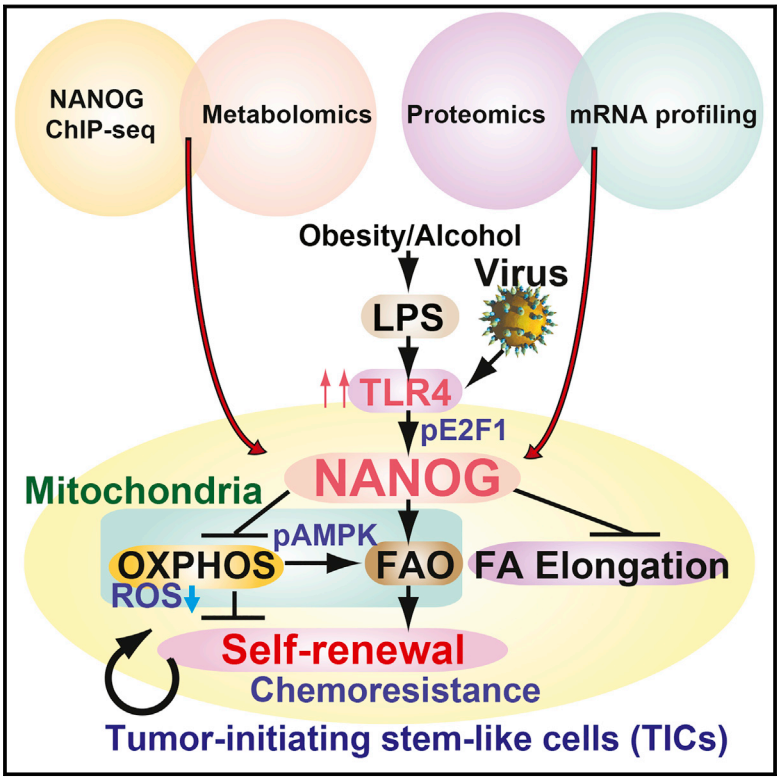
Article

Cell Metabolism

NANOG Metabolically Reprograms Tumor-Initiating Stem-like Cells through Tumorigenic Changes in Oxidative Phosphorylation and Fatty Acid Metabolism

Graphical Abstract



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In Brief

Chen et al. show that the pluripotency transcription factor NANOG contributes to liver cancer progression by reprogramming mitochondrial metabolism to promote self-renewal ability, tumor-initiation property, and chemoresistance of tumor-initiating stem-like cells (TICs). Restoration of OXPHOS activity and inhibition of fatty acid oxidation restores TIC susceptibility to chemotherapy drugs.

Highlights

- Stem cell marker NANOG is activated by the TLR4-E2F1 pathway
- NANOG ChIP-seq identifies target genes involved in OXPHOS and FAO
- Nanog represses OXPHOS and mitochondrial ROS in TICs
- Restoration of OXPHOS and inhibition of FAO restores TIC susceptibility to drugs

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NANOG Metabolically Reprograms Tumor-Initiating Stem-like Cells through Tumorigenic Changes in Oxidative Phosphorylation and Fatty Acid Metabolism

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SUMMARY

Stem cell markers, including NANOG, have been implicated in various cancers; however, the functional contribution of NANOG to cancer pathogenesis has remained unclear. Here, we show that NANOG is induced by Toll-like receptor 4 (TLR4) signaling via phosphorylation of E2F1 and that down-regulation of *Nanog* slows down hepatocellular carcinoma (HCC) progression induced by alcohol western diet and hepatitis C virus protein in mice. NANOG ChIP-seq analyses reveal that NANOG regulates the expression of genes involved in mitochondrial metabolic pathways required to maintain tumor-initiating stem-like cells (TICs). NANOG represses mitochondrial oxidative phosphorylation (OXPHOS) genes, as well as ROS generation, and activates fatty acid oxidation (FAO) to support TIC self-renewal and drug resistance. Restoration of OXPHOS activity and inhibition of FAO renders TICs susceptible to a standard care chemotherapy drug for HCC, sorafenib. This study provides insights into the mechanisms of NANOG-mediated generation of TICs, tumorigenesis, and chemoresistance through reprogramming of mitochondrial metabolism.

INTRODUCTION

Major risk factors for the third-deadliest cancer, hepatocellular carcinoma (HCC), are hepatitis C virus (HCV), alcoholism, and obesity (He et al., 2008; Okuda et al., 2002). Compelling evidence identifies a synergism between obesity/alcohol and HCV infection with the associated risk of developing HCC (Yuan et al., 2004). The risk of HCC increases from 8–12 to 48–54 by co-morbidities such as alcoholism or obesity (Yuan et al., 2004). Obesity and alcoholism increase gut permeability leading to endotoxemia, which in turn activates Toll-like receptor 4 (TLR4) in the liver with production of cytokines and an inflammatory response. This leads to subsequent development of obesity/alcohol-related liver disease (Hritz et al., 2008). Therefore, an in-depth understanding of the underlying molecular mechanisms regulating obesity/alcohol/HCV-induced hepatocarcinogenesis is essential for the development of improved therapeutics.

By using mice with liver-specific expression of the HCV NS5A protein, we demonstrated that mice fed alcohol for 12 months develop liver tumors in a TLR4-dependent manner (Chen et al., 2013). TLR4 is ectopically induced by the HCV viral protein NS5A in hepatocytes/hepatoblasts. Circulating endotoxin binds TLR4, activates hepatocytes/hepatoblasts, and induces the stem cell marker NANOG. This process generates TLR4/NANOG-dependent, chemoresistant tumor-initiating stem-like cells (TICs; CD133+), which can induce HCC in mice (Chen et al., 2013).

TICs are rare, highly malignant cells that are present in diverse tumor types and play a central role in tumorigenesis, malignant progression, and resistance to chemotherapy (Machida et al., 2009; Rountree et al., 2008). Sorafenib, a multi-kinase inhibitor, is the most commonly used monotherapy agent for the treatment of HCC; however, resistance to sorafenib eventually occurs in patients (Villanueva et al., 2008). We recently reported that treatment with sorafenib made TICs more susceptible to tumor growth retardation, with a decrease in tumor size by ~55% when combined with knockdown of NANOG-inducible proto-oncogenes (including YAP1, which induces antioxidant gene programs) (Chen et al., 2013). However, the underlying mechanism of chemoresistance and self-renewal of TICs remains incompletely understood.

We hypothesized that NANOG promotes self-renewal ability, tumor-initiation property, and chemoresistance of TICs through metabolic reprogramming. Our studies showed that oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) were NANOG-mediated oncogenic pathways through metabolic reprogramming as demonstrated by NANOG ChIP-seq analysis and metabolomic profiling.