MCT4 drives triple-negative breast cancer progression via lactate-induced histone lactylation and mapk pathway activation

Yang Peng[1], Qiuyan Luo[1], Han Li[1], Zehao cai[1] and Shengchun Liu[1]

[1]Department of Endocrine and Breast Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China 400016;

Abstract

Background: Triple-negative breast cancer (TNBC) frequently develops chemoresistance with poor outcomes. MCT4, a lactate efflux transporter overexpressed in glycolytic cancers, maintains metabolic homeostasis and correlates with aggressive phenotypes. Lactate serves as substrate for histone lactylation, an epigenetic modification regulating gene expression. However, MCT4's role in linking lactate metabolism to chemoresistance via epigenetic regulation remains unexplored.

Methods: We analyzed paired primary and chemoresistant TNBC tissues for lactylation levels. Functional studies used MCT4-knockdown MDA-MB-231 cells to assess paclitaxel sensitivity, glucose metabolism, proliferation, migration, and invasion. Xenograft models evaluated in vivo efficacy. CUT&Tag sequencing identified H3K9 lactylation targets and pathway mechanisms.

Results: Chemoresistant TNBC tissues showed elevated pan-lactylation, particularly histone H3. MCT4 was overexpressed and upregulated by hypoxia/paclitaxel. MCT4 knockdown reduced glucose uptake, suppressed proliferation/migration/invasion, and enhanced paclitaxel sensitivity in vitro and in vivo. Mechanistically, MCT4 depletion decreased H3K9 lactylation and downregulated MAPK signaling.

Conclusions: We reveal a novel MCT4-H3K9 lactylation-MAPK axis driving TNBC chemoresistance. Targeting MCT4 or lactylation-mediated epigenetic regulation represents a promising therapeutic strategy.

Introduction

Triple-negative breast cancer (TNBC) accounts for 15-20% of breast cancers and represents the most aggressive subtype with limited therapeutic options and poor prognosis (1). Cancer cells exhibit enhanced aerobic glycolysis (Warburg effect), resulting in excessive lactate production that promotes invasion and immune evasion (2). Monocarboxylate transporter 4 (MCT4) serves as the primary lactate efflux transporter, maintaining intracellular pH homeostasis and facilitating glycolytic flux (3). Recent discoveries revealed that lactate functions as a signaling molecule and drives histone lactylation, a post-translational modification regulating gene transcription (4). However, the mechanistic link between MCT4-mediated lactate metabolism and oncogenic signaling in TNBC remains unclear. This study investigates MCT4's role in TNBC progression and explores molecular mechanisms connecting lactate metabolism to cancer aggressiveness and chemotherapy resistance.

Methods and Materials

TNBC tissue microarrays were analyzed to assess pan-lactylation levels. MDA-MB-231 cells were subjected to hypoxia (1% O_2) and paclitaxel treatment, with MCT4 stably silenced using lentiviral shRNA. Sodium lactate (NaLa, 20 uM) was used to assess lactate's effect on chemoresistance. Cell proliferation, invasion, migration, glucose uptake (2-NBDG), and paclitaxel sensitivity (IC50) were assessed. Nude mice bearing control or MCT4-knockdown cells were treated with paclitaxel (10 mg/kg weekly, i.p.) to evaluate tumor growth and therapeutic response. Western blot analysis detected MCT4, pan-lactylation, H3K9 lactylation (H3K9la), and key MAPK pathway proteins including phosphorylated and total ERK and MEK. RNA sequencing was performed on control and MCT4-knockdown cells under normoxic or hypoxic conditions, with differentially expressed genes (DEGs) subjected to KEGG pathway enrichment analysis. CUT&Tag sequencing mapped genome-wide H3K9 lactylation distribution under hypoxic conditions, with peaks annotated to gene promoters and enhancers for pathway analysis. Statistical analysis used Student's t-test for two-group comparisons or one-way ANOVA with Tukey's post-hoc test for multiple groups, with $P < 0.05$ considered statistically significant. All experiments were performed in at least triplicate.

Results

Results1：Lactylation levels are elevated in chemoresistant TNBC and enhance paclitaxel resistance (A) Quantitative analysis revealed significantly elevated pan-lactylation levels in paired chemoresistant TNBC tissues compared to primary tumors. (B) Western blot analysis confirmed increased pan-lactylation in resistant tissues, with notably enhanced signal at ~17 kDa (putative histone H3). (C) Exogenous sodium lactate (20 u M NaLa) significantly increased paclitaxel IC50 values in MDA-MB-231 cells.

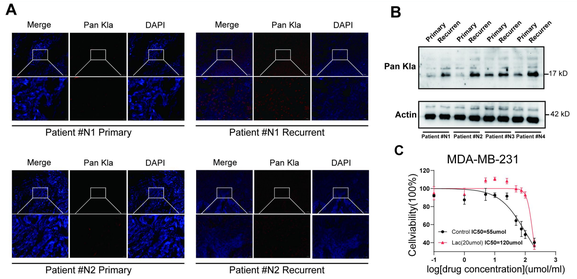


Figure 1: Lactylation promotes chemoresistance

Results

Results2： MCT4 is overexpressed in TNBC and regulates glucose metabolism (A) MCT4 expression was significantly elevated in TNBC tissues. (B) Both hypoxia and paclitaxel treatment upregulated MCT4 expression in MDA-MB-231 cells. (C) MCT4 knockdown significantly reduced glucose uptake capacity.

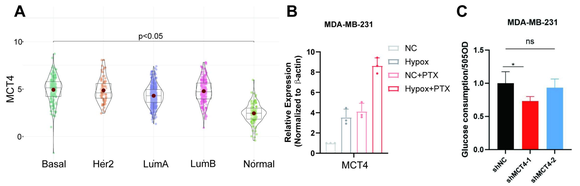


Figure 2: MCT4 is the key lactate transporter

Results3： MCT4 knockdown suppresses tumor progression and enhances paclitaxel sensitivity (A-B) MCT4 knockdown significantly inhibited cell proliferation, invasion, and migration. (C) MCT4 depletion markedly enhanced paclitaxel sensitivity in vitro. (D) In vivo xenograft studies confirmed that MCT4 knockdown delayed tumor growth and improved paclitaxel therapeutic response.

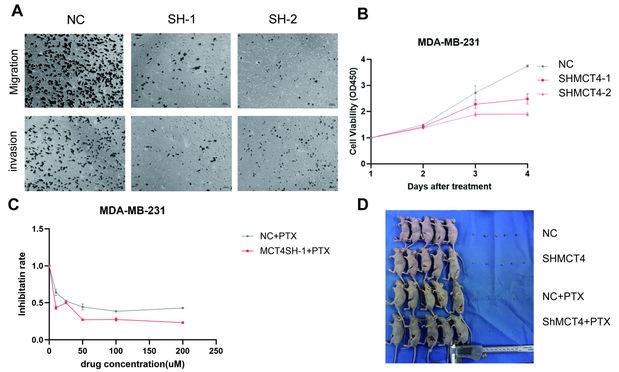


Figure 3: MCT4 is essential for TNBC progression

Results

Results4： MCT4 regulates chemoresistance through H3K9 lactylation-mediated MAPK pathway activation (A)H3K9 lactylation levels decreased following MCT4 knockdown. (B-C)CUT&Tag analysis in hypoxia-induced cells revealed that H3K9 lactylation-activated genes were enriched in MAPK signaling pathways. (D)Western blot confirmed that MCT4 knockdown downregulated key MAPK pathway proteins.

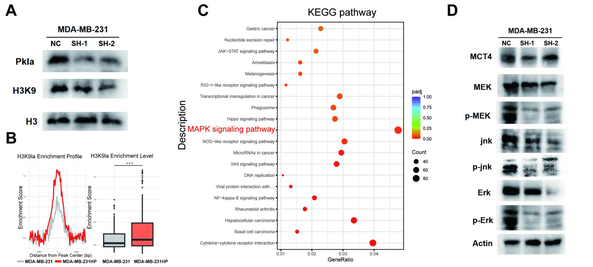


Figure 4: Mechanistic insights: MCT4-H3K9la-MAPK axis

Discussion

Our findings establish a metabolic-epigenetic mechanism of TNBC chemoresistance. Resistant tumors show elevated H3 lactylation, reflecting epigenetic reprogramming. MCT4 serves dual functions: exporting lactate for metabolic balance while maintaining intracellular pools for histone modification. CUT&Tag analysis revealed that H3K9 lactylation directly activates MAPK pathway genes at their promoters, driving pro-survival signaling. This MCT4-H3K9la-MAPK axis links lactate metabolism to transcriptional control of chemoresistance.

Limitations: Our study focused on H3K9 lactylation; other sites require investigation. The mechanism sustaining intracellular lactate despite MCT4-mediated export needs clarification. Immunocompromised mouse models cannot fully capture tumor-immune interactions.

Conclusions

We reveal a novel MCT4-H3K9 lactylation-MAPK axis driving TNBC chemoresistance. MCT4 bridges lactate metabolism and histone modifications, activating pro-survival signaling. Targeting this axis offers a rational approach to resensitize resistant TNBC and improve patient outcomes.

Contact

<Yang Peng>
<Department of Endocrine and Breast Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China 400016>
Email:204750@hospital.cqmu.edu.cn
Website:NA
Phone:+86 15123310109/ +1 6193221285

References

1. Bianchini G, De Angelis C, Licata L, Gianni L. Treatment landscape of triple-negative breast cancer - expanded options, evolving needs. Nat Rev Clin Oncol. 2022;19(2):91-113.
2. Chen J, Cao X, Li B, et al. Warburg effect is a cancer immune evasion mechanism against macrophage immunosurveillance. Front Immunol. 2020;11:621757.
3. Halestrap AP, Wilson MC. The monocarboxylate transporter family--role and regulation. IUBMB Life. 2012;64(2):109-119.
4. Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, et al. Metabolic regulation of gene expression by histone lactylation. Nature. 2019;574(7779):575-580.

This presentation is the intellectual property of the author/presenter. Contact them at <<204750@hospital.cqmu.edu.cn>> for permission to reprint and/or distribute.

© Poster Template by Genigraphics® 1.800.790.4001 www.genigraphics.com