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Long non-coding RNA KCNQ1OT1 modulates oxaliplatin resistance in hepatocellular carcinoma through miR-7-5p/ ABCC1 axis

Huiqin Hu [a], Li Yang [a], Lijun Li [b], Changchun Zeng [c, *]

[a] Department of Chinese Medicine, Affiliated Longhua Central Hospital, Guangdong Medical University, Shenzhen, 518110, Guangdong, China
[b] Department of Quality Control, Affiliated Longhua Central Hospital, Guangdong Medical University, Shenzhen, 518110, Guangdong, China
[c] Department of Medical Laboratory, Affiliated Longhua Central Hospital, Guangdong Medical University, Shenzhen, 518110, Guangdong, China

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ABSTRACT

The underlying functions of long non-coding RNAs (lncRNAs) on chemoresistance in multiple cancers have been testified. However, the function and mechanism of lncRNAs on chemoresistance in hepatocellular carcinoma are still confused. In this study, we concentrated on the function and mechanism of KCNQ1OT1 on oxaliplatin resistance in hepatocellular carcinoma. Results showed that KCNQ1OT1 was significantly up-regulated in oxaliplatin-resistant HepG2 and Huh7 cells. Moreover, knockdown of KCNQ1OT1 inhibited the cell proliferation, migration, invasion and reduced the expression of drug-resistant gene (MRP5, MDR1, LRP1). Additionally, bioinformatics analysis and dual-luciferase reporter assay showed that miR-7-5p directly targeted the 3′-UTR of miR-7-5p and ABCC1 mRNA, indicating that KCNQ1OT1 regulated the expression of ABCC1 via endogenous sponging miR-7-5p. Conclusively, KCNQ1OT1 modulated oxaliplatin resistance in hepatocellular carcinoma through miR-7-5p/ABCC1 axis, indicating a novel approach for the treatment of hepatocellular carcinoma.

1. Introduction

Hepatocellular carcinoma (HCC) as the most common malignancy occurred primarily in patients with cirrhosis and chronic liver disease [1,2]. Although routine screening is making it possible for earlier diagnosis, the threat from hepatocellular carcinoma is growing in recent years [3]. Moreover, the molecular mechanism of HCC is still unclear and it is important to explore the pathogenesis of HCC for diagnosis and treatment.

Emerging evidence has showed that a growing number of long non-coding RNAs (lncRNAs) were involved in the progression of HCC [4]. For example, the aberrant upregulation of UCA1 inhibited miR-216b and activated FGFR1/ERK signaling pathway to promote the progression of HCC [5]. Moreover, AK021443 contributed the proliferation and migration of HCC cells via modulating epithelial-mesenchymal transition (EMT) [6]. Furthermore, the down-regulation of lncRNA-NEF inhibited the cell migration and EMT via cis-modulating EMT suppressor FOXA2 and deactivating Wnt/β-catenin signaling in HCC [7].

Several research have proved that oxaliplatin-based chemotherapy decreased mortality and prolonged survival in the treatment of patients with advanced HCC [8,9]. However, oxaliplatin resistance reduced the effectiveness in treatment of HCC and the resistance mechanism of oxaliplatin is still unclear. Previous studies have suggested that lncRNAs were involved in the chemotherapy resistance in HCC. For example, a series of lncRNAs were remarkable up-regulated in chemo-resistant HCC cells and tissues and played a vital role in HCC progression and oxaliplatin resistance [10]. Furthermore, lncARSR acted as a novel prognostic factor in HCC and enhanced doxorubicin resistance via regulating PTEN-PI3K/Akt pathway in HCC [11]. Additionally, knockdown of HULC enhanced the chemosensitivity via suppressing autophagy in HCC cells [12].

Previous studies have shown that KCNQ1OT1 played a carcinogenic role in a variety of tumors, such as melanoma [13], HCC [14], glioma [15], and so on. Additionally, KCNQ1OT1 acted as an oncogene in lung adenocarcinoma and associated with chemoresistance [16]. However, the biological function and clinical significance of KCNQ1OT1 are still confused in HCC.

Abbreviations: HCC, hepatocellular carcinoma; LncRNA, long non-coding RNA; miRNA, microRNA; qRT-PCR, quantitative real-time polymerase chain reaction; CCK-8, Cell Counting Kit-8.

* Corresponding author.
E-mail addresses: 721872905@qq.com (H. Hu), kkk6667@163.com (L. Yang), 43989344@qq.com (L. Li), zengchch@glmc.edu.cn (C. Zeng).

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In this study, the function and mechanism of KCNQ1OT1 on oxaliplatin resistance in HCC were investigated. Our results showed that KCNQ1OT1 modulates oxaliplatin resistance through miR-7-5p/ABCC1 axis in HCC, indicating that KCNQ1OT1 acted as a novel therapeutic target for HCC.

2. Materials and methods

2.1. Clinical samples

49 paired gastric cancer tissues and adjacent non-cancerous tissues were collected from the Affiliated Longhua Central Hospital of Guangdong Medical University between April 2003 and September 2014. Patients enrolled in the experiment had written the informed consent and the study was performed in accordance with the Ethics Committee of the hospital.

2.2. Cell lines

HCC cell lines (SMMC-7721, Huh7, SK-Hep-1 and HepG2) and normal liver cell lines (Lo-2) were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) with 10% foetal bovine serum (FBS, Gibco, USA) in a humidified atmosphere at 37 °C.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs were isolated by TRIzol reagent (Invitrogen, USA) according to manufacturer's instructions. Subsequently, a Reverse Transcription Kit (Takara, China) was used to reversely transcribed RNAs into cDNA according to manufacturer's instructions. Moreover, qRT-PCR was performed with SYBR Green PCR Master Mix (Takara, China) in a 7500 Fast PCR system (Applied Biosystems,

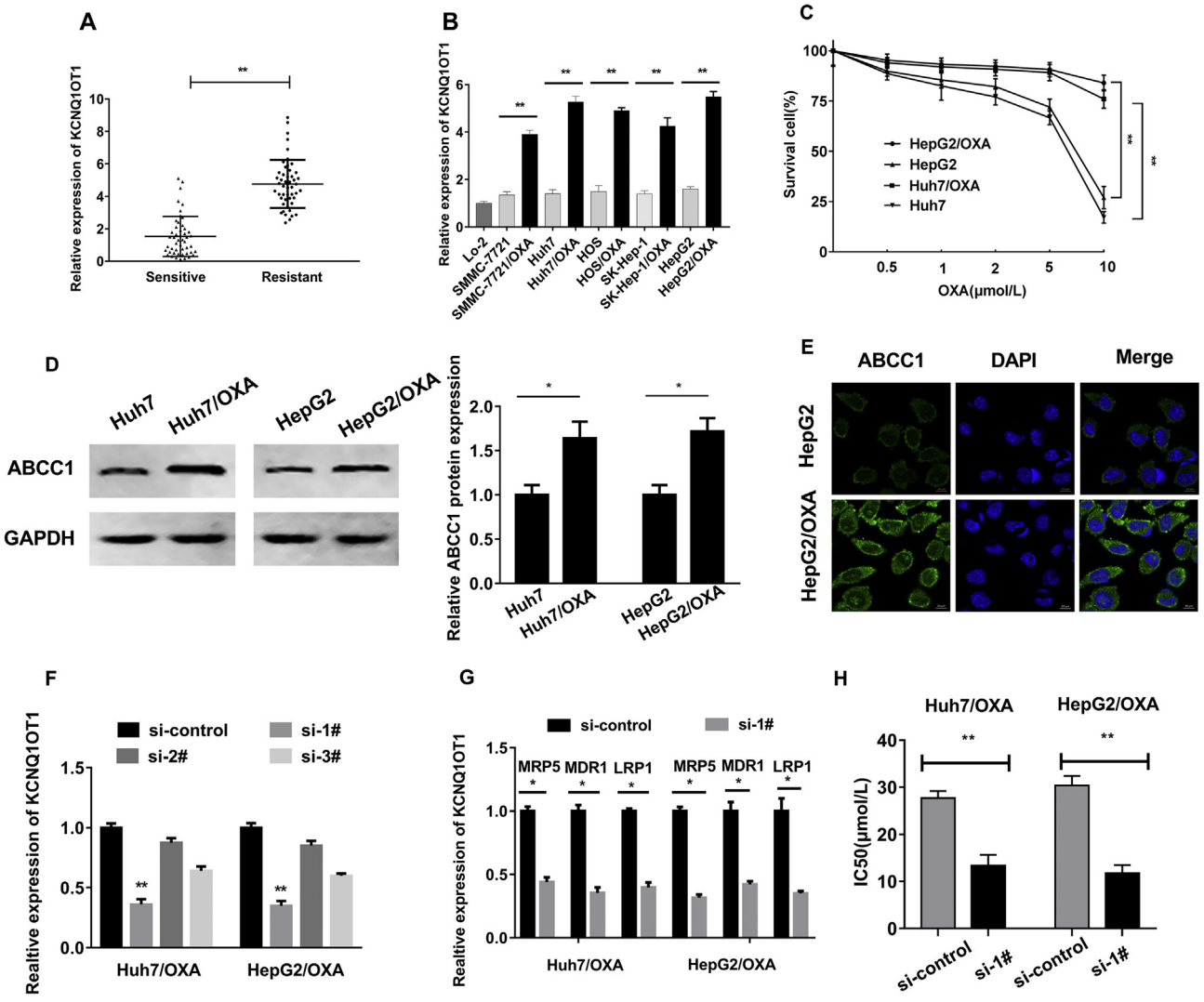

Fig. 1. The role of KCNQ1OT1 in oxaliplatin (OXA)-resistant hepatocellular carcinoma (HCC). A. KCNQ1OT1 was up-regulated OXA-resistant HCC tissues, compared with OXA-sensitive tissue tissues by qRT-PCR analysis. B. KCNQ1OT1 expression was up-regulated in human OXA-resistant HCC cell lines, compared with parental cell lines by qRT-PCR analysis. C. Cell survival rate of OXA-resistant HCC cells (HepG2/OXA, Huh7/OXA) and their parental cells dealt with an increasing concentration of OXA. D. Protein expression of ABCC1 in OXA-resistant HCC cells (HepG2/OXA, Huh7/OXA) and their parental cells by western blot analysis. E. ABCC1 protein in HepG2/OXA cells their parental cells by immunofluorescence analysis. F. The expression of KCNQ1OT1 in HepG2/OXA and Huh7/OXA cells transfected with si-KCNQ1OT1 (si-1#, si-2#, si-3#). G. The expression of drug-resistant gene (MRP5, MDR1, LRP1) by qRT-PCR analysis in HepG2/OXA and Huh7/OXA cells. H. IC50 value in HepG2/OXA cells. I. IC50 value in Huh7/OXA cells. Data are showed as the mean ± SD. *P < 0.05, **P < 0.01.

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