Circulating miRNA response to Kinesiological intervention in runners with musculoskeletal pain: a pilot study integrating expression and network analysis.

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Abstract

Background: Musculoskeletal pain is highly prevalent among runners and often impairs performance and quality of life. Exercise-based interventions, such as kinesiology-guided programs, are increasingly used to promote recovery and prevent recurrence, yet individual responses vary widely. Circulating miRNA may serve as molecular biomarkers to elucidate underlying mechanisms and predict therapeutic outcomes. This study aimed to assess changes in circulating miRNAs in runners following a kinesiology-based intervention and to explore their potential involvement in pain-related biological processes.

Methods: Seventeen long-distance runners with musculoskeletal pain underwent a six-week kinesiology-based intervention. Pain intensity and physical activity were assessed pre- and post-intervention. Plasma levels of four inflammation- and muscle-related miRNAs (hsa-let-7a-5p, hsa-miR-133b, hsa-miR-146a-5p, and hsa-miR-155-5p) were quantified using qRT-PCR. Bioinformatic analyses were conducted to explore molecular networks involving the most responsive miRNAs.

Results: A general trend of miRNA downregulation was observed post-intervention, with a statistically significant reduction in hsa-let-7a-5p ($p = 0.035$) and a near-significant decrease in hsa-miR-133b ($p = 0.068$). No significant associations were found between miRNA changes and pain remission in regression analysis. However, integrative network and pathway analyses revealed the involvement of hsa-let-7a-5p and hsa-miR-133b in molecular pathways related to inflammation, tissue remodeling, and neuroimmune signaling.

Discussion: The observed modulation of hsa-let-7a-5p and hsa-miR-133b suggests that these miRNAs may be sensitive to physiological changes induced by kinesiological intervention, potentially reflecting systemic adaptations rather than directly mediating pain remission. The lack of correlation with pain reduction may be due to the small sample size or the multifactorial nature of pain modulation. Nonetheless, the bioinformatic evidence highlights plausible biological mechanisms that merit further investigation.

Conclusions: These findings suggest that hsa-let-7a-5p and hsa-miR-133b could reflect biological adaptations to functional recovery, even if not predictive of clinical outcomes. Further research is needed to validate their role in

musculoskeletal rehabilitation and to assess their potential utility in guiding personalized exercise-based strategies.

Keywords: microRNA, musculoskeletal pain, kinesiology, runners, bioinformatic analysis, molecular pathways

1. Introduction

Musculoskeletal pain is a widespread and often debilitating condition, affecting an estimated 1.71 billion people globally [1]. It encompasses discomfort in muscles, tendons, ligaments, and bones, significantly impairing physical function and overall well-being [2]. This condition is particularly prevalent among athletes [3], with runners being especially affected due to the repetitive, high-impact nature of the activity [4]. In runners, musculoskeletal pain compromises performance, training continuity, and quality of life [5,6].

Contributing factors such as muscle weakness, misalignment, and excessive joint loading can lead to microtraumas and inflammation over time [7–9].

Given its multifactorial nature, musculoskeletal pain requires therapeutic strategies that not only alleviate symptoms but also restore function and prevent recurrence [6]. Among non-pharmacological approaches, general exercise is widely recognized as one of the most effective interventions, as it promotes tissue repair and modulates pain perception [10,11]. When implemented within a structured framework, this modulation may enhance mobility and engagement in active recovery, targeting underlying factors such as muscular imbalances or poor posture through improved strength, flexibility, and neuromuscular control [6,12].

In this context, kinesiology represents a structured and non-invasive approach, particularly in its preventive and adapted applications. Through personalized programs focused on postural control, flexibility, and strength, kinesiology may support symptom relief and promote long-term musculoskeletal health. However, despite its increasing use in functional recovery settings, the evidence supporting its specific efficacy for pain reduction remains limited [3,13,14].

Importantly, individual responses to exercise-based interventions can vary widely, influenced by factors such as genetic variability, baseline fitness, and the nature of the pain condition [15]. This heterogeneity highlights the need for biomarkers capable of predicting and monitoring therapeutic response.

Among the most promising candidates are circulating microRNAs (miRNAs), due to their stability in bodily fluids [16,17] and their ability to reflect physiological and pathological states [18–20]. MiRNAs are small (~22 nucleotides), non-coding RNA molecules that regulate gene expression by binding to the 3′-untranslated region of

messenger RNA (mRNA), thereby repressing translation [21]. They are involved in key biological processes, such as inflammation, tissue regeneration, and pain modulation [22–24], making them particularly relevant in the context of musculoskeletal disorders [25,26].

In particular, hsa-let-7a-5p has been shown to suppress inflammation by targeting pro-inflammatory cytokines and modulating immune cell function [27]. Hsa-miR-133b is associated with muscle regeneration, influencing key processes such as myogenesis and muscle repair [28,29]. Additionally, hsa-miR-146a-5p and hsa-miR-155-5p are known to regulate immune responses and inflammatory pathways [30,31].

Beyond miRNAs, long non-coding RNAs (lncRNAs), transcripts longer than 200 nucleotides, also regulate gene expression through diverse mechanisms [32–34], including interaction with miRNAs via competitive endogenous RNA networks [35,36]. The interplay between miRNAs and lncRNAs suggests their combined suggests a synergistic role in fine-tuning gene regulation during inflammation and recovery. However, their potential as predictive biomarkers in response to exercise-based functional interventions remains largely unexplored.

This pilot study investigated whether a kinesiology-guided functional recovery program [37,38] could modulate circulating miRNAs in runners with musculoskeletal pain and explored the potential relevance of such molecular changes in the context of pain-related biological processes. Understanding how circulating miRNAs respond to exercise-based interventions in individuals with musculoskeletal pain may provide insight into the molecular mechanisms underlying symptom resolution and tissue recovery. Additionally, *in silico* prediction of miRNA targets and their interactions with lncRNAs was performed to reconstruct a gene regulatory network and provide preliminary insight into the pathways potentially involved in the therapeutic response.

2. Materials and methods

2.1 Study design and participants

This pre-post pilot study is part of the MiMuS project *("microRNAs as biomarkers of musculoskeletal pain in long-distance runners"*), whose protocol and case-control study were previously detailed [26,39].

Seventeen long-distance runners, all aged 35 or older, affiliated with the Lecce branches of the Italian Federation of Athletics and holding valid medical certificates for competitive sports, participated in this study. Exclusion criteria included the presence of chronic diseases, current pregnancy, or having given birth or weaned a child within the past six months.

Pain intensity and physical activity levels were collected through the Numerical Rating Scale (NRS) [40] and the short version of the International Physical Activity Questionnaire (IPAQ) [41], respectively. Both questionnaires were administered at both the beginning and the end of the intervention.

The study protocol was approved by the Ethics Committee of the Lecce Local Health Authority (ASL/LE) (deliberation no. 0000108, February 2, 2023) and was retrospectively registered at ANZCTR (https://www.anzctr.org.au/TrialSearch.aspx) under the registration number ACTRN12625000120471.

2.2 Kinesiological intervention

Participants underwent a kinesiological intervention based on the Canali Postural Method (CPM), a technique designed to identify and correct compensatory postural patterns to prevent, reduce, or eliminate them. CPM emphasizes individualized gymnastic exercises, targeting not the painful area itself but rather peripheral muscular factors, such as imbalances or resistance, that may contribute to pain [37,38].

The intervention lasted six weeks, with three sessions per week. In each week, one session was conducted at the Posture and Movement Laboratory (MP-LAB) of the Institute of Clinical Physiology, under the supervision of a CPM-certified operator. The remaining two sessions were performed independently by the participants in their usual training environments.

During the first session at the MP-LAB, each participant underwent a postural assessment to identify misalignments and potential muscular causes contributing to discomfort or pain. The objective was to define a personalized exercise program

aimed at eliminating muscular resistances (e.g., excessively rigid leg flexor muscles) and/or balancing muscular dominance patterns (e.g., excessive recruitment of the quadriceps during abdominal exercises due to core muscle weakness). Exercises were selected based on the individual needs of each participant. Even when two individuals were assigned the same general exercise, the specific execution could differ in terms of angle adjustments or the inclusion of facilitatory modifications to optimize the outcome. For instance, an exercise targeting leg flexor muscle stretching (specifically the biceps femoris) could be performed in its standard form by one participant (Figure 1A), while another participant requiring modifications due to discomfort at the tendinous level in the popliteal region would perform a facilitated version (Figure 1B). Each participant was instructed to focus on the sensation of tension in the muscle belly rather than at the tendinous insertion. If discomfort or pain was reported in the popliteal region while performing the exercise in its standard form (Figure 1A), the facilitated version (Figure 1B) was recommended. This modification involved angle adjustments to redirect tension to the muscle belly, optimizing effectiveness while minimizing discomfort.

Fig. (1). Stretching of the leg flexor muscles at the MP-LAB in the standard form (A) and in the facilitated form (B).

During all MP-LAB sessions, participants were given a practical demonstration of the exercises and how to perform them independently. The correct execution was

verified weekly, during the session conducted at the MP-LAB, ensuring uniformity in the application of the protocol.

2.3 RNA isolation and quantification of miRNA

Before and after the kinesiological intervention, fasting venous blood samples were collected from participants in the morning, using EDTA-containing vacutainer tubes to prevent coagulation. Plasma was isolated by centrifuging at 1900 g for 10 minutes at 4°C, aliquoted, and stored at -80°C until RNA extraction.
Total RNA was extracted from plasma samples using the Quick-cfRNA™ Serum & Plasma Kit (Zymo Research, Orange, CA, USA) according to the manufacturer’s protocol. RNA concentration and purity were assessed with a NanoDrop spectrophotometer. Complementary DNA (cDNA) synthesis was performed with the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's recommended thermal cycling conditions: 16°C for 30 minutes, 42°C for 30 minutes, and 85°C for 5 minutes. Quantitative real-time PCR (qRT-PCR) was conducted using TaqMan Universal PCR Master Mix II and specific TaqMan miRNA Assays (Applied Biosystems, Foster City, CA, USA). The expression levels of hsa-miR-16-5p were used as an internal reference to normalize miRNA expression. All PCR reactions were performed in duplicates. Relative expression levels were calculated using the $2^{\wedge-\Delta\Delta Ct}$ method [42].
The miRNAs selected for this analysis — hsa-miR-146a-5p (Assay ID: 000468), hsa-miR-155-5p (Assay ID: 467534_mat), hsa-miR-133b (Assay ID: 002085), and hsa-let-7a-5p (Assay ID: 000377) — were the same as those previously analyzed in our case-control study [26], where they were identified as key regulators involved in the pathogenesis of pain and inflammation [25,43].

2.4 Functional and Network Analysis of miRNA-Associated Targets

To explore the regulatory roles of miRNAs, we first identified lncRNAs interacting with them using the miRNet database, a comprehensive and user-friendly platform that integrates microarray, RNA-seq, and RT-qPCR data to construct, visualize, and analyze miRNA-target interaction networks, thereby providing deeper biological insights [44].

Subsequently, experimentally validated mRNA targets were retrieved from miRTarBase (https://bio.tools/mirtarbase, accessed on 20 June 2024), considering only interactions supported by strong experimental evidence (e.g., reporter assays, western blotting) [45].

To explore the protein–protein interaction (PPI) networks associated with the miRNA-targeted mRNAs, the Search Tool for the Retrieval of Interacting Genes (STRING) database was used [46]. The Markov Clustering (MCL) algorithm was applied to group proteins into clusters based on interaction strength, thus identifying sets of functionally related or interconnected proteins.

To further elucidate the regulatory landscape, a lncRNA-miRNA-mRNA interaction network was constructed using Cytoscape (https://cytoscape.org/, accessed on 22 September 2021) [47]. Functional enrichment analysis was carried out using the Database for Annotation, Visualization, and Integrated Discovery (DAVID), assessing Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [48]. GO analysis included the three main categories: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). The most relevant biological functions and pathways were reported, with statistical significance determined by a p-value < 0.05 and False Discovery Rate correction applied for multiple testing.

2.5 Statistical analysis

Categorical variables were presented as absolute frequencies and percentages, while continuous variables were reported as either mean ± standard deviation or median with interquartile range (25th–75th percentile), depending on the distribution of the data. Normality was assessed using the Shapiro–Wilk test.

The significance of changes in miRNA expression levels was evaluated using the non-parametric Wilcoxon signed-rank test. Statistical significance was set at $p < 0.05$, and all tests were two-tailed. In cases where statistically significant pre-post differences were observed, univariate logistic regression analysis was performed to assess the association between miRNA variation and pain remission.

All statistical analyses were conducted using the open-source software R, version 4.2.0 [49].

3. Results

3.1 Characteristics of participants

The baseline characteristics of the 17 enrolled runners were summarized in Table 1.

Table 1. Subject characteristics at baseline (N = 17).

Male, N (%)	**Age years, mean (SD)**	**BMI (kg/m2), mean (SD)**	**Physical activity (METs/min/week) Total, median (25–75%)**
14 (82.4%)	50.9 (7.45)	23.6 (2.3)	10102 (8445–12240)

Of these, 14 participants (82.4%) completed the six-week intervention; among them, 11 were male. Three participants withdrew prior to the conclusion of the study due to reasons unrelated to the intervention.

Complete remission of pain was reported by six participants (42.9%), while all but one of the remaining participants reported a reduction in pain intensity.

Most participants (71.4%) maintained high levels of physical activity through the intervention period.

3.2 Analysis of the effects of the Kinesiological intervention on miRNA levels

Baseline levels of the four analyzed miRNAs were not significantly associated with pain remission following the intervention (all $p>0.25$). Consequently, the main analyses focused on changes in miRNA expression (pre–post intervention).

Figure 2 illustrated the variations in selected miRNAs following the kinesiological intervention. A general down-regulation in miRNA expression was observed post-intervention, except for hsa-miR-155-5p, which remained upregulated. Among the analyzed miRNAs, the change in plasma levels of hsa-let-7a-5p reached statistical significance ($p = 0.035$), while hsa-miR-133b showed a trend toward significance ($p = 0.068$). Conversely, no significant changes were observed for hsa-miR-146a-5p ($p = 0.14$) and hsa-miR-155-5p ($p = 0.46$).

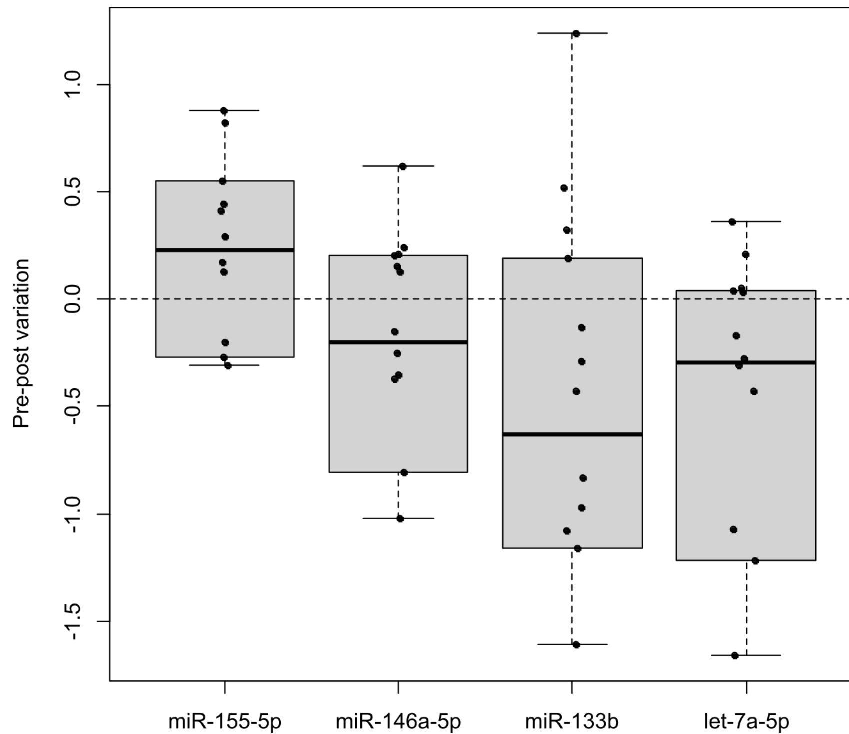


Fig. (2). Box plot of the pre-post variation of plasma miRNAs.

Moreover, none of the miRNAs showing statistically significant or borderline variation emerged as independent predictor of pain remission in the logistic regression analysis (p >0.25). Although these miRNAs were not identified as independent predictors of clinical pain remission, this does not preclude their potential biological involvement in treatment response mechanisms. The lack of statistical significance in the logistic regression may reflect limitations in sample size or clinical heterogeneity, rather than the absence of a true biological effect. Therefore, to further explore their potential mechanistic roles, a bioinformatic analysis was conducted focusing on hsa-let-7a-5p and hsa-miR-133b, the two miRNAs that showed statistically significant or near-significant changes following the intervention.

3.3 Integrative Functional and Network Analysis of hsa-let-7a-5p and hsa-miR-133b Targets

Target prediction for hsa-let-7a-5p and hsa-miR-133b was performed using the miRNet database, which identified 53 lncRNAs associated with hsa-let-7a-5p and 19 lncRNAs associated with hsa-miR-133b (Table 2).

Table 2. The lncRNA-miRNA pairs predicted by miRNet database (the lncRNA names are arranged in alphabetical order).

ID	lncRNA
hsa-let-7a-5p	ARHGAP27P1-BPTFP1-KPNA2P3, CARMN, CDKN2B-AS1, DRAIC, G2E3-AS1, HCG18, HEIH, HELLPAR, HOXA11-AS, IER3-AS1, IQCH-AS1, KCNQ1OT1, LINC00265, LINC00294, LINC00665, LINC00885, LINC00894, LINC00963, LINC01001, LINC01678, LINC01806, LINC01978, LINC02242, LINC02381, LINC02432, LMCD1-AS1, MEG8, MIR29B2CHG, MIR99AHG, MIRLET7BHG, MUC20-OT1, NEAT1, NUTM2A-AS1, OIP5-AS1, OLMALINC, RPARP-AS1, SLC9A3-AS1, SNHG12, SNHG16, SNHG4, STAG3L5P-PVRIG2P-PILRB, THSD4-AS1, TMEM147-AS1, TMPO-AS1, TRG-AS1, TTC28-AS1, TTTY15, UBL7-AS1, VASH1-AS1, XIST, ZNF337-AS1, ZNF436-AS1, ZNF571-AS1
hsa-miR-133b	AGAP11, DLEU1, FGD5-AS1, HCG18, KCNMA1-AS1, KCNQ1OT1, LHX5-AS1, LINC00847, LINC01278, LINC01995, LINC02381, LINC02432, LINC02538, MCM3AP-AS1, MIR4697HG, NEAT1, NOP14-AS1, TMEM147-AS1, ZEB1-AS1

To investigate potential downstream molecular effects, miRTarBase, the largest repository of experimentally validated miRNA–mRNA interactions, was queried. Only mRNA targets validated through high-confidence experimental methods (e.g., Western blot, qRT-PCR, luciferase assays) were retained, resulting in the identification of 20 validated targets for hsa-miR-133b and 23 for hsa-let-7a-5p (Table 3), which were subsequently used for functional enrichment analysis.

Table 3. Target genes of miRNAs predicted by miRTarBase database (the target names are arranged in alphabetical order).

ID	Target
hsa-let-7a-5p	*AGO-01, AAK1, ARG2, CCND2, CCR7, E2F2, EWSR1, EZH2, HMGA1, HMGA2, HRAS, IL6, ITGB3, KRAS, NF2, NKIRAS2, NRAS, PAK1, PRDM1, RAB40C, RRM2, UHRF1, UHRF2*
hsa-miR-133b	*AAK1, AKT1, BCL2L1, BCL2L2, CDC42, CTGF, CXCR4, EGFR, FAIM, FGFR1, FOXL2, FSCN1, GLI1, HCN4, KCNH2, MCL1, PRDM1, RHO4, STK3, TAGLN2*

PPI networks were constructed using the STRING database to explore protein-level interactions. For hsa-let-7a-5p, the resulting network comprised 22 nodes and 46 edges (PPI enrichment p-value: 3.55e−11); for hsa-miR-133b, the network included 19 nodes and 39 edges (p = 1.03e−07). Clustering was performed using the MCL algorithm based on STRING interaction scores, revealing distinct functional modules (Figure 3).

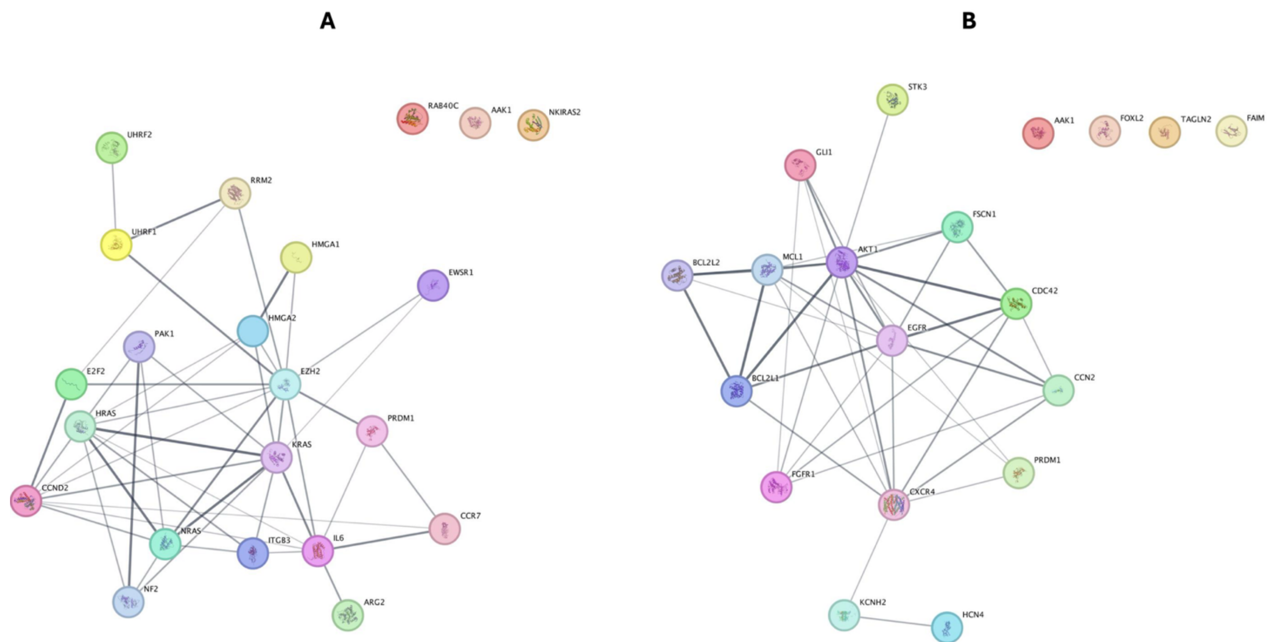


Fig. (3). A) target mRNAs of hsa-let-7a-5p organized by string interactions clusters; B) target mRNAs of hsa-miR-133b organized by string interactions clusters.

A comprehensive lncRNA–miRNA–mRNA interaction network was then reconstructed by integrating the miRNA–mRNA and miRNA–lncRNA interactions. The final network consisted of 109 nodes and 115 edges (Figure 4), revealing shared regulatory elements such as *AAK1*, *PRDM1*, HCG18, KCNQ1OT1, LINC02381, LINC02432, NEAT1, and TMEM147-AS.

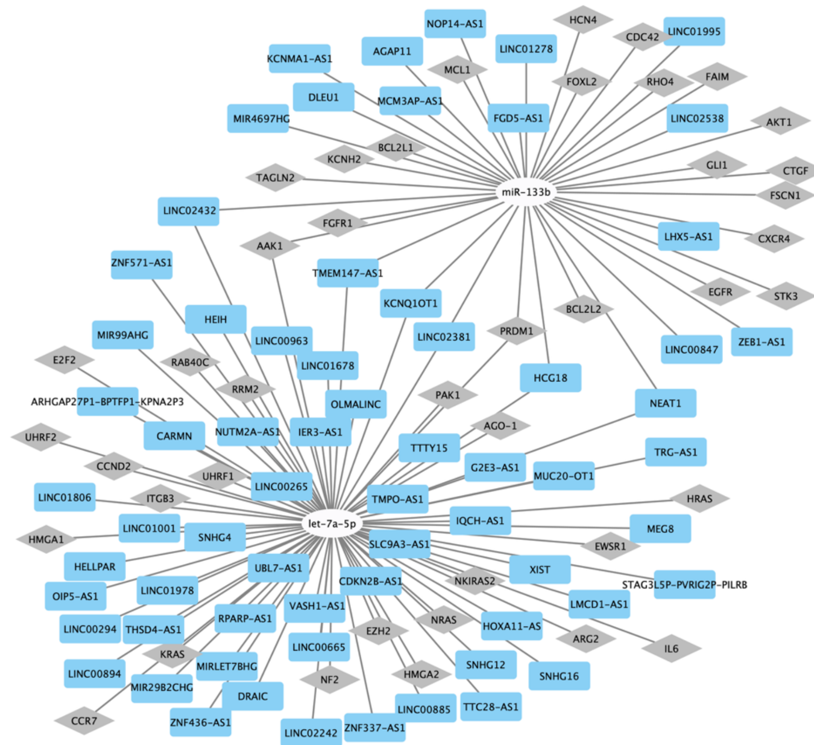
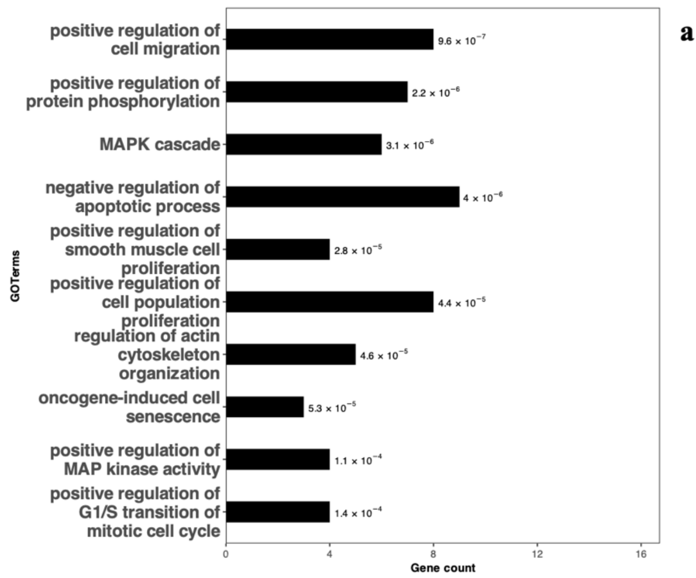


Fig. (4). LncRNA-miRNA-mRNA networks related to hsa-miR-133b and hsa-let-7a-5p (ellipses represents miRNAs, diamonds represent target genes, and round rectangles represent lncRNAs).

Functional annotation was carried out using GO and KEGG pathway enrichment analyses. The top 10 enriched GO terms in each category were shown in Figure 5 and Table S1 in the Supplementary Materials. In the BP category (Figure 5A), enriched terms included processes related to cell proliferation and migration (e.g., positive regulation of cell migration, smooth muscle cell proliferation), signal transduction (MAPK cascade, positive regulation of protein phosphorylation), cell survival and cycle control (G1/S transition of the mitotic cell cycle, negative regulation of apoptosis), as well as cytoskeleton remodeling and senescence (regulation of actin cytoskeleton, oncogene-induced senescence). In the MF category (Figure 5B), enriched functions included GTPase activity, GDP/GTP binding, cis-regulatory DNA binding, protein–protein interaction domains (e.g., BH/BH3 domain binding), and ion channel activity. The CC category (Figure 5C) revealed localization of targets in structures involved in survival and inflammation

(e.g., Bcl-2 protein complex), cell motility (focal adhesion, lamellipodium, ruffle membrane), gene regulation (nucleoplasm, cytoplasm, cytosol), and synaptic signaling (glutamatergic synapse), suggesting roles in pain modulation and neuroplasticity.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MAPK cascade
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negative regulation of apoptotic process
4 × 10−6
positive regulation of smooth muscle cell proliferation
2.8 × 10−5
positive regulation of cell population proliferation
4.4 × 10−5
regulation of actin cytoskeleton organization
4.6 × 10−5
oncogene-induced cell senescence
5.3 × 10−5
positive regulation of MAP kinase activity
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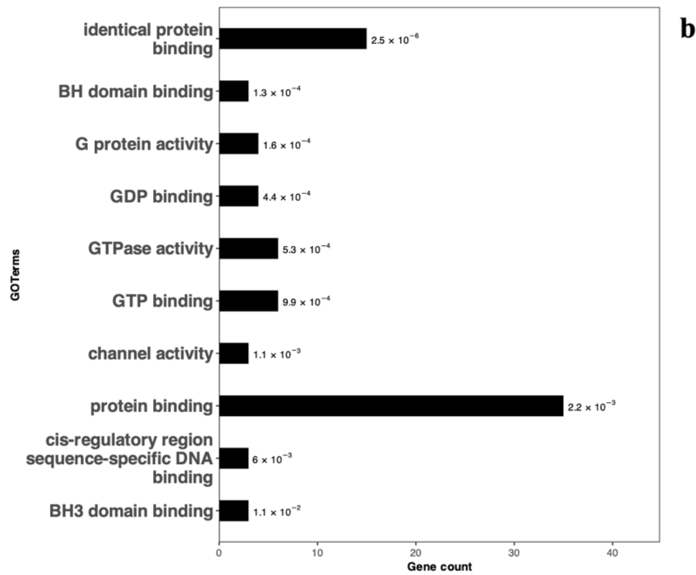
b
GOTerms
identical protein binding
2.5 × 10−6
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GDP binding
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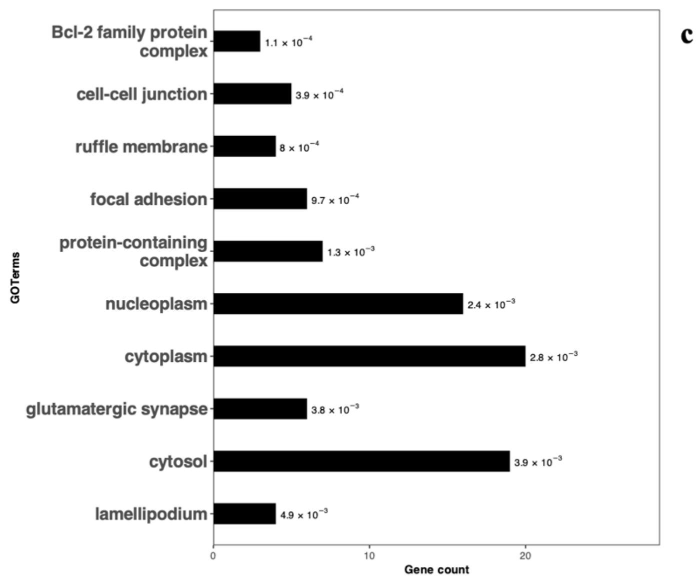
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nucleoplasm
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cytoplasm
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glutamatergic synapse
3.8 × 10−3
cytosol
3.9 × 10−3
lamellipodium
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0
10
20
Gene count

Fig. (5). Top 10 enriched GO terms for hsa-miR-133b and hsa-let-7a-5p: (a) Biological Process, (b) Molecular Function, (c) Cellular Component. Terms are ranked by p-value (descending). Bar length indicates the number of associated genes.

Finally, KEGG pathway analysis identified 27 enriched pathways, with the most significant ones presented in Figure 6 and Table S2 in the Supplementary Materials. These included pathways related to human diseases (e.g., MicroRNAs in cancer, Pathway in cancer, Melanoma), cellular processes (e.g., regulation of actin cytoskeleton), signal transduction (e.g., Ras signaling pathway, MAPK signaling pathway), immune system regulation (e.g., Chemokine signaling pathway), and drug resistance (e.g., EGFR tyrosine kinase inhibitor resistance).

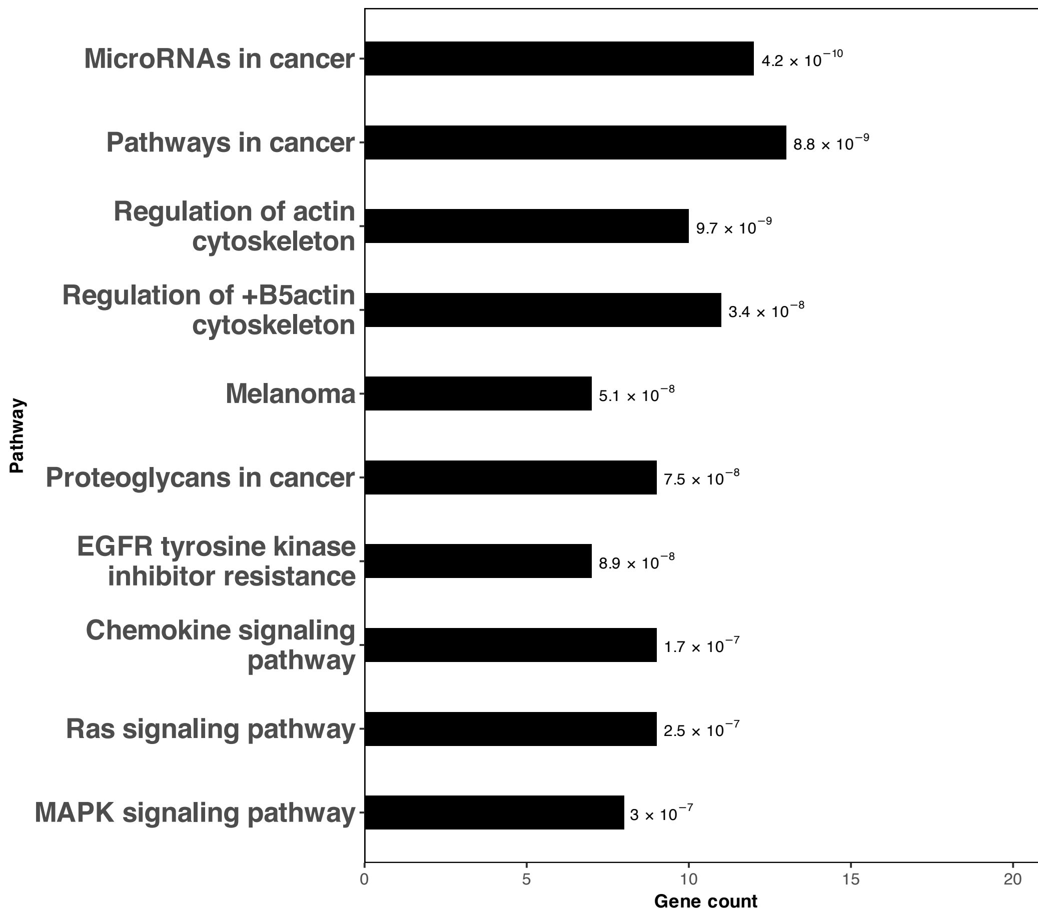

Fig. (6). Top 10 KEGG pathways ranked by significance (p-value). Bar length indicates the number of genes associated with each pathway.

Discussion

This exploratory study evaluated the expression of pain- and inflammation-related miRNAs following a kinesiology-based intervention in long-distance runners. While miRNAs have been extensively studied in the context of exercise physiology and inflammatory responses, their potential as biomarkers of therapeutic response to targeted rehabilitation interventions remains largely unexplored. This gap limits the development of personalized treatment strategies and objective molecular tools for monitoring recovery. The study aimed to assess whether specific circulating miRNAs could reflect or predict therapeutic outcomes, thereby offering molecular insights into the effectiveness of rehabilitation interventions.

The four miRNAs analyzed were selected based on prior evidence of their involvement in inflammatory pathways [25,26]. After six weeks of intervention, a general trend toward downregulation was observed, with hsa-let-7a-5p reaching a statistically significant decrease. This finding suggests a potential involvement of hsa-let-7a-5p in the molecular adaptations triggered by the intervention. However, none of the miRNAs showed significant associations with pain remission in logistic regression analyses, despite 42.9% of participants reporting complete resolution of pain. These results indicated that therapeutic response was likely influenced by multifactorial mechanisms beyond single biomarker expression, including individual variability in inflammatory profiles, neuroimmune interactions, and psychosocial factors such as pain perception and treatment expectation.

To date, few studies have assessed miRNA modulation in runners undergoing therapeutic interventions. Most available research has examined miRNA responses to general physical activity [50–52]. For example, a systematic review by da Silva et al. [53] identified several miRNAs, including hsa-miR-146a-5p and hsa-miR-155-5p, as being modulated by exercise and implicated in inflammation and muscle adaptation. The present study contributes to this body of evidence by evaluating miRNA expression in the context of a structured kinesiology-based program, emphasizing potential anti-inflammatory and tissue-adaptive effects.

Among the miRNAs evaluated, hsa-let-7a-5p emerged as the most responsive. Members of the let-7 family are known to play dual roles in initiating and resolving inflammation. Prior research reported increased circulating let-7 levels following

acute endurance exercise, suggesting a role in transient inflammatory activation [54]. In contrast, the present results may indicate a compensatory anti-inflammatory effect arising from repeated neuromuscular activation. These findings highlight the context-dependent nature of miRNA regulation, influenced by exercise type, duration, and intensity.

Hsa-miR-133b, a muscle-specific miRNA involved in myogenesis and regeneration, showed a non-significant but potentially meaningful trend toward downregulation. Literature on hsa-miR-133b modulation by exercise remains inconclusive, with some studies reporting no significant changes [55], and others showing increased levels after high-intensity training [56]. This variability may reflect differences in study design, exercise protocols, and individual biological responses.

Interestingly, hsa-miR-155-5p remained upregulated post-intervention, although with a slight reduction in fold change. This miRNA is known to regulate inflammation, nociception, and muscle repair. Its persistent expression may indicate ongoing tissue remodeling or a delayed resolution of inflammatory signaling. Similar patterns were reported in patients with chronic low back pain, where miR-155-5p expression remained elevated despite treatment [57]. Its role in macrophage activation, neuroinflammation, and pain signaling warrants further exploration in the context of rehabilitation [58–61].

To gain insight into the possible biological mechanisms underlying the observed changes, a bioinformatic analysis was conducted focusing on hsa-let-7a-5p and hsa-miR-133b. Several lncRNAs and mRNA targets were identified, forming regulatory networks potentially involved in therapeutic response. Notably, *AAK1* and *PRDM1* were identified as common targets. *AAK1* is involved in synaptic vesicle recycling and neuropathic pain [62,63], while *PRDM1* regulates immune cell differentiation and inflammatory responses [64,65]. Both are relevant to neuroimmune modulation in musculoskeletal pain. Additionally, shared lncRNAs such as NEAT1 [66,67] and HCG18 [68,69], known to modulate pain sensitization and inflammatory responses support the hypothesis of an integrated miRNA–lncRNA–mRNA regulatory system.

PPI network analysis further revealed tightly connected clusters of target genes, with significant enrichment in biological processes related to inflammation, tissue remodeling, and immune signaling. These results support the concept that the regulatory effects of miRNAs are exerted not through isolated genes but via coordinated modulation of functional gene networks.

GO and KEGG pathway analyses confirmed enrichment in key pathways such as MAPK signaling, apoptosis regulation, actin cytoskeleton organization, and chemokine signaling—processes central to musculoskeletal recovery and pain modulation [70–72]. Although several enriched KEGG pathways were associated with disease, including cancer and neurodegeneration, this likely reflects shared regulatory processes—such as inflammation, proliferation, and cell survival—that are activated in both pathological and physiological contexts [73]. The enrichment of chemokine signaling is particularly noteworthy given its role in immune cell recruitment, nociceptor sensitization, and local tissue inflammation

This study presents several limitations. First, the sample size was relatively small, and no formal a priori power analysis was performed. This was primarily due to the lack of robust literature data on which to base a reliable estimate of the expected effect size. As a result, the findings should be considered exploratory and hypothesis-generating, rather than confirmatory, and require validation in larger, adequately powered studies. The limited sample size reduced the ability to detect statistically significant effects and affects the generalizability of the results; however, this is in line with the pilot nature of the study. Future research involving larger, more diverse, and gender-balanced cohorts will be essential to confirm these findings and to better capture interindividual variability in miRNA expression and therapeutic response. Second, the demographic composition of the sample—predominantly male—may have introduced bias, especially considering known sex-related differences in pain perception and molecular regulation. Third, the follow-up period was limited to six weeks of intervention. While this duration was sufficient to observe short-term molecular and clinical changes, it may not have captured the long-term dynamics of miRNA regulation or the persistence of therapeutic effects. Longer follow-up periods are needed to determine whether the observed changes reflect transient responses or sustained physiological adaptations.

Finally, the bioinformatic analyses relied on *in silico* predictions of miRNA–lncRNA–mRNA interactions. Although these tools offer valuable mechanistic insights, they remain hypothetical without experimental validation. Future research should incorporate functional assays—such as luciferase reporter assays, RNA interference, or CRISPR-mediated gene editing—to verify the biological relevance of the predicted interactions in the context of musculoskeletal pain and rehabilitation.

Conclusion

In summary, these findings suggest that hsa-let-7a-5p and, to a lesser extent, hsa-miR-133b may participate in regulatory networks involved in inflammation, pain modulation, and musculoskeletal adaptation following kinesiology-based functional recovery programs. Although these miRNAs did not show a statistically significant association with clinical outcomes in the regression analysis, bioinformatic results suggest their involvement in interconnected molecular pathways relevant to tissue repair. These observations support their potential as indicators of underlying biological responses, even if not as standalone predictive biomarkers.

Future studies involving larger and more diverse cohorts, extended follow-up periods, and functional validation experiments will be essential to determine their clinical utility and to assess their role in guiding personalized post-rehabilitation exercise strategies.

LIST OF ABBREVIATIONS

miRNAs = microRNAs

lncRNAs = long non-coding RNAs

NRS = Numerical Rating Scale

IPAQ = International Physical Activity Questionnaire

CPM = Canali Postural Method

MP-LAB = Posture and Movement Laboratory

qRT-PCR = Quantitative real-time PCR

PPI = protein–protein interaction

STRING = Search Tool for the Retrieval of Interacting Genes

MCL = Markov Clustering

DAVID = Database for Annotation, Visualization, and Integrated Discovery

GO = Gene Ontology

KEGG = Kyoto Encyclopedia of Genes and Genomes

BP = Biological Process

MF = Molecular Function

CC = Cellular Component

AAK1 = Adaptor-associated protein Kinase 1

PRDM1 = PR/SET Domain 1

HCG18 = HLA Complex Group 18

KCNQ1OT1= KCNQ1 Opposite Strand/Antisense Transcript 1

LINC02381 = Long Intergenic Non-Protein Coding RNA 2381

LINC02432 = Long Intergenic Non-Protein Coding RNA 2432

NEAT1 = Nuclear Paraspeckle Assembly Transcript 1

TMEM147-AS = TMEM147 Antisense RNA 1

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committee of the Lecce Local Health Authority (ASL/LE) (deliberation no. 0000108, February 2, 2023).

HUMAN AND ANIMAL RIGHTS

Research Involving Humans

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CRediT AUTHOR STATEMENT

Maria Rosaria Tumolo: Conceptualization, Investigation, Writing - Original Draft, Visualization. **Egeria Scoditti:** Investigation, Writing - Review & Editing. **Antonella Bodini:** Formal analysis, Writing - Review & Editing. **Pierpaolo Mincarone:** Writing - Review & Editing. **Carlo Giacomo Leo:** Writing - Review & Editing. **Francesco Bagordo:** Writing - Review & Editing. **Luca Bertone:** Writing - Review & Editing. **Elisabetta De Matteis:** Writing - Review & Editing. **Tiziana Grassi:** Resources, Writing - Review & Editing, Supervision. **Saverio Sabina:** Conceptualization, Writing - Review & Editing, Supervision, Project administration.

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CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest related to the manuscript.

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