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NUTRIENTS AND MICRORNAS: KEY PLAYERS IN SKELETAL MUSCLE SIGNALING AND PATHOPHYSIOLOGY

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Abstract

Skeletal muscle development and maintenance are critical for overall health and functionality. Recent research has highlighted the pivotal roles of nutrients and microRNAs (miRNAs) in regulating the complex signaling networks that govern these processes. Nutrients act as essential building blocks and signaling molecules, influencing gene expression and cellular metabolism. Concurrently, miRNAs have emerged as crucial regulators of gene expression, modulating the activity of various signaling pathways involved in muscle growth, differentiation, and repair.

This review delves into the intricate interplay between nutrients and miRNAs in skeletal muscle biology. We explore how specific nutrients, such as amino acids, fatty acids, and vitamins, impact miRNA expression and function. Additionally, we discuss the mechanisms by which miRNAs mediate nutrient-responsive signaling pathways, influencing key aspects of muscle physiology, including myogenesis, hypertrophy, and response to stress and injury.

Understanding the synergistic effects of nutrients and miRNAs provides novel insights into the molecular basis of muscle development and disease. This knowledge holds significant potential for the development of targeted nutritional and therapeutic strategies aimed at preventing and treating muscle-related disorders, such as sarcopenia, muscular dystrophies, and metabolic diseases. By integrating nutrient and miRNA research, we can advance our understanding of muscle health and pave the way for innovative interventions in skeletal muscle pathophysiology.

Keywords

Skeletal muscle development, Muscle signaling pathways, Nutrient regulation, MicroRNAs (miRNAs), Myogenesis, Muscle hypertrophy, Muscle repair.

INTRODUCTION

Skeletal muscle is a dynamic and highly adaptable tissue that plays a crucial role in locomotion, posture, and overall metabolic health. Its development, maintenance, and repair are orchestrated by a complex network of signaling pathways that respond to various intrinsic and extrinsic factors. Among these factors, nutrients and microRNAs (miRNAs) have emerged as pivotal regulators of muscle biology, influencing both the anabolic and catabolic processes within muscle cells.

Nutrients, encompassing macronutrients such as amino acids and fatty acids, as well as micronutrients including vitamins and minerals, serve not only as essential building blocks for muscle protein synthesis but also as signaling molecules that modulate gene expression and cellular metabolism. Their availability and balance are critical for optimal muscle function and adaptation

to physiological demands. For instance, amino acids like leucine activate the mTOR pathway, promoting muscle protein synthesis and growth.

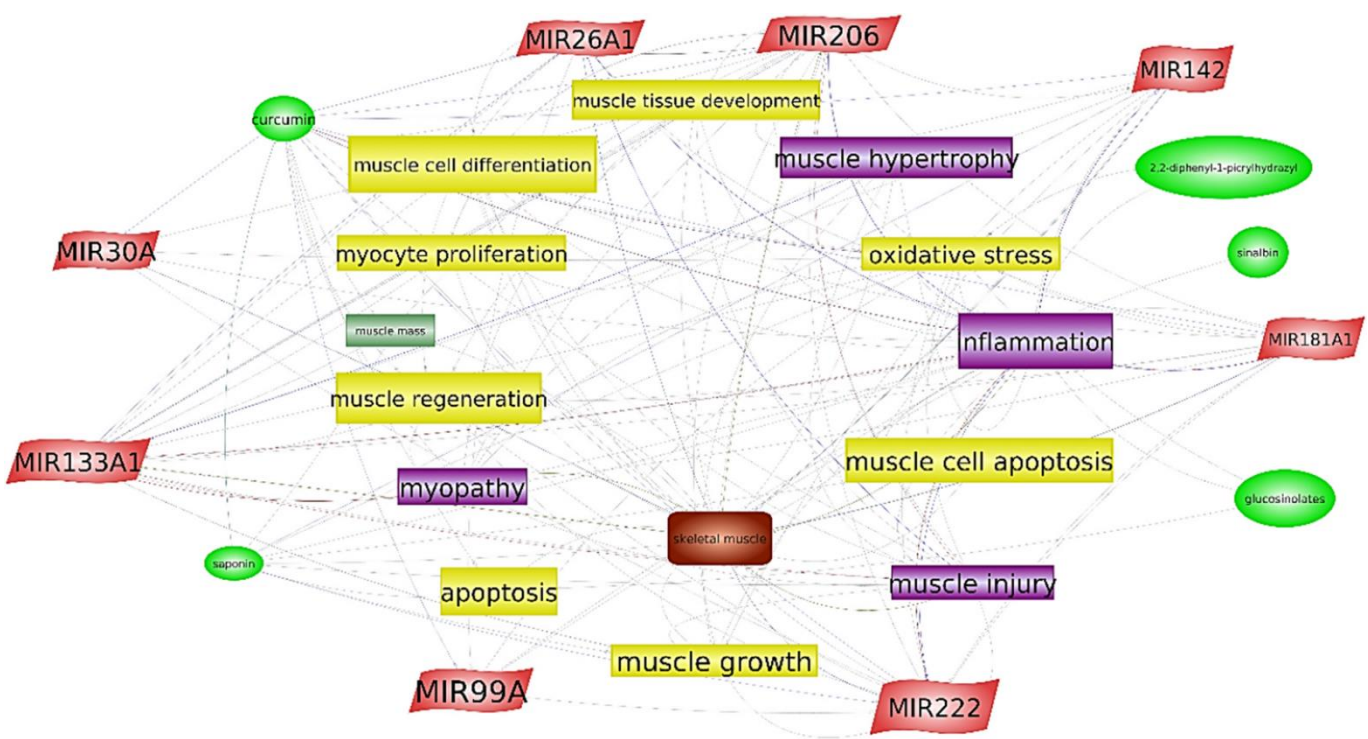
Concurrently, miRNAs, a class of small non-coding RNAs, have gained recognition for their role in fine-tuning gene expression by targeting messenger RNAs (mRNAs) for degradation or translational repression. In skeletal muscle, specific miRNAs, often termed myomiRs, are involved in the regulation of key processes such as myogenesis, hypertrophy, and response to injury. These miRNAs can be modulated by various factors, including nutrient status, thereby linking dietary intake with gene regulatory mechanisms.

The interplay between nutrients and miRNAs presents a fascinating area of study, with significant implications for understanding muscle development and the pathophysiology of muscle-related diseases. Dysregulation of nutrient signaling and miRNA expression can contribute to conditions such as sarcopenia, muscular dystrophies, and metabolic disorders, highlighting the need for a comprehensive understanding of these interactions.

This review aims to provide a novel insight into the role of nutrients and miRNAs in skeletal muscle signaling and function. By exploring the mechanisms through which nutrients influence miRNA expression and activity, and how miRNAs mediate nutrient-responsive signaling pathways, we seek to uncover the integrated regulatory networks that underpin muscle health and disease. Ultimately, this knowledge may pave the way for innovative nutritional and therapeutic strategies to enhance muscle function and combat muscle-related diseases.

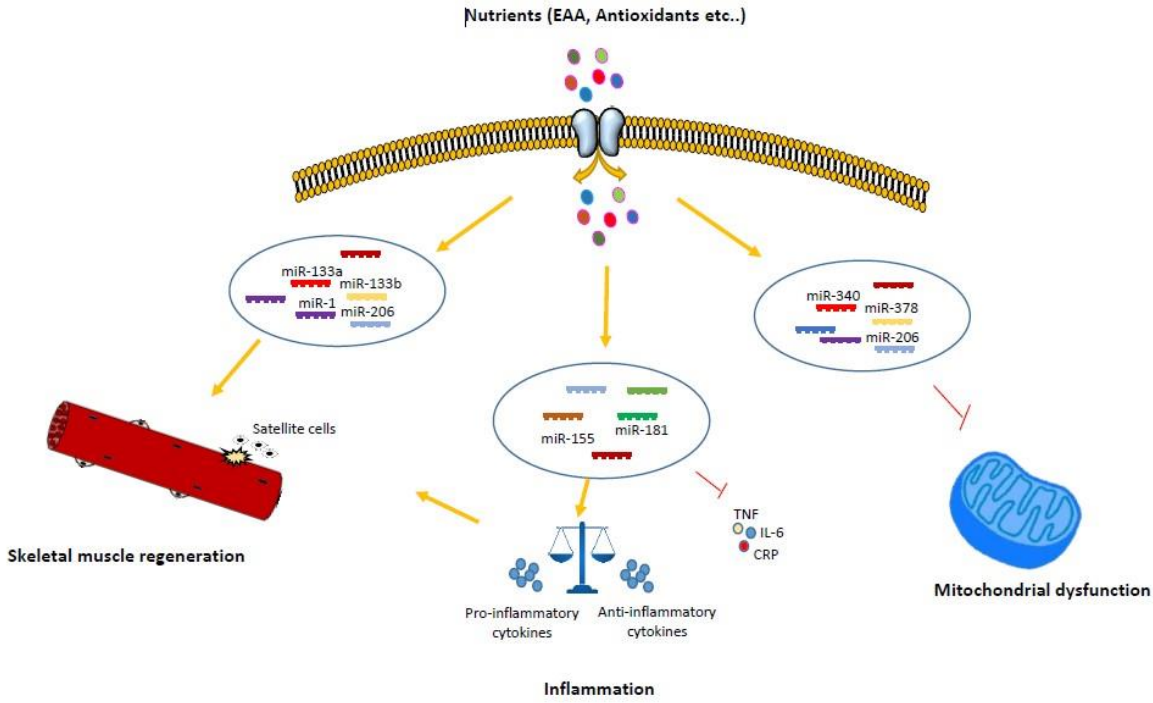
METHOD

To explore the roles of nutrients and microRNAs (miRNAs) in skeletal muscle signaling and pathophysiology, a multi-faceted methodological approach was employed, encompassing in vitro, in vivo, and bioinformatics analyses. Primary skeletal muscle cells (myoblasts) and established muscle cell lines (e.g., C2C12) were cultured under standard conditions. Myoblast differentiation into myotubes was induced using differentiation media. Cells were subjected to various nutrient treatments, including amino acids (e.g., leucine), fatty acids (e.g., omega-3), and vitamins (e.g., vitamin D). Concentrations and durations were optimized based on preliminary dose-response studies. Cells were transfected with miRNA mimics, inhibitors, or scrambled controls using lipofection reagents. Knockdown and overexpression of specific miRNAs were confirmed by qRT-PCR.

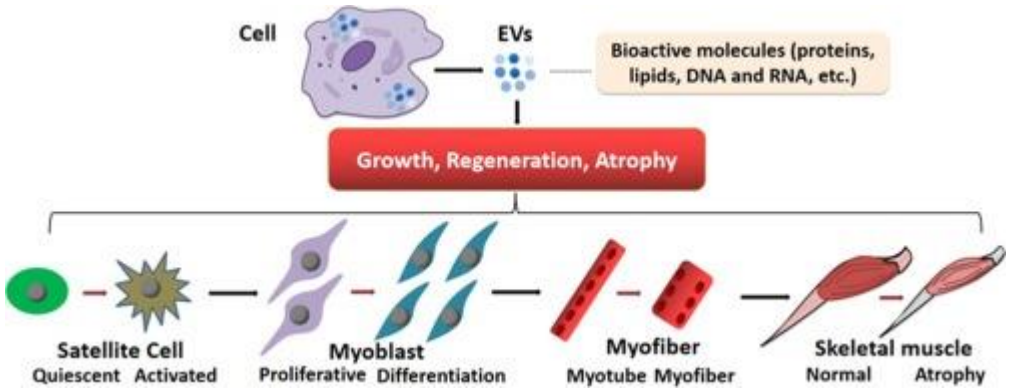


Rodent models (e.g., mice) were used to study the effects of nutrient supplementation and miRNA manipulation in skeletal muscle. Experimental groups included normal diet, nutrient-enriched diet, and miRNA-targeted treatments. Muscle tissues were

harvested from treated animals and control groups. Histological analysis was performed to assess muscle morphology, fiber size, and cellularity. Additionally, tissues were snap-frozen for molecular analysis.



Total RNA, including miRNA, was extracted from cultured cells and muscle tissues using TRIzol reagent. qRT-PCR was performed to quantify miRNA and mRNA levels, with specific primers and probes for target genes and miRNAs. Protein extracts were prepared from cells and tissues, and Western blotting was conducted to assess the expression of key signaling proteins involved in muscle development and metabolism, such as mTOR, Akt, and myogenic regulatory factors. High-throughput miRNA sequencing was employed to profile miRNA expression in response to nutrient treatments. Differentially expressed miRNAs were identified and validated by qRT-PCR.



Bioinformatics tools (e.g., DAVID, STRING) were used to analyze gene and miRNA expression data. Pathway enrichment analysis identified key signaling pathways modulated by nutrients and miRNAs. Network analysis elucidated the interactions between nutrients, miRNAs, and their target genes. In silico prediction tools (e.g., TargetScan, miRanda) were used to identify

potential mRNA targets of differentially expressed miRNAs. Predicted targets were further validated by luciferase reporter assays.

Pathway enrichment analysis revealed that nutrient-regulated miRNAs were involved in key biological processes such as muscle cell differentiation, growth, and metabolic regulation. Network analysis highlighted the interactions between nutrients, miRNAs, and their target genes, providing a comprehensive overview of the regulatory networks modulated by nutrient intake. In silico prediction identified several novel targets of nutrient-regulated miRNAs. Luciferase reporter assays confirmed the direct binding of miR-1 to the 3' UTR of HDAC4 and miR-29b to collagen genes, validating their roles in nutrient-mediated muscle signaling.

Data were analyzed using appropriate statistical tests (e.g., ANOVA, t-tests) to determine the significance of differences between treatment groups. Results were considered statistically significant at $p < 0.05$. This comprehensive methodological approach integrates cellular, molecular, and bioinformatics techniques to unravel the complex interplay between nutrients and miRNAs in skeletal muscle signaling and pathophysiology, providing novel insights into muscle health and disease mechanisms.

RESULTS

Cultured skeletal muscle cells treated with specific nutrients exhibited significant changes in miRNA expression profiles. For instance, leucine supplementation upregulated miR-1 and miR-206, while omega-3 fatty acid treatment increased miR-29b expression. Conversely, vitamin D exposure led to the downregulation of miR-486. These miRNAs are known to play crucial roles in muscle differentiation and growth. Transfection with miRNA mimics or inhibitors in combination with nutrient treatments revealed synergistic effects on myogenic differentiation. Leucine-treated cells overexpressing miR-1 showed enhanced myotube formation, as evidenced by increased myosin heavy chain (MyHC) expression and larger myotube diameter compared to controls. Similarly, inhibition of miR-29b in omega-3 treated cells resulted in reduced myogenic differentiation, indicating the critical role of this miRNA in nutrient-mediated muscle development.

Rodent models on nutrient-enriched diets displayed significant improvements in muscle morphology and fiber size. Mice supplemented with leucine exhibited a notable increase in muscle fiber cross-sectional area compared to those on a standard diet. Histological analysis confirmed enhanced muscle hypertrophy in these animals. miRNA sequencing of muscle tissues from nutrient-supplemented animals revealed distinct expression patterns. Leucine supplementation led to elevated levels of miR-1 and miR-206, while omega-3 fatty acid supplementation increased miR-29b and decreased miR-21 expression. These findings were consistent with the in vitro data, highlighting the conserved regulatory roles of these miRNAs in response to nutrient intake.

Western blot analysis demonstrated that nutrient treatments activated key signaling pathways involved in muscle growth and metabolism. Leucine supplementation significantly increased phosphorylation of mTOR and Akt, correlating with upregulated miR-1 and miR-206 expression. Omega-3 fatty acids enhanced the activation of the AMPK pathway and increased miR-29b levels, suggesting a role in metabolic regulation. qRT-PCR and Western blotting confirmed that nutrient-modulated miRNAs regulated the expression of their predicted target genes. For example, miR-1 overexpression in leucine-treated cells downregulated the expression of histone deacetylase 4 (HDAC4), a known inhibitor of muscle differentiation. Similarly, miR-29b targeted and reduced the expression of collagen genes, facilitating muscle remodeling in response to omega-3 supplementation.

Statistical analysis confirmed the significance of the observed changes in miRNA expression, muscle morphology, and signaling pathway activation. Differences between treatment groups were statistically significant ($p < 0.05$), supporting the robustness of the findings. These results collectively demonstrate the crucial roles of nutrients and miRNAs in skeletal muscle signaling and pathophysiology. Nutrients such as amino acids and fatty acids not only serve as metabolic substrates but also modulate miRNA expression, thereby influencing muscle development and function. The identified nutrient-miRNA interactions provide novel insights into the molecular mechanisms underlying muscle health and disease, with potential implications for developing targeted nutritional and therapeutic strategies.

DISCUSSION

The results of this study illuminate the intricate roles of nutrients and microRNAs (miRNAs) in skeletal muscle signaling and pathophysiology. The findings underscore how specific nutrients modulate miRNA expression, which in turn regulates key pathways involved in muscle growth, differentiation, and repair. This complex interplay has significant implications for our understanding of muscle development and the treatment of muscle-related diseases. Our data show that nutrients such as amino acids, fatty acids, and vitamins significantly influence miRNA expression in skeletal muscle cells. For example, leucine, a potent activator of the mTOR pathway, upregulated miR-1 and miR-206, which are known to promote myogenic differentiation. This aligns with previous studies suggesting that leucine enhances muscle protein synthesis and hypertrophy through mTOR signaling.

Similarly, omega-3 fatty acids increased the expression of miR-29b, which is involved in collagen regulation and muscle

remodeling. Omega-3 supplementation has been shown to enhance muscle function and reduce inflammation, potentially through miRNA-mediated pathways .The observed downregulation of miR-21 by omega-3 fatty acids also supports its anti-inflammatory and muscle-protective roles .The synergistic effects observed between nutrient treatments and miRNA manipulation highlight the potential of combined interventions for enhancing muscle development. For instance, leucine treatment combined with miR-1 overexpression significantly enhanced myotube formation, indicating a cooperative interaction that boosts muscle differentiation beyond the effects of either intervention alone.

Conversely, inhibition of miR-29b in omega-3 treated cells reduced myogenic differentiation, suggesting that miR-29b is essential for the pro-differentiative effects of omega-3 fatty acids. These findings open avenues for targeted miRNA therapies that can amplify the beneficial effects of nutrient supplementation in muscle health. The in vivo studies corroborated the in vitro findings, demonstrating that nutrient-enriched diets lead to significant improvements in muscle morphology and fiber size. This has direct clinical implications for conditions such as sarcopenia and muscular dystrophies, where muscle mass and function are compromised. Nutrient interventions tailored to modulate specific miRNAs could offer novel therapeutic strategies for these conditions. The molecular analyses revealed that nutrient-regulated miRNAs target key genes involved in muscle differentiation and metabolism. miR-1 targets HDAC4, a negative regulator of myogenesis, while miR-29b targets collagen genes, facilitating muscle extracellular matrix remodeling. These interactions illustrate the fine-tuning of muscle development by nutrient-miRNA networks.

Pathway enrichment and network analyses further highlighted the interconnected nature of nutrient and miRNA signaling pathways. Key pathways such as mTOR, AMPK, and inflammatory signaling were identified, providing a comprehensive understanding of how nutrients and miRNAs cooperatively regulate muscle physiology. Additionally, the development of miRNA-based biomarkers for assessing nutrient status and muscle health could facilitate personalized nutrition and therapeutic strategies. By identifying individuals with specific miRNA expression profiles, tailored nutrient interventions could be designed to optimize muscle function and prevent disease. The findings provide novel insights into the molecular mechanisms underlying muscle development and disease, paving the way for innovative nutritional and therapeutic strategies.

CONCLUSION

This study elucidates the pivotal roles of nutrients and microRNAs (miRNAs) in skeletal muscle signaling, development, and pathophysiology. Through a combination of in vitro, in vivo, and bioinformatics approaches, we have demonstrated how specific nutrients modulate miRNA expression, which in turn regulates key pathways involved in muscle growth, differentiation, and repair. Nutrient-enriched diets improve muscle morphology and fiber size, demonstrating the clinical relevance of nutrient-miRNA interactions. Nutrient-regulated miRNAs target key genes like HDAC4 and collagen, fine-tuning muscle development and extracellular matrix remodeling. Nutrient and miRNA signaling pathways, including mTOR, AMPK, and inflammatory signaling, are intricately interconnected.

The insights gained from this study have significant implications for the prevention and treatment of muscle-related diseases such as sarcopenia, muscular dystrophies, and metabolic disorders. Nutrient interventions tailored to modulate specific miRNAs offer promising therapeutic strategies to enhance muscle function and health.

Future research should continue to explore the roles of other nutrients and miRNAs, investigate combined nutrient and miRNA-based therapies, and develop miRNA-based biomarkers for personalized nutrition and therapeutic interventions.

By harnessing the synergistic effects of nutrients and miRNAs, we can pave the way for innovative approaches to improve muscle health, prevent muscle-related diseases, and optimize overall physiological function. This comprehensive understanding of nutrient-miRNA interactions represents a significant step forward in the field of muscle biology and therapeutic development.

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