.
21. Quinn, S.R., and L.A. O'Neill. 2011. A trio of microRNAs that control Toll-like receptor signaling [J]. *International Immunology* 23(7): 421–425.

22. Koch, M., H.J. Mollenkopf, U. Klemm, and T.F. Meyer. 2012. Induction of microRNA-155 is TLR- and type IV secretion system-dependent in macrophages and inhibits DNA-damage induced apoptosis [J]. *Proceedings of the National Academy of Sciences of the United States of America* 109(19): E1153–E1162.
23. Schulte, L.N., A.J. Westermann, and J. Vogel. 2013. Differential activation and functional specialization of miR-146 and miR-155 in innate immune sensing [J]. *Nucleic Acids Research* 41(1): 542–553.
24. Kohanbash, G., and H. Okada. 2012. MicroRNAs and STAT interplay [J]. *Seminars in Cancer Biology* 22(1): 70–75.
25. Alexander, M., R. Hu, M.C. Runtsch, D.A. Kagele, T.L. Mosbruger, T. Tolmachova, M.C. Seabra, J.L. Round, D.M. Ward, and R.M. O'Connell. 2015. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin [J]. *Nature Communications* 6: 7321.
26. Nazari-Jahantigh, M., Y. Wei, H. Noels, S. Akhtar, Z. Zhou, R.R. Koenen, K. Heyll, F. Gremse, F. Kiessling, J. Grommes, C. Weber, and A. Schober. 2012. MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages [J]. *Journal of Clinical Investigation* 122(11): 4190–4202.
27. Steer, C.J., and S. Subramanian. 2012. Circulating microRNAs as biomarkers: a new frontier in diagnostics [J]. *Liver Transplantation* 18(3): 265–269.
28. Nouraee, N., and S.J. Mowla. 2015. MiRNA therapeutics in cardiovascular diseases: promises and problems [J]. *Frontiers in Genetics* 6: 232.
29. Zheng, Y., S. Xiong, P. Jiang, R. Liu, X. Liu, J. Qian, X. Zheng, and Y. Chu. 2012. Glucocorticoids inhibit lipopolysaccharide-mediated inflammatory response by downregulating microRNA-155: a novel anti-inflammation mechanism [J]. *Free Radical Biology and Medicine* 52(8): 1307–1317.
30. Chen, Y., W. Liu, T. Sun, Y. Huang, Y. Wang, D.K. Deb, D. Yoon, J. Kong, R. Thadhani, and Y.C. Li. 2013. 1,25-Dihydroxyvitamin D promotes negative feedback regulation of toll-like receptor signaling via targeting MicroRNA-155-SOCS1 in macrophages [J]. *The Journal of Immunology* 190(7): 3687–3695.
31. Boesch-Saadatmandia, C.L.A., A.E. Wagnera, A. Stachurskab, A. Jozkowiczb, J. Dulakb, F. Döringa, S. Wolfframc, and G. Rimbacha. 2011. Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155 [J]. *Journal of Nutritional Biochemistry* 22: 293–299.
32. Tili, E.M.J.-J., B. Adair, H. Alder, E. Limagne, C. Taccioli, M. Ferracin, D. Delmas, N. Latruffe, and C.M. Croce. 2010. Resveratrol decreases the levels of miR-155 by upregulating miR-663, a microRNA targeting JunB and JunD [J]. *Carcinogenesis* 31: 1561–1566.
33. Wagner, AEB-SC, J. Dose, G. Schultheiss, G. Rimbach. 2011. Anti-inflammatory potential of allylisothiocyanate-role of Nrf2, NF-κB and microRNA-155 [J]. *Journal of Cellular and Molecular Medicine* 1582-4934.
34. Kleemann, R., L. Verschuren, M. Morrison, S. Zadelaar, M.J. van Erk, P.Y. Wielinga, and T. Kooistra. 2011. Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models [J]. *Atherosclerosis* 218(1): 44–52.
35. Banerjee, N., S. Talcott, S. Safe, and S.U. Mertens-Talcott. 2012. Cytotoxicity of pomegranate polyphenolics in breast cancer cells in vitro and vivo - potential role of miRNA-27a and miRNA-155 in cell survival and inflammation [J]. *Breast Cancer Research and Treatment* 136(1): 21–34.
36. Marcucci, G., K.S. Maharry, K.H. Metzeler, S. Volinia, Y.Z. Wu, K. Mrózek, D. Nicolet, J. Kohlschmidt, S.P. Whitman, J.H. Mendler, S. Schwind, H. Becker, A.K. Eisfeld, A.J. Carroll, B.L. Powell, J.E. Kolitz, R. Garzon, M.A. Caligiuri, R.M. Stone, and C.D. Bloomfield. 2013. Clinical role of microRNAs in cytogenetically normal acute myeloid leukemia: miR-155 upregulation independently identifies high-risk patients[J]. *Journal of Clinical Oncology* 31(17): 2086–2093.
37. Gosain, A., and L.A. DiPietro. 2004. Aging and wound healing [J]. *World Journal of Surgery* 28: 321–326.
38. van Solingen, C., E. Araldi, A. Chamorro-Jorganes, C. Fernández-Hernando, and Y. Suárez. 2014. Improved repair of dermal wounds in mice lacking microRNA-155 [J]. *Journal of Cellular and Molecular Medicine* 18(6): 1104–1112.
39. Diegelmann, R.F., and M.C. Evans. 2004. Wound healing: an overview of acute, fibrotic and delayed healing [J]. *Frontiers in Bioscience* 9: 283–289.
40. Reed, M.J., L. Cooper, W. Wood, B. Stramer, and P. Martin. 2004. Wound healing and inflammation: embryos reveal the way to perfect repair [J]. *Philosophical Transactions of the Royal Society of London* 359: 777–784.
41. Mescher, A.L., and A.W. Neff. 2005. Regenerative capacity and the developing immune system [J]. *Advances in Biochemical Engineering and Biotechnology* 93: 39–66.
42. Yang, L.L., J.Q. Liu, X.Z. Bai, L. Fan, F. Han, W.B. Jia, L.L. Su, J.H. Shi, C.W. Tang, and D.H. Hu. 2014. Acute downregulation of miR-155 at wound sites leads to a reduced fibrosis through attenuating inflammatory response [J]. *Biochemical and Biophysical Research Communications* 453(1): 153–159.
43. Salisbury, J.R. 1993. The pathology of the human notochord [J]. *The Journal of Pathology* 171: 253–255.
44. Fuchs, B., I.D. Dickey, M.J. Yaszemski, C.Y. Inwards, and F.H. Sim. 2005. Operative management of sacral chordoma [J]. *Journal of Bone and Joint Surgery (American)* 87: 2211–2216.
45. Osaka, E., A.D. Kelly, D. Spentzos, E. Choy, X. Yang, J.K. Shen, P. Yang, H.J. Mankin, F.J. Hornicek, and Z. Duan. 2015. MicroRNA-155 expression is independently predictive of outcome in chordoma [J]. *Oncotarget* 6(11): 9125–9139.
46. Mitchell, P.S., R.K. Parkin, E.M. Kroh, B.R. Fritz, S.K. Wyman, E.L. Pogosova Agadjanyan, A. Peterson, J. Noteboom, K.C. O'Briant, A. Allen, D.W. Lin, N. Urban, C.W. Drescher, B.S. Knudsen, D.L. Stirewalt, R. Gentleman, R.L. Vessella, P.S. Nelson, D.B. Martin, and M. Tewari. 2008. Circulating microRNAs as stable blood-based markers for cancer detection [J]. *Proceedings of the National Academy of Sciences of the United States of America* 105: 10513–10518.
47. Creemers, E.E., A.J. Tijsen, and Y.M. Pinto. 2012. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? [J]. *Circulation Research* 110(3): 483–495.
48. Olson, E.N. 2014. MicroRNAs as therapeutic targets and biomarkers of cardiovascular disease [J]. *Science Translational Medicine* 6(239): 239.
49. Mao, Y., R. Mohan, S. Zhang, and X. Tang. 2013. MicroRNAs as pharmacological targets in diabetes [J]. *Pharmacological Research* 75: 37–47.
50. Romaine, S.P., M. Tomaszewski, G. Condorelli, and N.J. Samani. 2015. MicroRNAs in cardiovascular disease: an introduction for clinicians [J]. *Heart* 101(12): 921–928.