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Title: Mitochondrial defect-responsive gene signature in liver-cancer progression

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Keywords: gene signature; hepatocellular carcinoma; mitochondrial defect; retrograde signal; transcription regulator

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Perspective to: Lee et al. (2015), Identification of a mitochondrial defect gene signature reveals *NUPRI* as a key regulator of liver-cancer progression, Hepatology DOI.10.1002

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Running title: Mitochondrial gene signature in liver cancer

Abbreviations: common mitochondrial defect (CMD); nuclear protein 1 (*NUPRI*); granulin (*GRN*); hepatocellular carcinoma (HCC)

Abstract

Mitochondrial respiratory defects are a key bioenergetics feature of hepatocellular carcinoma (HCC) cells. However, their involvement and roles in HCC development and progression remain unclear. Recently, we identified 10 common mitochondrial defect (CMD) signature genes that may be induced by retrograde signaling-mediated transcriptional reprogramming in response to HCC mitochondrial defects. HCC patients with enriched expression of these genes had poor prognostic outcomes, such as shorter overall survival and recurrence-free survival. Nuclear protein 1 (*NUPRI*), a key transcription regulator, was up-regulated by Ca^{++} -mediated retrograde signaling. *NUPRI*-centric network analysis and a biochemical promoter-binding assay demonstrated that granulin (*GRN*) is a key downstream effector of *NUPRI* for the regulation of HCC cell invasiveness; association analysis of the *NUPRI-GRN* pathway supported this conclusion. Mitochondrial respiratory defects and retrograde signaling thus play pivotal roles in HCC progression, highlighting the potential of the *NUPRI-GRN* axis as a novel diagnostic marker and therapeutic target for HCC.

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Aerobically growing cells produce most of their energy through oxidative phosphorylation, which consists of five respiratory complexes. Mitochondria, the “powerhouses” of the cell, have their own independent genome (mitochondrial DNA), which encodes 13 mRNAs for respiratory complex subunits, 22 mitochondrial tRNAs, and two rRNAs; these components support oxidative phosphorylation. The main function of mitochondria is therefore considered to be ATP production, although mitochondria also engage in other metabolic processes, including β -oxidation of fatty acids, the citric acid cycle, and heme synthesis.

Mitochondrial respiratory defects and activated glycolysis are the most striking metabolic hallmarks of cancer cells. Although accumulating evidence has emphasized the importance of

efficient glycolytic ATP generation and subsequent lactic acidosis in acquiring tumor-cell activity (growth and invasiveness) and in forming the tumor microenvironment, it remains unclear whether mitochondrial defects are merely an epiphenomenon accompanied by inevitable hypoxia due to fast tumor growth or whether these defects play causative roles in the course of cancer development.

Bioinformatics-based analysis of differentially expressed genes employed three independently designed cellular models of respiratory defects: hepatocellular carcinoma (HCC) cells harboring a respiratory defect (a tumoral defect), cells subjected to pharmacological inhibition of respiration (a functional defect), and cancer cells depleted for mitochondrial DNA (a genetic defect). This approach revealed 10 common mitochondrial defect (CMD) genes that were up-regulated in response to respiratory defects. Surprisingly, enriched expression of the CMD signature was closely correlated with poor clinical outcomes in HCC patients, such as shorter overall survival and recurrence-free survival, as evidenced by prognostic correlation analysis of two independent HCC cohorts. These findings emphasize that mitochondrial respiratory defects and subsequent transcriptional reprogramming play key roles in the progression of HCC.

Mitochondria communicate continuously with the nucleus through ‘mitochondrial retrograde signaling’ to maintain mitochondrial homeostasis and to reconfigure cellular metabolism or function in response to mitochondrial stress. This retrograde signaling includes the release of second messengers, such as reactive oxygen species and Ca^{++} , alteration of NAD^+/NADH and ADP/ATP ratios, transmission of this signal to several cytosolic signal transducers, and activation of transcription factors or cofactors that switch on transcriptional reprogramming (Fig. 1, left and center panels). Although increasing evidence indicates the existence of multiple molecular mechanisms of retrograde signaling in diverse pathologies, few studies have examined these transcriptional responses in order to identify the actual effectors, especially in cancer progression. These effectors may include secondary effectors that in turn induce additional transcriptional reprogramming. It is important to identify the secondary transcription factors or regulators induced by primary transcriptional activation because single secondary transcription factors/regulators can contribute to a variety of phenotypes during tumor progression. Interestingly, the genes encoding three secondary transcriptional regulators, *NUPR1*, *NFIX*, and *NFE2L1*, were included in the set of 10 CMD genes (Fig. 1, right panel), suggesting their involvement in HCC progression. *NUPR1* expression was induced by Ca^{++} signaling triggered by the respiratory defect, increasing HCC cell invasiveness via the activation of granulin (*GRN*) promoter activity. These observations suggest that *NUPR1* and *GRN* may constitute novel therapeutic targets for advanced HCC. Future detailed studies of *NFIX* and *NFE2L1* will elucidate mitochondrial retrograde responses during HCC progression.

HCC is one of the most common tumors worldwide; it often develops in conjunction with chronic viral hepatitis and/or cirrhosis. Unfortunately, no effective targeted therapy has been developed for HCC, unlike for other cancers such as breast cancer (trastuzumab), chronic myelogenous leukemia (imatinib), and lung cancer (gefitinib). Although sorafenib, a multikinase inhibitor, has recently been approved as a standard first-line treatment for patients with advanced HCC, few patients respond to this drug and its effect lasts only a few months, probably due to pre-existing or acquired chemoresistance. Therefore, it is important to identify novel therapeutic targets for HCC. One of the key roles of the liver is the metabolism and clearance of chemicals, including alcohols; hence, the liver is highly

susceptible to drug toxicity within the patient's lifespan. Drug-induced hepatitis and acquired chemoresistance may contribute to HCC progression. On the other hand, liver cells contain >1000 mitochondria, and many HCC cells harbor defective mitochondria, implying their functional dependency on mitochondria and a pathogenic link to mitochondrial defects. It is urgent and critical to elucidate the detailed molecular mechanisms underpinning mitochondrial defects and subsequent transcriptional reprogramming, and to explore how these mechanisms are involved in HCC progression and chemoresistance, thereby uncovering novel therapeutic targets and strategies for HCC. Our recent study of a mitochondrial defect-responsive gene signature via bioinformatics-based analysis of diverse cell models and human HCC cohorts will guide future investigations.

Acknowledgments

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Figure Legend

Figure 1. Schematic model for the molecular involvement of mitochondrial defects and their responses in HCC progression. Several primary (1o) transcription factors (TFs) or transcription regulators (TRs) are activated by mitochondrial defect-triggered retrograde signaling and in response to this signal diverse secondary TFs and TRs, and effector genes are expressed, contributing to HCC progression.

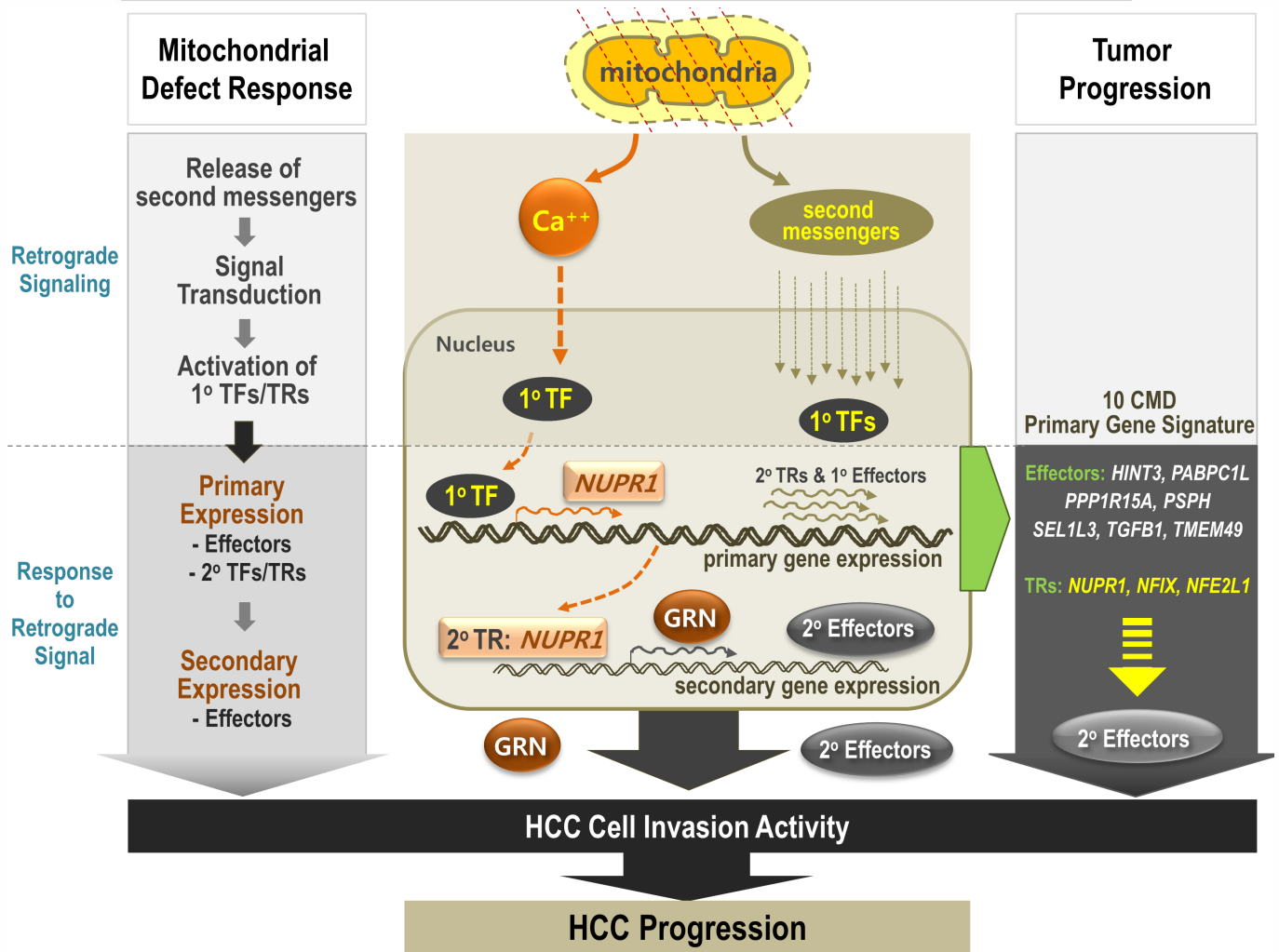


Fig. 1