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Oncofetal SALL4-Driven Tumorigenesis Is Highly Dependent on Oxidative Phosphorylation, Revealing Therapeutic Opportunities

See "New high-throughput screening identifies compounds that reduce viability specifically in liver cancer cells that express high levels of SALL4 by inhibiting oxidative phosphorylation, by Tan JL, Li F, Yeo JZ, et al, on page 1615.

Oncofetal genes are factors that regulate normal embryonic and fetal development, but these factors can become aberrantly reactivated in tumorigenesis. The oncofetal gene SALL4 plays an important role in a subtype of aggressive hepatocellular carcinoma (HCC), and blocking the action of this protein could have an enormous therapeutic impact on HCC. In this issue of *Gastroenterology*, Tan et al[1] find that SALL4 drives tumorigenesis by binding and transcriptionally activating many oxidative phosphorylation (OXPHOS) genes, thus creating a therapeutic opportunity for HCC.

Liver cancer is the second leading cause of cancer mortality worldwide, owing to limited therapeutic options. One of the most important hallmarks of cancer is metabolic reprogramming. During tumorigenesis, cancer cells often rewire their bioenergetic metabolism by switching from mitochondrial OXPHOS to aerobic glycolysis despite the presence of oxygen, a phenomenon commonly known as the Warburg effect. However, in this issue of *Gastroenterology*, Tan et al[1] showed that SALL4$^+$ cells in HCC defy this common trend in cancer cell metabolism by driving and depending on mitochondrial OXPHOS instead.

SALL4 is a zinc finger transcription factor that regulates pluripotency and self-renewal in embryonic stem cells,[2,3] and its expression is mostly extinguished by birth. However, SALL4 is reactivated in plasma cell myeloma, acute lymphoblastic leukemia, breast cancer, lung cancer, colorectal cancer, and liver cancer,[4,5] thus qualifying it as an oncofetal gene. SALL4 plays an especially important role in the extensive network of molecular pathways involved in hepatocarcinogenesis.[5] Indeed, by developing an endogenous-isogenic chemical genetic screening platform, Tan et al identified 4 natural compounds that selectively suppress SALL4$^+$ cancer cells but not SALL4$^-$ cancer cells. In particular, the mitochondrial adenosine triphosphate (ATP) synthase inhibitor oligomycin showed a strong potency in suppressing SALL4-dependent HCC with very low doses in vitro and in vivo. Importantly, the low doses used in these settings did not cause any of the severe muscular, respiratory, or convulsion toxicity effects typically observed at high doses in mice. These discoveries should stimulate exploration of these compounds in the clinic.

Chromatin immunoprecipitation sequencing and RNA sequencing profiles showed that SALL4 directly bound and increased the transcription of many mitochondrial OXPHOS genes. To further elucidate the mechanism of SALL4 in tumorigenesis, Tan et al. silenced SALL4 in SALL4$^+$ cancer cells, which led to the downregulation of many OXPHOS genes, including ATP5D, ATP5E, ATP5G2, and NDUFA3 among others, and a functional decrease in mitochondrial oxygen consumption and OXPHOS. Through liquid chromatography–mass spectrometry metabolomics profiling, they also observed that SALL4 significantly increased the malate-aspartate shuttle-related metabolites, the NAD$^+$/NADH ratio, and ATP, consistent with changes in the cytosolic-mitochondrial redox balance and increased OXPHOS. Several of the glycolysis intermediates and urea cycle metabolites were also perturbed, although these were more likely owing to indirect effects coupled with the increase in mitochondrial OXPHOS.

SALL4 is a key transcription factor and marker of several stem cell types and cancers. Both stem cells and cancer cells share a common requirement for high proliferative capacity, which is typically fueled by the Warburg effect. The Warburg effect primarily operates in highly proliferative cells to rapidly build up glycolytic intermediates for proliferation, while minimizing reactive oxygen species–induced damage. These anabolic intermediates can be shunted into nucleotide, amino acid, or lipid synthesis.[6] However, Tan et al[1] showed that many steady-state glycolysis intermediates and basal lactate production are significantly decreased in SALL4$^+$ cancer cells, although the maximal glycolytic capacity is only slightly decreased, suggesting that SALL4$^+$ cancer cells actually possess a latent capacity for the Warburg effect, but this latent capacity is suppressed at steady state. One likely

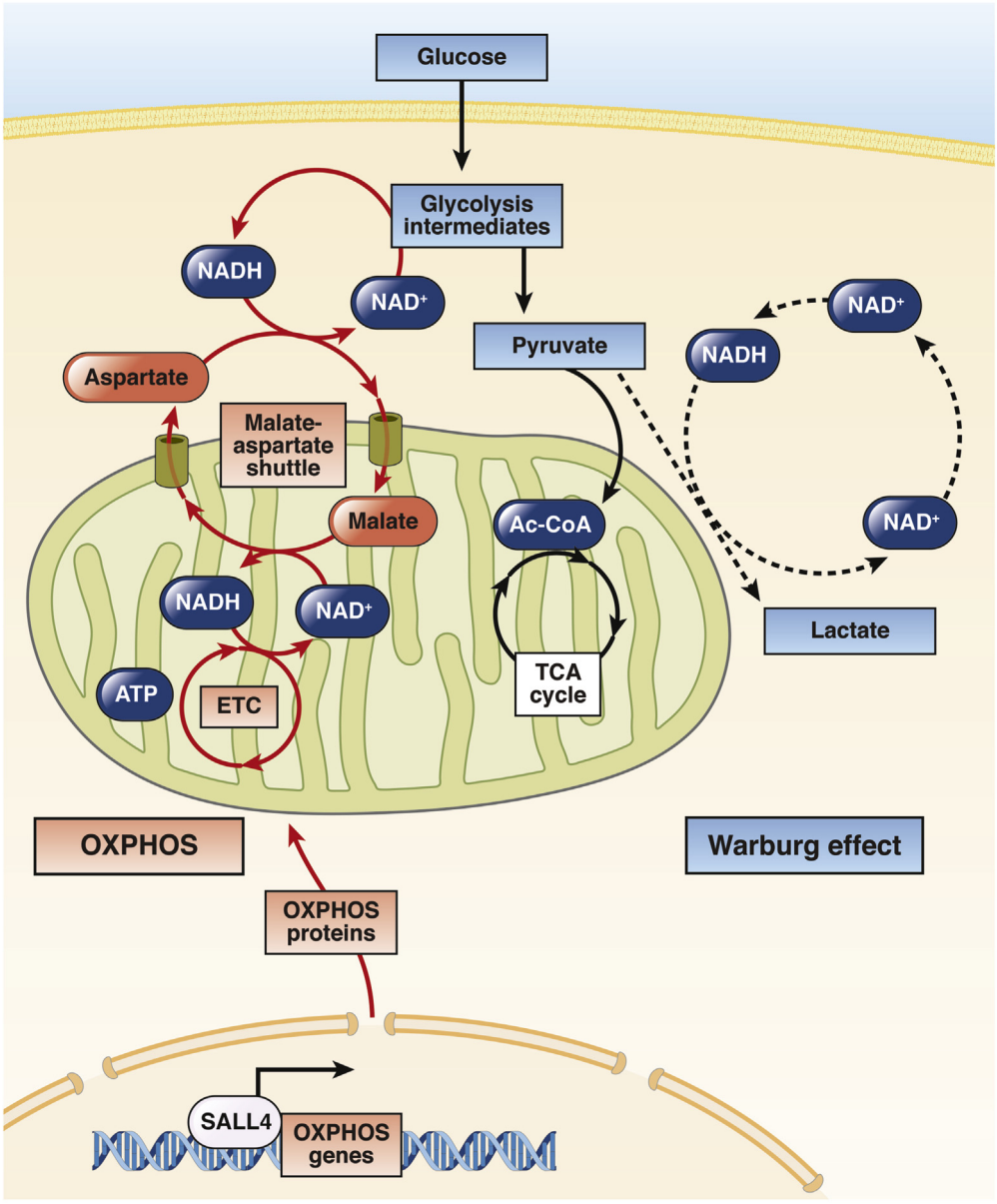

Figure 1. Metabolism in SALL4-dependent hepatocellular carcinoma (HCC) cells. Tan et al[1] have shown that whereas most cancer cells prefer to upregulate and depend on aerobic glycolysis in the Warburg effect, SALL4-dependent cancer cells demonstrate a preferential dependence on mitochondrial OXPHOS. Ac-CoA, acetyl-coenzyme A; ETC, electron transport chain; NAD, nicotinamide adenine dinucleotide; OXPHOS, oxidative phosphorylation.

explanation is that SALL4 induction of OXPHOS and mitochondrial ATP led to the allosteric suppression of basal glycolysis rates. A key step in the Warburg effect is the pyruvate-lactate conversion step, which is necessary to recycle the rate-limiting NAD^+ coenzyme and keep the redox reactions in glycolysis running rapidly. SALL4 induction of the malate-aspartate shuttle might have replaced the pyruvate-lactate step to recycle the rate-limiting NAD^+ and keep glycolysis active, albeit at slower rates (Figure 1).

But why did SALL4 induce mitochondrial OXPHOS? Previous work on metabolic reprogramming in tumorigenesis had already shown that mitochondria can sustain cancer cell survival and proliferation, not only because they generate ATP by OXPHOS, but also because of their involvement in many biochemical pathways, such as glutaminolysis, nucleotide metabolism and de novo lipogenesis, which lead to precursors for biomass accumulation. Moreover, pyruvate derived from glycolysis, fatty acids and amino acid can also supply substrates to the TCA cycle to sustain mitochondrial ATP production in cancer cells.[7-9] These findings behoove us to look deeper into cancer metabolism to discover more metabolic vulnerabilities for cancer.

More questions remain to be answered, of course. For example, why exactly do SALL4-dependent HCC cells have such high sensitivity to low doses of OXPHOS inhibitors, when most healthy normal cells are also dependent on mitochondrial OXPHOS for most of their ATP? Are there differences in total OXPHOS flux, reactive oxygen species clearance, or metabolic flexibility? Do other oncofetal genes, such as LIN28 and the IGF2BPs,[10] also similar patterns of OXPHOS during fetal development and cancer? Finally, how

does tumor heterogeneity in oxygen distribution relate to the heterogeneity in SALL4^{+} progenitors and OXPHOS induction, and the hypothesized cancer phenomenon of cancer stem cells?[11] Answers to these questions will not only further extend our understanding of cancer metabolic reprogramming, but also push the development of innovative therapies.

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A Dive Into the Deep Heterogeneity of Hepatocellular Carcinoma

See "Genomic and epigenomic features of primary and recurrent hepatocellular carcinomas," by Ding X, He M, Chan AWH, et al, on page 1630.

Over the past decade, high-throughput genomic data has significantly improved our understanding of the molecular heterogeneity of cancer. Different layers of tumor heterogeneity have been described: (1) genetic and molecular diversity among tumors from different patients, (2) spatial intratumor heterogeneity within each tumor, and (3) temporal heterogeneity with molecular variation that occurs during tumor evolution.

Hepatocellular carcinoma (HCC) is a heterogeneous and aggressive malignancy that arises from a single tumor cell through the sequential selection of genomic and epigenomic alterations driving the genetic diversity within tumors. This molecular heterogeneity contributes to the high risk of cancer recurrence and potentially to the primary and secondary resistance to systemic targeted therapies.[1] Most of the studies on tumor heterogeneity in HCC have focused on the identification of distinct molecular subtypes which are associated with different etiological and clinicohistopathologic features. Accordingly, 2 major classes, each integrating several molecular subgroups have been identified: a proliferative class characterized by chromosomal instability and an enrichment of *TP53* gene mutations and a nonproliferative class that includes less aggressive and more differentiated tumors characterized by chromosomal stability and enriched in *CTNNB1* activating mutations.[2,3] Furthermore, these studies delineated the genetic/epigenetic landscape of HCC through the identification of recurrent genomic/epigenomic alterations in 6 major signaling pathways: telomere maintenance, Wnt/ß-catenin, cell cycle regulation, oxidative stress, epigenetic modifiers, AKT/mTOR, and MAP kinase pathways.[4,5] Although there is a