Mitochondrion-Nucleus PDH1 Transfer Drives Epigenetic NANOG Reprogramming of Tumor-Initiating Cells in Hepatocellular Carcinoma

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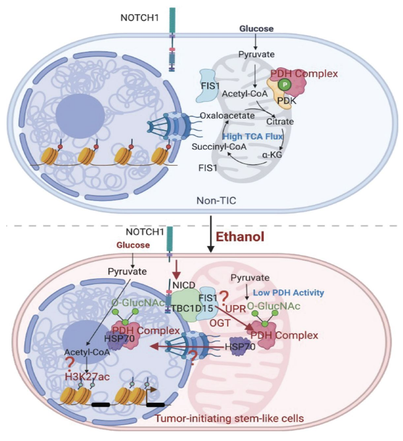
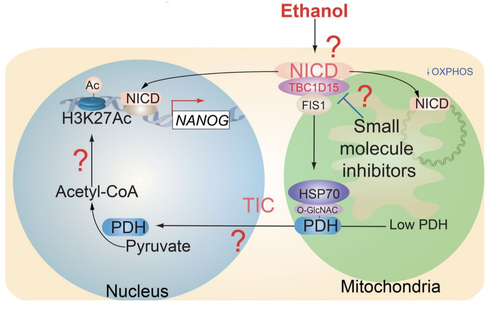
Abstract

Alcohol misuse promotes the development of hepatocellular carcinoma (HCC), which is the third leading cause of malignancy-related mortality in the world. Therapeutic efficacy for HCC is limited due to the therapy resistance and recurrence in which tumor-initiating stem-like cells (TICs) are implicated. The oncoprotein TBC1D15 is induced by TICs and is responsible for the degradation of p53 (a tumor suppressor gene), as well as the activation of the NOTCH1 receptor, which causes the release of an intracellular domain (NICD1). The mitochondrial fission protein 1 (FIS1) regulates mitochondrial activity and HCC metastasis. The TCA enzyme pyruvate dehydrogenase (PDH1) translocates to the nucleus to generate acetyl-coA and stimulate H3K27 acetylation, which epigenetically enhance NANOG expression. NANOG is a transcription factor that maintains the pluripotency of TICs and allows them to continually self-renew. NICD1, TBC1D15, and FIS1 form a tripartite complex that initiates PDH1 nuclear transfer.



Hypothesis: Mito-nucleus crosstalk drives sustained NANOG upregulation initiated by NICD1-TBC1D15-FIS1 interaction, which allows for the nuclear translocation of PDH1 and generation of pyruvate-derived acetyl-CoA. Targeting this interaction is predicted to be therapeutic for alcohol-associated HCC.

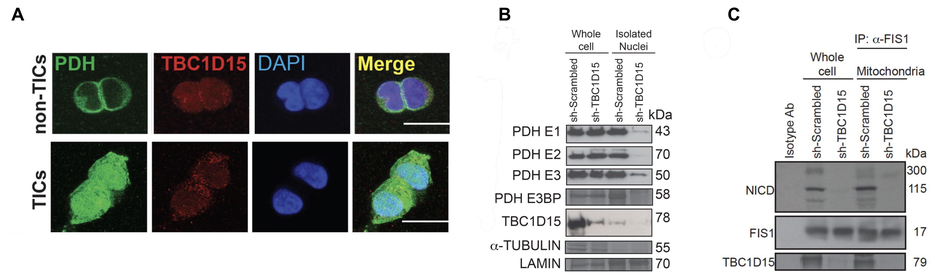


Objective: To dissect the molecular mechanisms of the mito-nucleus transfer of PDH1.

Novelty and Impact: Our study investigates the novel epigenetic regulation of how TICs' self-renewal is maintained and promoted by PDH1 translocation from the mitochondria to the nucleus via the interactions between NICD1, TBC1D15, and FIS1. A translational outcome of the study is the identification of new inhibitors that target the NICD1-TBC1D15-FIS1 interaction and suppress the self-renewal of TICs.

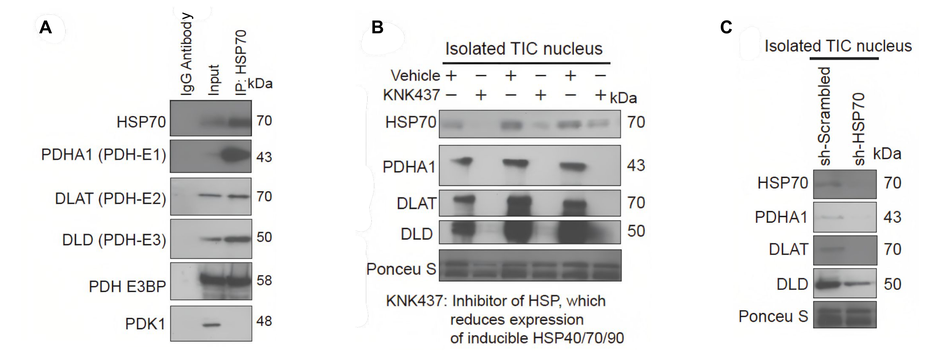
Results

Figure 1: NOTCH Intracellular Domain (NICD) interacts with mitochondria fission protein 1 (FIS1) in TICs in a manner dependent on TBC1D15



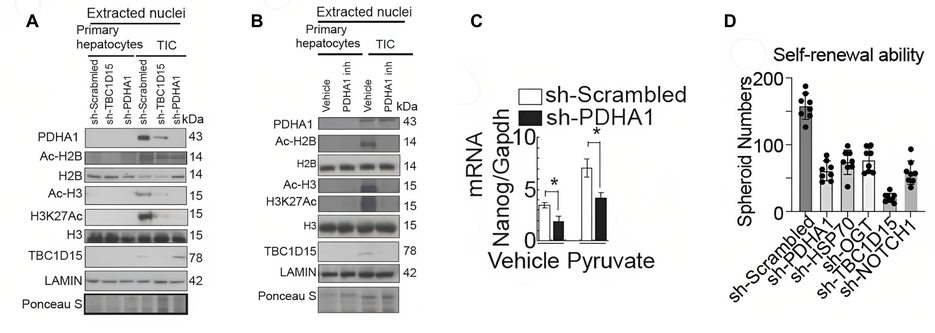
(A) IF staining reveals nuclear localization of PDH1 in TICs. **(B)** IB detects nuclear presence of PDH1 subunits which is diminished by TBC1D15 KD. **(C)** Co-IP analysis reveals TBC1D15 silencing reduces FIS1-NICD interaction.

Figure 2: Mitochondrial heat shock protein HSP70 binds to PDH complex



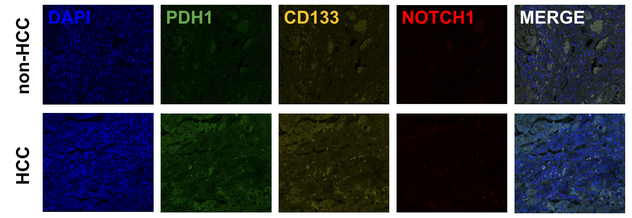
(A) HSP70 interacts with PDH subunits in TICs. **(B)** KNK437 treatment reduces nuclear levels of PDH1 subunits. **(C)** HSP70 KD reduces nuclear PDH1 subunits.

Figure 3: A novel epigenetic mechanism of PDH1-dependent H3K27ac upregulation for NANOG expression in TICs



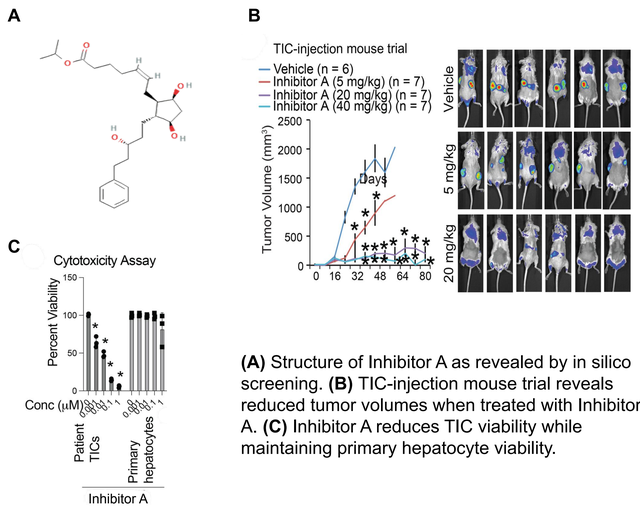
(A) TBC1D15 and PDHA1 KD reduce nuclear PDHA1 and histone acetylation. **(B)** PDHA1 inhibitor treatment reduces histone acetylation including H3K27Ac in TICs. **(C)** NANOG mRNA expression is reduced by PDHA1 KD in both basal and pyruvate-stimulated TICs. **(D)** TIC self-renewal is suppressed by KO of PDHA1 as shown by KD of HSP70, OGT, TBC1D15, and NOTCH1, which are required for PDHA1 nuclear transfer.

Figure 4: PDH1, CD133, and NOTCH1 in patient tissues



IHC staining reveals elevated levels of PDH1, CD133, and NOTCH1 in HCC vs non-HCC patient tissues.

Figure 5: Inhibitor A inhibits TIC self-renewal



(A) Structure of Inhibitor A as revealed by in silico screening. **(B)** TIC-injection mouse trial reveals reduced tumor volumes when treated with Inhibitor A. **(C)** Inhibitor A reduces TIC viability while maintaining primary hepatocyte viability.

Summary

We can conclude the following:

- PDH1 translocates to the nucleus, and this is dependent on TBC1D15.
- NICD1-TBC1D15-FIS1 interaction is essential for PDH1 nuclear translocation.
- HSP70 binds to PDH1 and is crucial in PDH1 nuclear translocation.
- NANOG upregulation is dependent on PDH1 nuclear translocation.
- Higher concentrations of PDH1, NOTCH1, and CD133 are observed in HCC vs. normal patient tissues.
- Inhibitor A has shown promising results in the mouse trial and cytotoxicity assay. However, the data from the mouse trial was generated from tumors induced in the rear flank area due to accessibility.

Moving forward, we could conduct another experiment to test the effect of Inhibitor A on hepatocellular carcinoma in mice. We could also target the O-GlcNAcylation of PDH1 because this interaction promotes PDH nuclear transfer.

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