Meta-Analysis

Therapeutic Potential of microRNA-29 in Combating Collagen Fibrosis-Induced Skeletal Muscle Atrophy

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ABSTRACT

Fibrosis-induced skeletal muscle atrophy, driven by excessive extracellular matrix (ECM) deposition and abnormal collagen accumulation, represents a critical barrier to muscle regeneration. MicroRNA-29 (miR-29) has been shown to effectively suppress fibrosis by directly targeting collagen synthesis genes, such as COL1A1 and COL3A1, while preserving key ECM components like laminin and fibronectin. While various delivery methods have been explored, including Adeno-associated viruses (AAVs), nanoparticles, lipid-based transfection reagents, and electroporation, off-target effects remain a significant challenge. Preclinical studies have demonstrated the efficacy of miR-29, particularly when combined with other therapies, in reducing collagen deposition and restoring gastrocnemius muscle strength in murine models, offering preliminary success for miR-29 in treating skeletal muscle disorders like Duchenne muscular dystrophy. Nevertheless, the limited translation to success in clinical trials reflects the technology's ongoing challenges in targeted delivery, safety, and dosage optimization. This paper provides updates on the current state of the literature on the use of miR-29. The results underscore the need for further research to overcome these barriers, particularly in the context of skeletal muscle fibrosis, to fully unlock the therapeutic potential of miR-29.

Keywords: microRNA; muscle atrophy; collagen fibrosis; gene therapy; skeletal muscle

INTRODUCTION

Muscles are significant contributors to the overall health of humans due to their key role in mobility, strength, and metabolic functions (1, 2). Muscle degeneration is a significant public health concern, particularly as the global population ages. By 2050, it is projected that nearly a quarter of America's population will be over 65 years old (3), leading to an increased prevalence of age-related diseases such as sarcopenia, characterized by the progressive loss of skeletal muscle mass and strength (4). The consequences of this include not only impaired motility and reduced quality of life but also the source of marked increases in morbidity and mortality among afflicted populations (4, 5). Collagen deposition and fibrosis inhibit the skeletal muscle's innate capacity for regeneration (6). Collagen fibrosis is the

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excessive accumulation of collagen in the extracellular matrix (ECM). Collagen fibrosis contributes to muscle degeneration by disrupting the normal structure and function of skeletal muscle tissue. This excessive collagen accumulation increases the stiffness of the muscle tissue, reducing its elasticity and limiting its ability to contract and relax efficiently. As a result, muscle functionality declines, and the tissue becomes hypotrophic (7). Current therapeutic strategies, including both physical therapy and pharmacological interventions, have achieved limited success in restoring or preventing muscle degeneration due to their multifactorial origin and complex pathogenesis (8).

There is emerging evidence that microRNAs (miRNAs) are small non-coding RNA molecules that modulate gene expression at the post-transcriptional level, having therapeutic potentials in pathways associated with muscle degeneration (9). Indeed, specific miRNAs show great promise in controlling fibrosis, promoting muscle regeneration, reducing inflammation, and even supporting reinnervation in various contexts of muscle atrophy (10). miRNAs can target specific genes with key muscle repair or collagen synthesis functions. MiRNAs like miR-206 and miR-29 have shown therapeutic efficacy in murine muscular dystrophy and fibrotic muscle injury models, respectively (11). MiRNA therapy is a suitable approach to addressing skeletal muscle atrophy. Since miRNAs are regulators of many cellular processes, they offer a special therapeutic method in which the pathological molecular pathways responsible for muscle degeneration are specifically modulated. In addition, skeletal muscle is a great target for miRNA due to its capacity for sustained transgene expression. This is largely due to the post-mitotic and terminally differentiated nature of muscle fiber nuclei, which prevents frequent cell division and subsequent loss of the transgene. Furthermore, muscle fibers are not entirely destroyed even when localized damage occurs, allowing for continued transgene production over time (12).

This review discusses the role and effectiveness of miRNAs, specifically miR-29 in addressing collagen fibrosis-induced skeletal muscle atrophy. Based on recent studies in the field of miRNAs involved in muscle atrophy, I will give an extensive review of the potential of miRNAs regarding maintaining muscle function and regeneration and preventing degeneration. It also puts into perspective the future directions of miRNA-based therapies, discussing challenges in targeted delivery and stability, to translate miRNA treatments into clinical applications for patients with muscle atrophy.

METHODS

In this review, I conducted a comprehensive evaluation of studies related to the application of miRNA therapy for muscle fibrosis, with a specific focus on the regulation of collagen synthesis. Literature searches were performed using Google Scholar and PubMed. While no time restrictions were applied, studies published within the last 30 years were prioritized to capture both foundational and contemporary research. The keywords used in the search included “miRNA” and “muscle atrophy,” along with more specific terms such as “collagen synthesis” and “Mir-29” to ensure a targeted approach. All study types were considered, including *in vitro* experiments, animal models, and clinical trials, regardless of sample size or study design. This review consists of fibrosis resulting from various muscle injuries and surgeries, without limiting the scope to any specific condition.

RESULTS

Mechanism

miR-29 is a family of four miRNAs that are naturally produced by human genes located on chromosome 7 (miR-29a and miR-29b-1) and 1 (miR-29b-2 and miR-29c). MiR-29 is proven to regulate over 20 extracellular matrix (ECM)-relating genes, including collagen (13, 14). MiR-29 has emerged as a promising regulator of ECM collagen fibrosis, particularly by directly repressing the expression of type I and type III collagen. Type III collagen is a major component of muscle tissue, while type I collagen is more abundant in connective tissues such as tendons, ligaments, bones, and skin. The regulation of these collagens by miR-29 underscores its role in controlling ECM composition in skeletal muscles. It is also found that MiR-29 is found in less abundance in fibrotic skeletal muscle tissue.

The link between miR-29 and collagen deposition is well established. Therefore, it is proven that restoring or increasing the level of miR-29 may play a role in mediating collagen fibrosis (15). Studies have shown that miR-29 binds to the 3′ end of untranslated regions (UTRs) of these collagen mRNAs, specifically, COL1A1 and COL3A1, which encode type I and type III collagens, respectively, reducing translation and thus leading to downregulation of excessive collagen production. Reducing collagen deposition also mitigates the symptoms of muscle fibrosis—excessive ECM matter accumulation (16). In addition, selectively targeting fibrotic collagen expression, miR-29 preserves the structural integrity of ECM and thus does not interfere with its normal functioning. It is

shown that miR-29 does not influence the expression of laminin and fibronectin, which are key contributors to the physical structures of ECM (17).

Although miR-29 restoration or enhancement has great potential as a treatment for muscle fibrosis, targeting miR-29 alone may have its limitations. The TGF-β signaling pathway has a pivotal role in determining the deposition of collagen that facilitates the development of fibrosis. High concentrations of TGF-β induce suppressive effects on miR-29 expression, consequently entering a feedback loop potentially opposed to the beneficial results following miR-29 therapy (18). So an effective treatment against fibrosis might imply a restoration of miR-29 along with the simultaneous decrease in TGF-β to break the negativity associated with this feedback loop. Many therapeutic strategies targeting reductions of TGF-β signaling are under different developmental and clinical stages, specifically in human cancer treatments using monoclonal antibodies (19); another through small molecule inhibition, targeting TGF-β ligand and their receptors (20, 21). By combining the augmentation of miR-29 with the inhibition of TGF-β, this approach could more effectively reduce collagen accumulation and restore normal muscle function.

The Wnt signaling pathway and TGF-β signaling play converse roles in the regulation of fibrosis and production of the ECM. Active Wnt signaling maintains a balance within the ECM through the induction of genes such as c-Myc and Cyclin D1, which promote the proliferation of fibroblasts and the synthesis of collagen. Conversely, disturbed or inactive Wnt signaling may cause excessive production of collagen, contributing to the development of cardiac, skin, and muscular fibrosis (22). MiR-29 indeed significantly modulates ECM balance as it targets major constituents of the Wnt pathway, including the Wnt ligands, receptors, and intracellular signaling molecules. Overexpression of miR-29 downregulates such Wnt components to lower the production of the ECM and hence maintain homeostasis within the latter and prevent fibrosis (23).

Delivery Methods for miR-29 Therapy

A common miR-29 delivery method used is Adeno-associated virus (AAV). AAV is an ideal viral vector candidate for gene therapy due to its ability to infect most human cells. In addition, AAV has low pathogenicity and negligible immune response due to its requirement for a co-infector (24). However, AAV has some limitations. Its short genome size means that the transgene must be less than 4.5 kb in length, which is more than sufficient for miR-29 which is less than 0.2 kb (25). Although all serotypes of AAV (1-9) can infect every kind of cell in the human body, specific serotypes have comparative advantages in infecting certain types of human cells due to natural tropism. Studies have shown successful delivery of miR-29 with various types of AAV to hepatocytes, heart, and liver (26-28). AAV2, which presents a natural tropism toward infecting skeletal muscle cells is the most suitable for miR-29 delivery for anti-muscle fibrosis purposes. Since AAV delivers miR-29 templates, they can remain effective in the form of long-term expression and will not be diluted until the host cells divide. Fittingly, the muscle cells do not divide upon maturation, making it a suitable target for AAV miR-29 delivery. However, there are challenges to this delivery method. When a generic promoter is used in recombinant AAV (rAAV) expression cassettes, off-target effects can occur as the miR is delivered to unintended tissues. An endogenous promoter specific to the tissue should be used to improve accuracy. However, the challenge remains as their size may exceed the rAAV packaging limit (29).

Another delivery method of miR-29 is through nanoparticles. Unlike AAV which delivers the template into human cells which then transcribe the RNA, nanoparticles directly deliver miRNA mimics to tissues. Several types of nanoparticles are involved in miR mimics delivery, including inorganic nanoparticles (iron oxides, silica, gold), organic nanoparticles (polymers like PEI, PLGA), and natural polymers (hyaluronic acid). Nanoparticles have the advantage of shielding delivery agents from the environment to reduce degradation (30). The main issues with current nanoparticle drug delivery are its low accuracy and encapsulation efficiency.[31] Researchers have sought to address this issue with ligands specific to receptors in the targeting tissue. In the context of muscle, nanoparticles fortified with muscle-homing peptide M12 have increased muscle selective uptake both *in vitro* and *in vivo* (30, 32). Current nanoparticle miR-29 mimicry delivery has only been conducted on body tissues that are not skeletal muscle: exosome-mediated miR-29 transfer to the kidney, conjugated anionic lipopolyplex nanoparticles miR-29b to bone marrow, hyaluronic-based hydrogel miR-29b to the bone, and miR-29b loaded gold nanoparticles to endoplasmic reticulum (33-36). Studies on nanoparticle miR-29 mimicry delivery to skeletal muscle are still limited.

An alternative way to deliver miR mimics is by using lipid-based transfection reagents. Lipid-based transfection reagents such as Lipofectamine™ and DharmaFECT™ are commonly employed to facilitate the delivery of miR-29.

These reagents form complexes with the miRNA mimics, which are subsequently internalized by cells through endocytosis. Their widespread use *in vitro* is attributed to their ease of application, efficiency, and capability to transfect a diverse range of cell types, including muscle cells (37, 38). Yang *et al*. utilized Lipofectamine RNAiMAX Reagent to deliver miR-29b-3p to SH-SY5Y (cloned cells derived from a neuroblastoma cell line) aiming to address neuronal dysfunction (39). Neuhuber *et al*. (2002) demonstrated that lipid-based transfection reagents can achieve transfection efficiencies of up to 50% in primary skeletal muscle cultures when optimized (40).

Electroporation is another method to deliver miRNA mimics, which involves applying an electrical field to cells or tissues to temporarily increase the permeability of the cell membrane, allowing for the uptake of exogenous miRNAs. Skeletal muscle is a good target for electroporation due to its extensive network of capillaries (12). However, electroporation may cause muscle necrosis, which typically emerges from day 7 to day 14 after the procedure (41). Furthermore, electroporation will face limitations if the target skeletal muscle region is too large since the electrode is small in size. The small range between the electrodes, usually around 1 cm, will limit the transfer of genes to a large area of tissues, such as degenerated human gastrocnemius muscle (12). Nevertheless, miR-29c delivered to the tibialis anterior through electroporation increased muscle mass by 40% in the mice model, proving a preliminary success in combating muscle atrophy (42).

Preclinical and Clinical Studies

Studies have proven miR-29's effectiveness in anit-fibrosis and even muscle regrowth (13, 14). In research conducted by Su *et al*, four treatment groups: miR-29a, human umbilical cord mesenchymal stem cells (uMSCs), combined miR29a and uMSCs, and a control group, were tested on the bupivacaine (BPVC)-induced gastrocnemius muscle injury in male C57BL/6 mice. Following the injection of BPVC, it was observed that miR-29a expression was consistently suppressed in the injured group compared to the saline sham group across 3-day, 5-day, and 21-day post-injury. This suppression was specific to miR-29a, as no significant changes were detected in the expression levels of miR-29b and miR-29c between the BPVC and sham groups. Compared to the control group, both miR-29a alone (47%) and stem cells alone (62%) significantly improved blood perfusion recovery. However, their combination achieved 97% recovery, demonstrating a synergistic effect to yield the most substantial recovery. In the control group, mice exhibited severe fibrosis, characterized by markedly elevated ECM component levels such as COL1A1, COL3A1, and fibronectin. The levels of tissue inhibitors of metalloproteinases (TIMPs) that hinder ECM degradation are also observed. Fibrotic tissue occupies 57% of the gastrocnemius muscle area after BPVC injury, compared to only 8% in the sham group. Treatment with miR-29a alone resulted in a 51% reduction in BPVC-induced collagen deposition. The fibrosis area was reduced to approximately 27% of the gastrocnemius muscle by down-regulating COL1A1, COL3A1, and fibronectin. Similarly, transplantation of uMSCs alone demonstrated a 61% reduction in fibrosis, lowering the fibrotic tissue area to approximately 22.14%. The combination therapy of miR-29a and uMSCs exhibited a synergistic therapeutic effect, with a 74% reduction in fibrosis, reducing the fibrotic area to around 15% (43).

Moreover, a study by Heller *et al*. (2017) demonstrated that treating Duchenne muscular dystrophy (DMD) mouse models with miR-29 and micro-dystrophin led to significant recovery in the gastrocnemius muscle. The miR-29 treatment alone reduced the collagen deposition by 18% and increased specific (force generated per unit of cross-sectional area of muscle) and absolute force (total force output of the muscle unit) compared to untreated mice. However, when miR-29 is combined with micro-dystrophin therapy, results indicate a substantial increase in both specific and absolute muscle force compared to either treatment alone or no treatment at all. Remarkably, the muscle function of the treated mice showed no significant differences when compared to wild-type mice, underscoring the versatility and cooperative potential of miR-29 and micro-dystrophin in restoring muscle strength (44).

The clinical evaluation of MRG-201, a synthetic mimic of miR-29, represents a significant advancement in fibrosis research. Two trials, phase 1 between November 2015 and January 2017, and phase 2 between August 2018 and December 2023, conducted by MiRagen Therapeutics, explore the safety, tolerability, and efficacy of MRG-201 for fibrotic conditions, specifically keloid formation (45, 46).

The phase 1 trial was a double-blind, placebo-controlled, single- and multiple-dose escalation study involving 54 healthy male volunteers aged 18–65. This trial assessed the safety and pharmacokinetics of escalating doses (0.5 mg, 1 mg, 2 mg) administered via intradermal injection. While no detailed quantitative data on results has been published, the study confirmed that MRG-201 was

well-tolerated, with no serious adverse events reported. The pharmacodynamic activity demonstrated reduced collagen expression and delayed fibroplasia (development of fibrotic tissue) onset at injection sites, establishing the foundation for further efficacy studies.

The phase 2 trial was a double-blind, placebo-controlled study involving 6 cohorts of 12-16 subjects, in total of 96 participants, with a history of keloid formation. Participants received six doses of MRG-201 (5.3 mg) over two weeks at one excisional wound site and a placebo at another wound site, serving as their own controls. The primary endpoint was the percentage of confirmed keloid formation at 24 and 52 weeks, assessed using the modified Vancouver Scar Scale. At 52 weeks, keloid formation was observed in 100% of placebo-treated sites but reduced to 67% in MRG-201-treated sites. Adverse events were minimal, with no wound-related complications.

DISCUSSIONS

Muscle fibrosis, characterized by excessive ECM deposition and collagen overproduction, limits rehabilitation outcomes and causes muscle atrophy (13, 14). Emerging research highlights the regulatory role of microRNAs, notably miR-29, in modulating collagen synthesis pathways. The interplay between miR-29 and TGF-β and Wnt pathways is pivotal in fibrosis regulation (20-23). Restoration or overexpression of miR-29 in preclinical and clinical models has demonstrated significant antifibrotic (43, 45, 46). The most prevalent method used to deliver miR-29 is through the Adeno-associated virus although alternative methods such as nanoparticles, lipid-based transfection reagents, and electroporation are also used.

Therapeutic Potential of miR-29

The therapeutic potential of miR-29 is clearly promising in the context of skeletal muscles proven by its evident antifibrotic properties. Its mRNA targets and corresponding protein expressions are well-studied, providing a strong foundation for its application in fibrosis treatment. The reduction in collagen production achieved through miR-29 therapy has been shown to significantly decrease collagen deposition while addressing pathologies involved in muscle fibrosis. Notably, combination therapies, such as working alongside human umbilical cord mesenchymal stem cells and micro-dystrophin therapy, have exhibited synergistic effects in reducing fibrotic tissue and enhancing muscle (43, 44). However, all miR-29 trials in the context of skeletal muscles remain at the preclinical level on murine models. This can be attributed to various reasons including lack of funding and ethical concerns around using human models. This limitation underscores the need for future clinical trials to explore miR-29's application in skeletal muscle fibrosis and its role in mitigating muscle atrophy.

Preclinical and Clinical Trials

Currently, there are only two clinical studies on miR-29, both conducted by miRagen Therapeutics (MRG-201). Although both trials examined and verified miR-29's potential in collagen regulation, their focus was on epidermal keloids rather than skeletal muscles. The absence of clinical trials specifically targeting miR-29's application in skeletal muscle fibrosis renders its safety and efficacy inconclusive in this context. Moreover, the miRagen trials recruited a minimal number of participants—54 and 96 for trial one and trial two, respectively (45, 46). Furthermore, detailed data from the first trial is not publicly available, limiting transparency and hindering a comprehensive understanding of miR-29's therapeutic potential. Expanding clinical trials with a broader participant base and transparent reporting is essential to address these gaps.

Limitations

One significant limitation lies in the delivery methods for miR-29. While Adeno-associated viruses (AAVs) are a commonly employed vector, challenges such as off-target effects. Although the use of specific serotypes like AAV2 exhibits a natural tropism for skeletal muscle cells that improves targeting, it does not have the ability to target a specific muscle region Furthermore, the choice of promoters remains a critical issue. Generic promoters can lead to non-specific expression, whereas endogenous promoters, though more precise, may exceed the AAV packaging capacity. Similarly, nanoparticle-based delivery, although advantageous in protecting miR-29 mimics from degradation, faces the hurdle of low targeting accuracy. Although muscle-specific ligands M12 peptides have shown promise in increasing skeletal muscle tissue selectivity, their application only differentiates muscle cells from non-muscle cells, not the location of the skeletal muscle cells. This leads to expected off-target effects as non-fibrotic regions unintendedly receive miR-29 mimics.

Moreover, while miR-29 selectively targets fibrotic collagens (e.g., types I and III) without affecting ECM structural proteins like laminin and fibronectin, its broader effects on muscle tissue homeostasis remain inadequately

characterized. Any unintended disruption to ECM balance could impair muscle function or exacerbate recovery challenges, highlighting the need for comprehensive studies to delineate its downstream effects.

Finally, the translational gap between preclinical findings and human application presents a significant obstacle. *In vitro* and animal models, while valuable, fail to capture the full complexity and heterogeneity of human muscle fibrosis. Variability in miRNA expression between individuals introduces another layer of uncertainty, necessitating large-scale clinical trials to validate both the safety, efficacy, and dosage management of miR-29 therapies. Additionally, further research on diverse populations is also required to approve miR-29's application in pediatric and prenatal settings. In conclusion, mir-29's therapeutic potential is promising, yet more research needs to be done to address the aforementioned current limitations to fully unlock its therapeutic potential.

CONCLUSION

MicroRNA-29 has demonstrated promising efficacy in mitigating fibrosis-induced muscle atrophy, particularly by addressing abnormal collagen deposition. Despite its theoretical success, the practical application of miR-29, whether through template or mimic delivery, remains constrained by significant gaps in research. Clinical studies are particularly needed to refine delivery strategies, optimize dosage, and address safety concerns to advance miR-29 from experimental therapy to clinical use. Challenges such as the limitations of current delivery methods, variability in efficacy, and uncertain dosage management underscore the complexity of translating miR-29 therapies into clinical practice. Targeted miR-29 research in the specific context of skeletal muscle fibrosis will be essential for uncovering its full therapeutic potential for effective treatments for muscle fibrosis and atrophy.

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DECLARATION OF CONFLICT OF INTERESTS

Evan (Yiwen) Sun declares no conflicts of interest.

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