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NFκB mediated elevation of KCNJ11 promotes tumor progression of hepatocellular carcinoma through interaction of lactate dehydrogenase A

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ABSTRACT

It has been well documented that changes in ion fluxes across cellular membranes is fundamental in maintaining cellular homeostasis. Dysregulation and/or malfunction of ion channels are critical events in the pathogenesis of diverse diseases, including cancers. In this study, we focused on the study of K^+ channels in hepatocellular carcinoma (HCC). By data mining TCGA cohort, the expression of 27 K^+ channels was investigated and KCNJ11 was identified as a key dysregulated K^+ channels in HCC. KCNJ11 was differentially expressed in HCC and predicted a poor prognosis in HCC patients. Inhibition of NFκB signaling suppressed KCNJ11 expression in HCC cells. Knockdown of KCNJ11 expression inhibited cell proliferation, promoted cell apoptosis, and reduced cell invasive capacity. Mechanistically, we found that KCNJ11 promotes tumor progression through interaction with LDHA and enhancing its enzymatic activity. Pharmacological inhibition of LDHA largely compromised the oncogenic function of KCNJ11 in cell proliferation, cell apoptosis, and cell invasion. Collectively, our data, as a proof of principle, demonstrate that KCNJ11 acts as an oncogene in HCC though forming a complex with LDHA and suggest that targeting KCNJ11 can be developed as a candidate tool to dampen HCC.

1. Introduction

Over the past decade ion channels have been demonstrated to be aberrantly expressed in cancer cells and contribute to multiple malignant phenotypes of cancer cells, such as unlimited proliferation, metabolic reprogramming, apoptosis resistance, drug resistance, stimulation of neo-angiogenesis, and cell migration and invasiveness [1–3]. Ca^{2+}, Na^+, K^+, and Cl^- channels are essential regulators of cell proliferation and tumor progression [4–8]. Interestingly, many ion channel modulators are FDA-approved drugs and available in clinical use [9,10]. Therefore, ion channels are proposed as potential targets in cancer treatment.

K^+ channels have the greatest amount of diversity among ion channels in the plasma membrane. K^+ channels can crosstalk with multiple cellular signaling cascades and regulate a wide range of physiologic processes by adjusting the intracellular K^+ concentration, creating the membrane potential, regulating cell volume, and enhancing Ca^{2+} signaling [1]. Many studies have shown that expression of certain channels correlating with cancer stage and ectopic expression of K^+ channels occurs in human cancers [11–14].

Based on their activation mechanisms and domain structure, K^+ channels can divided into four main classes: calcium-activated K+ channels (KCa), voltage-gated K^+ channels (Kv), inward-rectifier K^+ channels (Kir), and two-pore-domain K^+ channels (K2P). KCNJ11 is an integral membrane protein and inward-rectifier type potassium channel [15,16]. Mutations in KCNJ11 can lead to familial persistent hyperinsulinemic hypoglycemia of infancy (PHHI), an autosomal recessive disorder characterized by unregulated insulin secretion [17]. Defects in KCNJ11 may also contribute to autosomal dominant non-insulin-dependent diabetes mellitus type II (NIDDM),

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transient neonatal diabetes mellitus type 3 (TNDM3), and permanent neonatal diabetes mellitus (PNDM) [18]. KCNJ11 plays an important role in the regulation of insulin secretion by β cells and pathogenesis of diabetes mellitus [19]. However, little is known about its roles in cancers.

In the present study, we fully addressed the expression profile of K^+ channel genes in hepatocellular carcinoma (HCC). As a result, KCNJ11 was identified as a key dysregulated K^+ channels and was regulated by NFκB signaling in HCC. We then confirmed the oncogenic roles of KCNJ11 in cell proliferation, cell apoptosis, and cell invasion by loss-of-function study and gain-of-function study. Finally, we validated the interaction between KCNJ11 and LDHA by co-immunoprecipitation and showed that LDHA partially mediates the pro-tumor effects of KCNJ11 in HCC.

2. Materials and methods

2.1. Cell culture and reagent

The human HCC cell lines Hep3B, MHCC-97H, MHCC-97L, Huh7, SUN-423, and HepG2 were purchased from Shanghai Institute of Cell Bank. The non-malignant THLE-2 cells were preserved in our hospital. Cells were all cultured in DMEM (Gibico) medium containing 10% fetal bovine serum (FBS, Gibco, USA), 1% streptomycin-penicillin (Sigma, Shanghai, China), 2 mM glutamine, and 10 mM HEPES buffer at 37 °C in a 5% CO_2 atmosphere. The LDHA inhibitor FX11 and pathway inhibitors (LY294002, U0126, Rapamycin, JSH-23, XAV-939, and FLI-06) were all purchased from Selleck (Shanghai, China).

2.2. Clinical samples

Tumor tissue samples used in this study were obtained from the Rizhao City People's Hospital. Samples from these patients were collected between 2012 and 2015 and used for quantitative real-time PCR. The commercial HCC tissue microarray (OD-CT-DgLiv01-012) was purchased from Shanghai Outdo Biotech Inc. All patients enrolled were provided with written informed consent and this experimental procedure was approved by the Ethics Committee of Qingdao University.

2.3. Data mining of TCGA

Level 4 gene expression data were downloaded for Liver Hepatocellular Carcinoma (LIHC) from TCGA, which were processed by Broad Institute's TCGA workgroup. The RNA-seq level 4 gene expression data contain upper quartile-normalized and log2-transformed RNA-seq by expectation maximization (RSEM) values summarized at gene level. Specifically, genes expressed in less than 80% of the samples were removed.

2.4. Cell transfection

For transient transfection, cells were plated at 60–70% confluence in 60 mm dishes. Two specific siRNAs target KCNJ11 were synthesized from GenePharma (Shanghai, China). Control siRNA targeting no known gene sequence was used as the negative control. Lipofectamine® RNAiMAX reagent (ThermoFisher Scientific, #13778030) was used to conduct siRNA transfection according to the manufacturer's protocol. For stable transfections, the KCNJ11 coding region was inserted into pcDNA3.1 (GenePharma) and transfected into Hep3B and MHCC-97L cells to stably overexpress KCNJ11. Cells stably transfected with the pcDNA3.1 empty expression vector (Invitrogen) were used as negative control. Positive clones were selected with G418 (Invitrogen, USA).

2.5. RNA extraction and real-time PCR

Total RNA from tissue or cell samples isolated using Trizol (TaKaRa, Dalian, China) according to the manufacturer's instruction. Total RNA (1μg) was converted to cDNA using PrimeScript Reverse Transcriptase (TaKaRa, China). Quantitative real-time PCR was performed on the ABI 7900 Prism HT (Applied Biosystems, USA), followed by melting curve analysis. The $2^{-\triangle\triangle Ct}$ method was used to assess the gene expression fold change among groups. Three independent experiments were performed. The primers used in this study were shown as follows. KCNJ11, 5′-TGATCCTCATCGTGCAGAACA-3′ (forward) and 5′-ACCCACACGTAGCATGAAGC-3′ (reverse); LDHA, 5′-ATGGCAACTCTAAAGGATCAGC-3′ (forward) and 5′-CCAACCCCAACAACTGTAATCT-3′ (reverse); ACTB, 5′-CATGTACGTTGCTATCCAGGC-3′ (forward) and 5′-CTCCTTAATGTCACGCACGAT-3′ (reverse).

2.6. Immunohistochemistry and immunofluorescence

Immunohistochemical staining were performed using routinely described methods. For antibody staining, tissue-containing slides were deparaffinized, rehydrated in an alcohol series, and stained with a primary antibody against KCNJ11 (ab79171; diluted at 1:200, Abcam). To define KCNJ11 expression, samples without any staining as 1 score and other samples were defined as low (2 scores), medium (3 scores), or high (4 scores) levels of expression. The scoring system was evaluated by two independent individuals who were blinded to the slides and the clinical data examined. For immunofluorescence staining, samples of treated cells were stained with anti-KCNJ11 (ab79171; diluted at 1:200, Abcam), followed by incubation with donkey anti-rabbit Alexa Fluor 594 (1:400, Jackson ImmunoResearch, #711-585-152). Positive cells were quantified by confocal microscopy and analyzed by ImagePro Plus software.

2.7. Western blotting

Whole cell extracts were prepared in a lysis buffer, and cellular protein was measured using a BCA protein assay kit (Pierce, Bonn, Germany). Total cellular protein were separated by 10% SDS-PAGE (10% acrylamide) and transferred to a nitrocellulose membrane (Millipore, Billerica, MA, USA). After blocking in 5% skim milk for 1 h, the membranes were incubated with primary antibodies against KCNJ11 (ab79171; diluted at 1:1000, Abcam), LDHA (ab208093; diluted at 1:1000, Abcam), and β-actin (1:1000; A4700, Sigma Aldrich) overnight at 4 °C. After washing three times with TBST, the membrane was incubated with the HRP-conjugated secondary antibody for 1 h at room temperature and proteins bands were visualized using enhanced chemiluminescence (Pierce).

2.8. Cell proliferation, apoptosis, and invasion assay

For cell proliferation assay, the Cell Counting Kit-8 (CCK-8, Solarbio, Beijing, China) was used. HCC cells (3×10^3 per well) seeded in 96-well plates were incubated with for 5 days. At indicated time point, 10 μl of CCK-8 solution was added to each well, and the 96-well plate was incubated at 37 °C for 1 h. The absorbance at 450 nm was measured by a multi-label plate reader (Bio-Rad, Hercules, California USA). For cell apoptosis assay, caspase-3/7 activity assay and Annexin V/PI staining assay were conducted. For caspase-3/7 activity assay, cells were seeded on 96-well plates at a density of 7000 cells per well. After serum starvation for 48 h, cell number and caspase-3/7 activity were monitored on the same sample using CellTiter-Blue (Promega G8081) and Apo-ONE Caspase-3/7 assay (Promega G7790), respectively. The relative Caspase-3/7 activity was estimated as the ratio Apo-ONE/CellTiter-