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NANOG-Dependent Metabolic Reprogramming and Symmetric Division in Tumor-Initiating Stem-like Cells

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Abstract

Alcohol abuse synergistically heightens the development of the third most deadliest cancer hepatocellular carcinoma (HCC) in patients infected with hepatitis C virus (HCV). Ectopically expressed TLR4 promotes liver tumor-igenesis in alcohol-fed HCV Ns5a or Core transgenic mice. CD133+/CD49f + tumor-initiating stem cell-like cells (TICs) isolated from these models are tumorigenic have p53 degradation via phosphorylation of the protective protein NUMB and its dissociation from p53 by the oncoprotein TBC1D15. Nutrient deprivation reduces overexpressed TBC1D15 in TICs via autophagy-mediated degradation, suggesting a possible role of this oncoprotein in linking metabolic reprogramming and self-renewal.

Keywords

HCC; Cancer stem cells; Tumor-initiating stem-like cells (TICs); NUMB

8.1 Introduction

Major risk factors for HCC are HCV, HBV, alcoholism, and obesity [12, 31]. Alcoholic liver disease (ALD) and viral hepatitis (HBV and HCV) are associated with development of hepatocellular carcinoma (HCC) [30] as more than 170 million people are infected with HCV worldwide [30,31,45]. HCV proteins (Nucleocapsid Core and others) are linked to transformation through overproduction of reactive oxygen species which may cause mitochondrial or nuclear DNA damage [19, 28, 31]. The core protein also inhibits microsomal triglyceride transfer protein activity and VLDL secretion [32], which contributes the genesis of fatty liver. The core also induces insulin resistance in mice and cell lines, and this effect may be mediated by degradation of insulin receptor substrates (IRS) 1 and 2 via up regulation of SOCS3 [16] in a manner dependent on PA28 γ 73, or via IRS serine phosphorylation [5]. HCV-induced mechanisms promotes HCC risk with non-alcoholic fatty liver disease (NAFLD) (9). HCV/HBV infection, ALD, and NAFLD share common pathophysiological events such as oxidant stress, organelle stress, and metabolic dysregulation which may contribute to their oncogenic activities.

Refractoriness to chemotherapy after HCC treatment is challenging via genesis of tumor-initiating stem cell-like cells (TICs) or so-called cancer stem cells (CSCs). Stem cells have three major characteristics, self-renewal, asymmetric division (clonality), and plasticity. Forty percent of HCC are assumed to have clonality and to originate from progenitor/stem cells [1, 34, 39, 48]. CD133+/CD49f + cells in liver tumors correlate with tumorigenesis and the expression of “stemness” genes, such as Wnt/ β -catenin, Notch, Hedgehog/SMO, and Oct3/4 [6, 9, 40]. Indeed, CD133+/CD49f + HCC TICs are chemoresistant [35], survive during an initial therapy. Although an encouraging therapeutic response may be seen, survived TICs eventually establish a clonal expansion and tumor recurrence. This chemoresistance may be caused by the plasticity of TICs with dysregulated signaling and gene expression. Several oncogenic signaling pathways are activated in HCC or TICs, including PI3K/AKT [24], signal transducer and activator of transcription 3 (STAT3) [43, 46], and hedgehog [37, 38] while defective tumor suppressor transforming growth factor-beta (TGF- β) pathway is also implicated [18, 29]. Another pivotal mechanism is asymmetric division of TICs producing dormant daughter cells which are less sensitive to chemotherapeutic drugs.

8.2 Synergistic Risk Factors for Alcohol-Associated HCCs

Co-existence of alcohol abuse or obesity, increases the HCV risk of developing HCC by additional eight fold, culminating to an overall 45–55 fold increase in the risk as compared to normal subjects (10,11). As alcohol and obesity continue to dominate as leading life-style factors for disease burdens around the world (12), heightened HCC incidence caused by synergistic interactions of these factors with hepatitis viruses, represents the most predictable and devastating global health issue. Compelling evidence identifies a synergism between obesity/alcohol and HCV infection with the associated risk of developing HCC [47]. The risk of HCC increases from 8–12 to 48–54 by co-morbidities such as alcoholism or obesity [47]. 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 [13]. Therefore, to develop better therapeutics, the underlying molecular mechanisms regulating obesity/alcohol/HCV-induced hepatocarcinogenesis should be elucidated.

8.3 Genesis of TICs Induced by Alcohol Exposures

Liver-specific expression of the HCV NS5A protein in mice fed alcohol for 12 months develop liver tumors in a TLR4-dependent manner [11]. Circulating endotoxin binds CD14-TLR4 complex, 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 [11].

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 [25, 35]. 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 [41].

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 protooncogenes (including YAP1, which induces antioxidant gene programs) [11]. However, the underlying mechanism of chemoresistance and self-renewal of TICs remains incompletely understood.

8.4 TLR4/NANOG-Dependent TICs Give Rise to Tumors

Mouse-derived tumors contain double-positive cells for NANOG and CD 133 or CD49f (24). TLR4 silencing reduces heightened expression of stemness genes and cell proliferation [10]. CD133+/CD49f + cells are TLR4/NANOG-dependent TICs and that Tlr4 is a putative proto-oncogene involved in the genesis/maintenance of TICs and liver tumor in HCV Tg models. Hepatoblastic HCC subtype with poor prognosis has a gene expression profile with markers of hepatic oval cells [3, 8, 20, 44]. HCC often recurs after chemotherapy due presumably to the presence of chemo-resistant TICs [33].

8.5 Metabolic Reprogramming and TIC Self-Renewal

Toll-like receptor 4 (TLR4) signaling phosphorylates E2F1 to transactivate NANOG. Down-regulation of Nanog reduces tumor progression. NANOG ChIP-seq identified genes associated with NANOG-dependent mitochondrial metabolic pathways to maintain tumor-initiating stem-like cells (TICs). The causal roles of NANOG in mitochondrial metabolic reprogramming occurred through the inhibition of oxidative phosphorylation (OXPHOS) with decreased production of mitochondrial ROS and activation of fatty acid oxidation (FAO), which was required for self-renewal and drug resistance [10]. Restoration of OXPHOS activity and inhibition of FAO rendered TICs susceptible to a standard care chemotherapy drug, sorafenib [10].

Complementary NANOG ChIP-seq and metabolomics studies of TICs demonstrated that NANOG induced by TLR4 suppressed mitochondrial OXPHOS and activated FAO, thus inhibiting OCR and ROS production. This conferred a tumor chemoresistant state which could be abrogated by NANOG-targeted gene silencing. Our findings demonstrated a NANOG-dependent downstream effect on mitochondrial function in TICs that contributed to the mechanism of chemotherapy resistance [10]. These metabolic reprogramming promoted self-renewal/oncogenesis, and explained how NANOG activation inhibited therapy-mediated apoptosis by quenching ROS production. Restoration of OXPHOS and activation of decreased FAO reduces tumorigenic capacity of TICs and increases susceptibility to chemotherapy [10].

As TICs rely on active FAO for their maintenance and function, FAO inhibitor suppresses self-renewal of leukemia-initiating cells (LICs) [36]. We experimentally reversed the effects of FAO gene silencing and restored the original TIC phenotype by overexpression of FAO genes. Thus the fate of stem cells is metabolically switched by FAO [14]. Potential mechanisms by which elevation of FAO maintains self-renewal ability include: (i) shunting of long-chain FA away from lipid and cell membrane synthesis; (ii) downregulation of ROS through production of NADPH to avoid loss of TICs; and (iii) reduction of metabolic

resistance to chemotherapy. By these criteria, NANOG function could be construed to serve as a gatekeeper for FAO activity.

8.6 Cell Fate Determinant NUMB and Oncogenesis in TICs

Stem cell populations are maintained through self-renewing divisions in which one daughter cell commits to a particular fate while the other retains the multipotent characteristics of its parent. Tumor-initiating cells (TICs) contribute to oncogenesis and progression to treatment-refractory metastatic disease [22, 23, 42] with the heightened expression and activation of a pluripotency-associated transcription factor (TF) network [15]. The p53 tumor suppressor regulates pluripotency and stem cell division. Genetic deletion or shRNA-mediated depletion of p53 enhances cellular reprogramming to the pluripotent state [4, 17, 27] and p53 can directly repress the expression of pluripotency-associated TFs [21]. p53 is also required to maintain asymmetry and cell polarity in proliferating stem cells and interacts directly with the NUMB protein. A polarity determinant NUMB is distributed asymmetrically in dividing stem cells and is segregated into the daughter cell which undergoes differentiation. The association with a tumor suppressor Numb stabilizes p53 [7, 26]. Regulation of the assembly or stability of the Numb-p53 complex mediates TIC-derived oncogenesis. NUNB-associated oncoproteins MDM2 E3 ubiquitin ligase destabilize the Numb-p53 complex and promote proteolysis of p53 [2]. The NUMB, a tumor suppressor, in conjunctions with another tumor suppressor protein p53, preserves this property and acts as a barrier against deregulated expansion of Tumor-associated stem cells. In this context, NUMB-p53 interaction plays a crucial role to maintain the proper homeostasis of both stem cells, as well as differentiated cells. As the molecular mechanism governing the assembly and stability of the NUMB-p53 interaction/complex are poorly understood, we tried to identify the molecule/s govern this process. Using cancer cell lines, tumor-initiating cells (TICs) of liver, the mouse model and clinical samples, we identified that phosphorylations of NUMB destabilize p53 and promotes self-renewal of TICs by pluripotency-associated transcription factor NANOG dependent manner. NANOG phosphorylates NUMB via aPKC ζ , through the direct induction of Aurora A kinase (AURKA) and the repression of an aPKC ζ inhibitor, LGL-2. Phosphorylation of NUMB by aPKC ζ destabilizes the NUMB-p53 interaction, p53 proteolysis and to deregulate self-renewal in TICs Fig. 8.1.

8.7 Conclusions and Discussions

We successfully isolated CD133+/CD49f + TICs which activate a unique TLR4-NANOG pathway as an integral component for their self-renewal and tumorigenic activities. These TICs are also identified in HCC sections of alcoholic HCV patients by immunostaining and isolated from such patients to validate induction of TLR4-dependent stemness genes and transformation. Based on this renewed concept, our studies have offered two novel insights into the molecular mechanisms of NANOG-mediated p53 degradation by disengagement from the protective NUMB protein via TBC1D15 interaction. Posttranslational modification of NUMB by NANOG-AURKA-aPKC ζ pathway is an important event in TICs self-renewal and tumorigenesis. Hence, our work identifies the NANOG-NUMB-p53 signaling axis is an important regulatory pathway for TICs event in TICs self-renewal and liver tumorigenesis and suggest a therapeutic strategy by targeting NUMB-phosphorylation. However, further in

depth in vivo and clinical studies are warranted to verify this suggestion. These studies are now exploring potential mechanistic connections to metabolic programming known to occur in cancer cells and TICs in promoting and maintaining “stem cell fate” via molecular, genetic, and epigenetic mechanisms.

NANOG maintains chemotherapy resistance of TICs involving not only the direct activation of self-renewal via stemness genes, but also the subsequent metabolic reprogramming in these cells leading to amplification of TIC oncogenic activity and their overall survival. Our data showed that NANOG reprogramming of mitochondrial metabolism was indeed responsible for human TIC oncogenicity and chemoresistance. The metabolic bases of altered cell functions and cell fate in TICs define potentially new approaches for chemosensitization and elimination of TICs for more efficacious HCC therapies. These studies have led to a paradigm shift in our understanding the underlying basis of alcohol/HCV-associated cancer, thus facilitating future development of new personalized treatment strategies targeted towards NANOG+ TICs arising from obesity, alcohol, or HCV-related HCC. The studies provide insights into the mechanisms of NANOG-mediated generation of TICs, tumorigenesis and chemo-resistance due to metabolic reprogramming of mitochondrial functions.

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Fig. 8.1.
Tumor initiation and genesis of TIC through environmental factors and virus infection