Lactate generated by LDHB suppression decreases PDH-mediated mitochondrial respiratory activity in hepatoma cell

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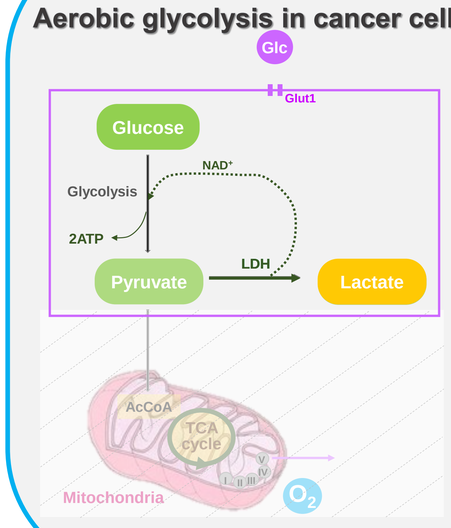
ABSTRACT

Aerobic glycolysis and mitochondrial dysfunction are distinctive metabolic hallmarks of solid tumor. However, it is unclear how these phenomena are developed during tumorigenesis. To maintain aerobic glycolysis, continuous generation of NAD^+ by lactate dehydrogenase (LDH) is essential. Recently, we reported that LDH5 isoenzyme formed by LDHB suppression is involved in increased glycolytic lactate production and mitochondrial respiratory defects in hepatoma cells. In this study, we aimed to investigate how LDHB suppression is linked with mitochondrial respiratory dysfunction. We have suggested that LDHB suppression might control mitochondrial respiration through posttranslational modification of respiratory related protein, thus, we have attempted to focus on pyruvate dehydrogenase (PDH) which is regulated by phosphorylation. Interestingly, LDHB knockdown effectively increased phosphorylation of PDH, indicating its inactivation. Treatment of lactate was increased PDH phosphorylation with lowering pH. This result implies that PDH phosphorylation is correlated with acidification, but is not lactate-specific event. Collectively, our results suggest that lactate-mediated PDH inactivation is the key mechanism to induce mitochondrial dysfunction in LDHB-suppressed hepatoma cell.

INTRODUCTION

Aerobic glycolysis in cancer cell



K_M value for Glucose
Basal blood glucose level is approximately 5mM
GLUT1 – 1mM GLUT2 – 15~20mM
GLUT3 – 1.7mM GLUT4 – 4.6mM

GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis
Am J Pathol. (2009) **174**, 1544-1552

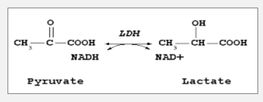
Mitochondrial DNA mutations in human disease
Nat Rev Genet. (2005) **6**, 389-402

Mitochondrial respiratory chain complexes: apoptosis sensors mutated in cancer?
Oncogene. (2011) **30**,:3985-4003

Aerobic Glycolysis: Meeting the Metabolic Requirements of Cell Proliferation.
Annu Rev Cell Dev Biol. (2011) **27**, 441-464

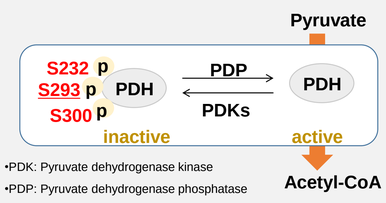
WHY DO CANCERS HAVE HIGH AEROBIC GLYCOLYSIS?
NATURE REVIEWS. (2004) **4**, 891-899

Lactate dehydrogenase (LDH)



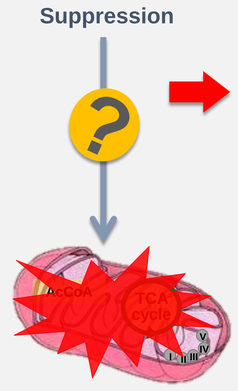
Pyruvate ⇄ Lactate
LDH1 (LDHB)
LDH5 (LDHA)

Pyruvate dehydrogenase



•PDK: Pyruvate dehydrogenase kinase
•PDP: Pyruvate dehydrogenase phosphatase

LDHB Suppression



- Alterations in expressions of respiratory proteins
- Post-translational modification of respiration-related enzymes

Pyruvate dehydrogenase(PDH)

RESULT

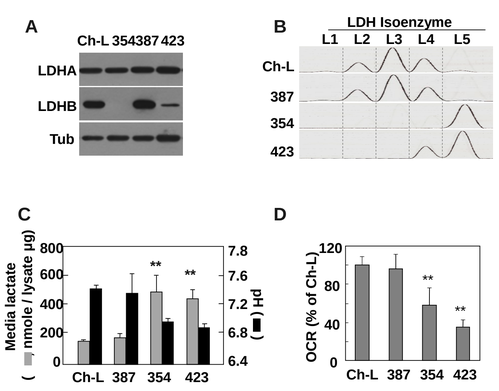


Figure 1. Decreased LDHB expression-mediated lactic acidosis is reversely associated mitochondrial respiratory activity.

Chang cell clone (Ch-L) and three different SNU hepatoma cell lines (SNU-387, SNU-354, and SNU-423) were cultured for 2 days to maintain exponentially growing state.
A. Western blot analysis for LDHA and LDHB expression.
B. In-gel activity profile of LDH isozymes was performed.
C. Lactate levels in media. Media lactate levels released from the cells for 2 days were estimated and expressed as lactate (nmole) released from 1 μg of cell lysate protein.
D. Endogenous cellular oxygen consumption rate was measured and its specificity for mitochondrial respiration was confirmed by adding 10mM KCN. [*, <0.05; **, <0.01 vs. Chang cells (control)].

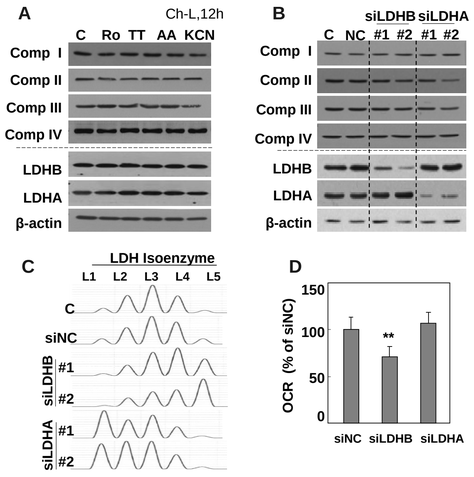


Figure 2. LDHB suppression is an upstream event of decreased mitochondrial respiration.

A. Ch-L was treated with 10 μM Rotenone (Ro), 400 μM TTFA (TT), 10 μM antimycin A (AA), 10 mM KCN (Kcn), 10 μM Oligomycin (Oli) for 12 hrs. Western blot analysis for mitochondrial respiratory complex subunits.
B-D. SNU-387 cells were transfected with siRNA for LDHB (siLDHB) and LDHA (siLDHA) for 3 days.
B. Western blot analysis for mitochondrial respiratory complex expression.
C. In-gel activity profile of LDH isozymes.
D. Cellular oxygen consumption rate.

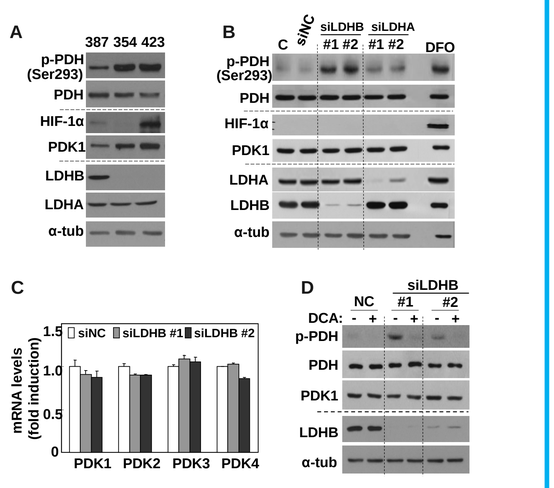


Figure 3. LDHB knockdown induces PDK-mediated PDH phosphorylation without HIF1-PDK1 induction.

A. Western blot analysis for phospho-PDH, HIF-1 and PDK1.
B-D. SNU-387 cells were transfected with siRNA for LDHB (siLDHB) and LDHA (siLDHA) for 3 days.
B. Western blot analysis for phospho-PDH, HIF-1 and PDK1.
C. Quantitative mRNA expression level of PDK isotypes.
D. Before harvest, cells were treated with 5 mM DCA for 1hr. Western blot analysis for phospho-PDH, HIF-1 and PDK1.

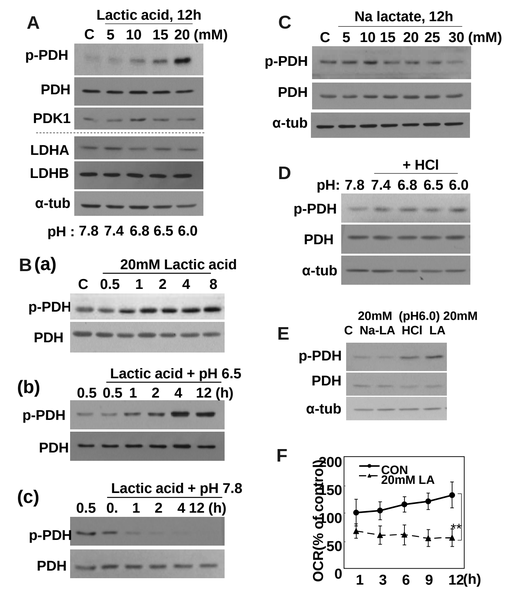


Figure 4. Lactic acidosis increases PDH phosphorylation.

A-E. Western blot analysis for phosphorylation status of PDH.
A. SNU-387 cells were treated with lactic acid for 12hrs. Phosphorylation of PDH increased in dose dependent manner. Western blot analysis for phospho-PDH, HIF-1 and PDK1.
B (a) SNU-387 cells were treated with 20 mM lactic acid in time-course. (b,c) SNU-387 cells were treated with 20 mM lactic acid and pH adjusted to 6.5 (b) and 7.8(c) by 100 mM NaOH.
C. SNU-387 cells was treated with 20 mM sodium lactate in time-course.
D. SNU-387 cells were treated with HCl media adjust to each pH (7.4, 6.8, 6.5, 6.0) with 5N HCl for 12hrs.
E. SNU-387 cells were treated with 20 mM lactic acid, pH 6.0 media, and 20 mM sodium lactate for 12hrs.
F. SNU-387cells were treated with 20 mM lactic acid and monitored with oxygen consumption rate (OCR) in time courses.

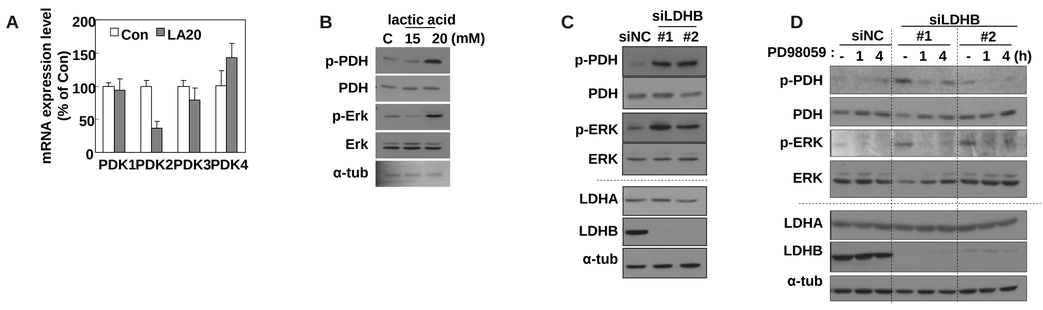


Figure 5. Erk signaling activation is involved with LDHB knockdown mediated PDH inactivation.

A-B. SNU-387 cells were treated with lactic acid for 12hrs.
A. Quantitative mRNA expression level of PDK isotypes.
B. Western blot analysis for phospho-PDH, phospho-ERK.
C-D. SNU-387 cells were transfected with siRNA for LDHB (siLDHB) for 3 days.
C. Western blot analysis for phospho-PDH, phospho-ERK.
D. Before harvest, cells were treated with 20μM PD98059 for indicated time. Western blot analysis for phospho-PDH, phospho-ERK.

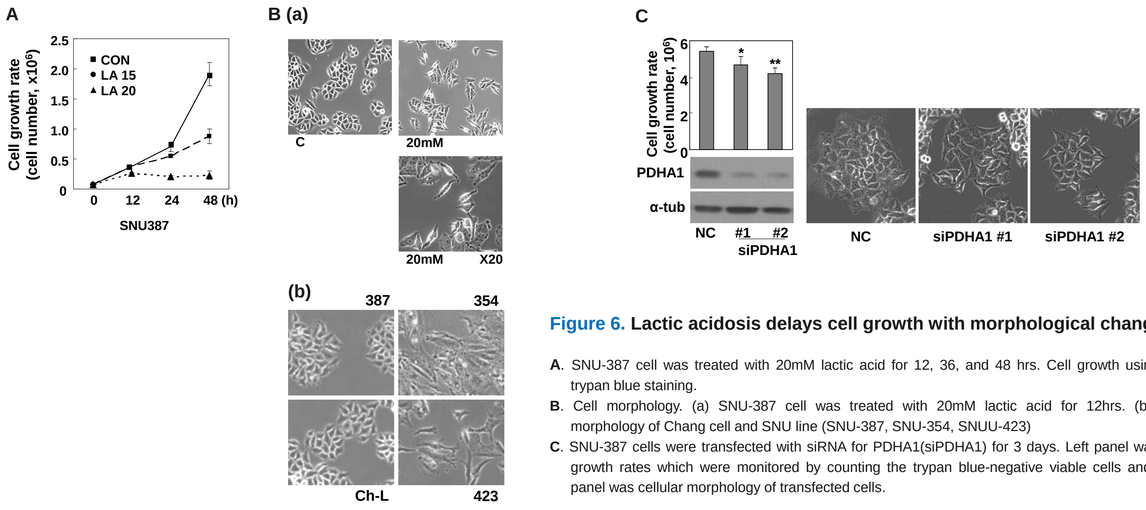
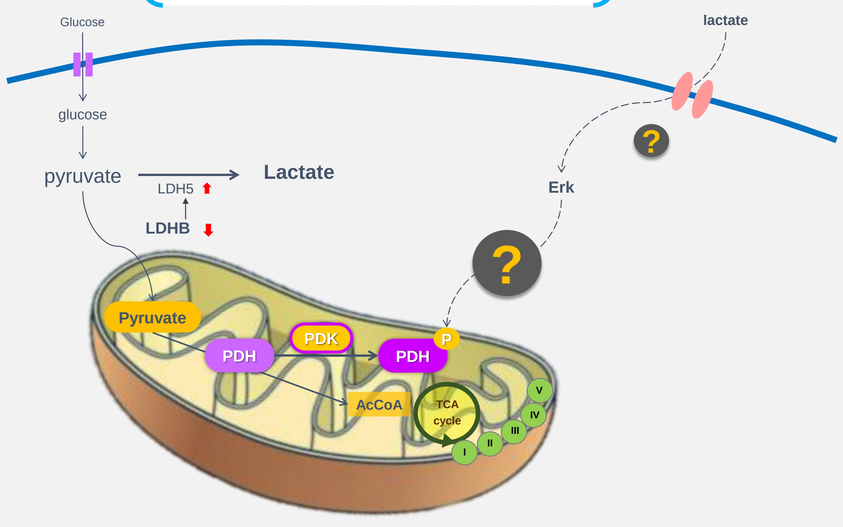


Figure 6. Lactic acidosis delays cell growth with morphological changes.

A. SNU-387 cell was treated with 20mM lactic acid for 12, 36, and 48 hrs. Cell growth using the trypan blue staining.
B. Cell morphology. (a) SNU-387 cell was treated with 20mM lactic acid for 12hrs. (b) Cell morphology of Chang cell and SNU line (SNU-387, SNU-354, SNUU-423)
C. SNU-387 cells were transfected with siRNA for PDHA1(siPDHA1) for 3 days. Left panel was cell growth rates which were monitored by counting the trypan blue-negative viable cells and right panel was cellular morphology of transfected cells.

CONCLUSION



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