Exosomal MiRNAs as Predictive Biomarkers for Metabolic Syndrome: Insights into Biological Adaptation in a High Altitude Hypoxic Environment

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Abstract: This study integrates human cohorts (n=60, 30 high-altitude [4300m] vs. 30 low-altitude [50m]) and C57BL/6 mouse models to elucidate regulatory mechanisms of metabolic adaptation via serum exosomal miRNAs under hypoxic stress. High-altitude exposure significantly improved metabolic health in humans (reduced BMI, FBG, HOMA-IR, Tch, TG, LDL-C; increased HDL-C) and mice (decreased weight gain, insulin, FBG, HOMA-IR, FFA, TG; $p<0.05$). High-throughput sequencing identified 57 differentially expressed miRNAs (22↑/35↓) in hypoxic mice, enriched in Ras/MAPK signaling and metabolic networks. Cross-species validation confirmed hsa-miR-5100 (AUC=0.9089), hsa-miR-184-3p (AUC=0.8233), and hsa-miR-122-5p (AUC=0.7521) as diagnostic biomarkers. We propose the first "exosomal miRNA-signaling pathway-metabolic phenotype" trinity framework, providing novel targets for hypoxia adaptation and metabolic disease intervention.

***Keywords*:** Exosomal miRNAs, High-altitude hypoxia, Metabolic adaptation, Hypoxic intervention biomarkers

1. Introduction

High-altitude hypoxia induces metabolic reprogramming by remodeling energy balance, including enhancing glycolysis, mitochondrial adjustments, and lipid optimization [1]. Prolonged hypoxia in animals fosters unique metabolic phenotypes with improved respiratory chain efficiency and oxidative stress tolerance [2]. Human hypoxic adaptation involves HIF signaling activation, coordinating transcriptional regulation of glycolytic enzymes and mitochondrial fission [3], while intermittent hypoxia enhances redox balance and modulates inflammation [4]. Gaps remain in understanding systemic regulatory networks and dynamic responses [5].

Exosomes serve as intercellular communicators under hypoxia, transmitting miRNAs that facilitate metabolic adaptation [6]. Hypoxia modifies EXO contents to promote adipose browning and distant organ reprogramming [7]. Tumor studies indicate hypoxia-induced exosomal miR-5100 remodels metabolic ecology via fibroblast activation [8], while adipose-derived miR-486-5p enhances tissue repair [9]. Serum exosomal miRNAs thus act as "molecular signaling hubs" integrating multi-tissue responses [10].

Exosomal miRNA omics reveal hypoxia modulates miRNA biosynthesis via RNA-binding proteins and epigenetic modifications [11]. Specific miRNAs regulate glucose/lipid metabolism through AMPK and PI3K/Akt pathways [12]. Despite advances, exosomal miRNA dynamics in high-altitude populations are underexplored, limiting biomarker/therapeutic development [13]. This study elucidates these mechanisms to advance prevention of high-altitude diseases and hypoxia-related metabolic disorders.

2. Methods

2.1 Clinical Sample Collection

Sixty healthy adult volunteers, with no significant findings on health examinations, were recruited at Qinghai Provincial People's Hospital. Participants had resided in fixed locations for the past five years. Based on permanent residence altitude, they were stratified into High-altitude (H-altitude, ≥3300 m) and Low-altitude (L-altitude, <3300 m) groups. All provided informed consent. Height, weight, body fat percentage, and waist-to-hip ratio were measured. Fasting venous blood was collected. Serum extracellular vesicles (EXO) were isolated and stored at -80°C.

2.2 Animal Model Construction

Forty 6-week-old male C57BL/6J mice were acquired and acclimatized. They were randomly assigned to two groups (n=20/group): the Plateau group, housed at a 4300 m altitude field laboratory, and the Control group, maintained at 50 m. Standard housing conditions were maintained for 8 weeks. General indicators were monitored. Post-modeling, retro-orbital venous blood was collected for biochemistry.Serum EXO were isolated and stored at -80°C.

2.3 Exosomal miRNA Sequencing and Clinical Validation

Serum exosomes were isolated from human and mouse samples using ExoQuick (SBI), validated by EM (Fig. 1A), and stored at -80°C. EXO-miRNAs underwent RNA quality assessment. Sequencing libraries were quality-checked, clustered, and sequenced. Clean reads were filtered from raw data. Bioinformatics identified differentially expressed miRNAs ($|\log2FC| \geq 1$, $p<0.05$) between altitude groups, followed by GO/KEGG enrichment. For qRT-PCR, miRNAs were isolated, with concentration and purity verified. Relative expression was calculated ($2^{-\Delta\Delta Ct}$) from triplicates (Ct SD <0.5).

2.4 Statistical methods:

Data are expressed as mean ± SD. Inter-group comparisons used the t-test. Receiver operating characteristic (ROC) analysis evaluated candidate miRNA predictive capacity for high-altitude hypoxia. Analyses were performed using GraphPad Prism 6.0, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Protective effects of high-altitude hypoxia on metabolism

The high-altitude (HA) human group (n=30, 3660 ± 486 m) exhibited significantly elevated RBC (5.33 ± 0.30 vs. 4.69 ± 0.37 × 10^{12}/L) and Hgb (160.6 ± 7.18 vs. 144.5 ± 13.68 g/dL) compared to the low-altitude (LA) group (n=30, 2268 ± 105 m), consistent with chronic hypoxia adaptation. Notably, the HA group demonstrated improved metabolic profiles, including lower BMI (22.27 ± 1.73 vs. 24.22 ± 1.86 kg/m²), FBG (4.83 ± 0.44 vs. 5.24 ± 0.57 mmol/L), fasting insulin (4.86 ± 0.29 vs. 5.42 ± 0.70 μIU/mL), HOMA-IR (1.04 ± 0.13 vs. 1.26 ± 0.20), TG (0.95 ± 0.78 vs. 1.73 ± 0.95 mmol/L), Tch (2.89 ± 0.79 vs. 3.50 ± 0.95 mmol/L), and LDL-C (1.98 ± 0.60 vs. 2.79 ± 0.65 mmol/L), alongside higher HDL-C (2.01 ± 0.28 vs. 1.13 ± 0.34 mmol/L) (Fig. 1B). No significant age or sex differences existed between groups ($p>0.05$).

Supporting these findings, mice modeled at 4300 m (vs. 50 m control) for 8 weeks showed significantly increased Hgb (150.25 ± 10.54 vs. 133.00 ± 10.71 g/L), reduced weight gain (4.95 ± 0.89 vs. 6.12 ± 0.90 g), lower FBG (5.73 ± 0.88 vs. 8.41 ± 0.97 mmol/L), fasting insulin (5.30 ± 0.64 vs. 5.77 ± 0.48 ng/mL), HOMA-IR (1.37 ± 0.17 vs. 2.15 ± 0.26), FFA (44.23 ± 5.77 vs. 58.39 ± 9.06 mmol/L), and TG (0.60 ± 0.01 vs. 0.10 ± 0.01 mmol/L) (Fig. 1C).

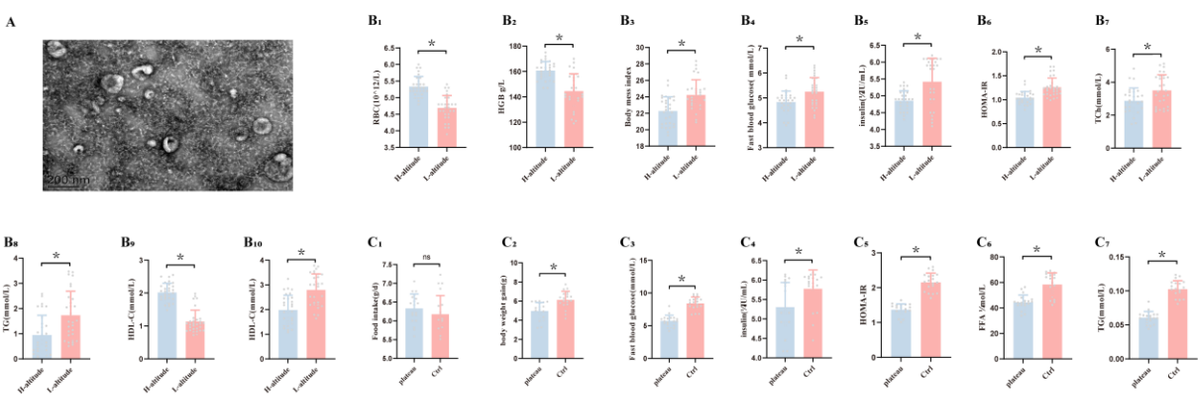

Figure 1. The adaptive metabolic responses to chronic hypoxia observed in high-altitude populations and animal models.

3.2 Enrichment profile and functional analysis of serum exosomal miRNAs at a high altitude

Serum exosomal miRNA analysis identified 815 miRNAs, with 588 (72.1%) shared between high-altitude (HA) and control groups, while 78 (9.6%) and 149 (18.3%) were exclusive to HA and controls, respectively. Differential expression analysis (threshold: $|\log2FC| \geq 1$, $-\log10(p) \geq 1.30$) revealed 22 significantly upregulated and 35 downregulated miRNAs in the HA group (volcano plot). Gene Ontology enrichment implicated roles in cellular metabolism, intracellular compartment development, and protein binding. KEGG analysis highlighted Ras/MAPK signaling and cancer-related proteoglycan

pathways. These findings indicate hypoxia dynamically reshapes exosomal miRNA profiles to regulate metabolic networks (Fig. 2).

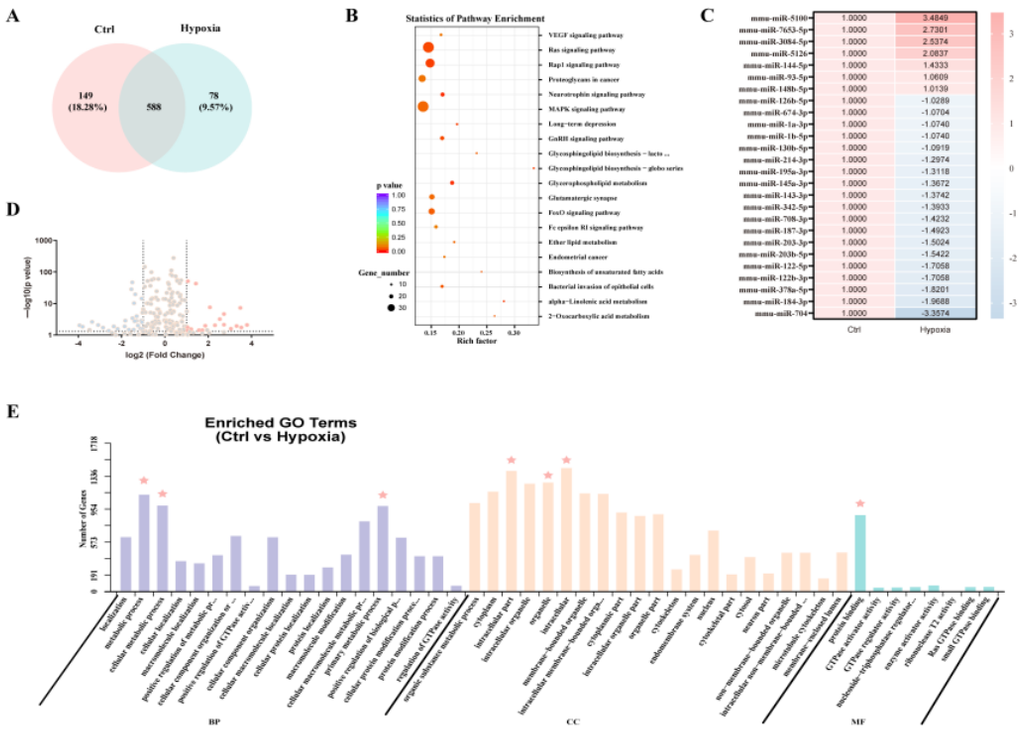

Figure 2. The differential expression and functional enrichment of serum exosomal miRNAs in high-altitude populations subjected to chronic hypoxia.

3.3 Cross-species validation of candidate miRNAs as biomarkers for metabolic adaptation to high altitude

significant upregulation of hsa-miR-5100 (2.35 ± 0.41 vs. 1.00 ± 0.22) and hsa-miR-3084-5p (1.82 ± 0.33 vs. 1.00 ± 0.18), and downregulation of hsa-miR-122-5p (0.48 ± 0.09 vs. 1.00 ± 0.15) and hsa-miR-184-3p (0.62 ± 0.12 vs. 1.00 ± 0.21) in the high-altitude group (vs. controls) via qRT-PCR (RIN>7, OD260/280=1.8–2.0). Six other miRNAs showed no significant differences; mmu-miR-704 lacked a human ortholog (Fig. 3A). ROC analysis identified hsa-miR-5100 as a high-performance biomarker (AUC=0.9089; 95% CI: 0.8317–0.9861), with 83.33% sensitivity and 86.67% specificity at FC=1.626 (Fig. 3B).

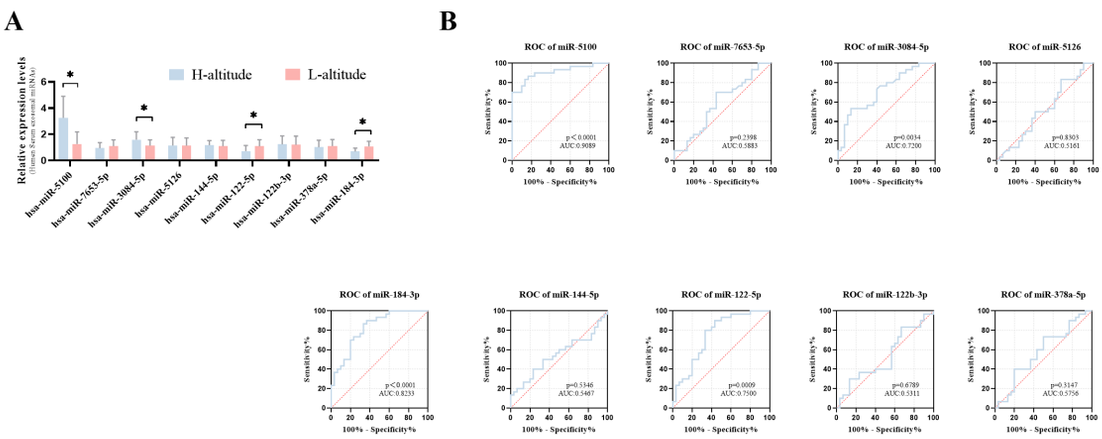

Figure 3. The validation of hypoxia-associated exosomal miRNA biomarkers in human cohorts and their diagnostic potential.

4. Conclusion

Chronic high-altitude hypoxia enhances metabolic health (improved FBG, HOMA-IR, lipids) in humans and mice via HIF signaling activation and energy metabolism reprogramming [1]. HIF-1α reduces ROS by promoting PDK1-mediated glycolysis and DRP1-dependent mitochondrial fission, while suppressing lipogenic enzymes to boost lipid β-oxidation. Downregulation of miR-122-5p attenuates hepatic lipid accumulation via KIF5B/AMPK [12], indicating integrated HIF-driven and exosomal miRNA-mediated adaptations [6].

Hypoxia significantly alters serum exosomal miRNA profiles, with enriched Ras/MAPK signaling and cellular metabolic processes. These miRNAs regulate proteoglycan pathways to optimize vascular function and energy allocation. Conservation of mechanisms exists across species [14].

Candidate biomarkers show translational promise: hsa-miR-5100 improves metabolic balance by targeting G6PC/CAAP1 [15]; downregulated miR-122-5p enhances insulin sensitivity via SIRT1/IL1RN [16]; reduced miR-184-3p alleviates metabolic stress via CRTC1 [17]. Novel miR-3084-5p requires further validation. These enable non-invasive adaptation assessment and inform therapies for hypoxia-linked disorders.

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