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IDH1 mutation correlates with a beneficial prognosis and suppresses tumor growth in IHCC

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ABSTRACT

Background: Isocitrate dehydrogenase 1/2 (IDH1/2) mutations have been reported in intrahepatic cholangiocarcinoma (IHCC). However, the prognosis of a single IDH1 mutation and impact of mutant IDH1 on IHCC tumor growth remain unclear.

Methods: A total of 85 IHCC tumor samples were sequenced. Prognosis and clinicopathological correlation were analyzed. The role of mutant IDH1 in IHCC tumor growth was measured by cell proliferation assay, colony formation assay in soft agar, and xenograft tumor models. Akt, ERK, p38 MAPK, and JNK signaling, which commonly affect tumor growth, were examined by Western blotting to explore the potential mechanism.

Results: IDH1 mutations correlated with a beneficial prognosis and smaller tumor size. Mutant IDH1 exhibited a growth-inhibitory effect on IHCC cell lines *in vitro* and *in vivo*. Akt signaling was suppressed in IHCC cell lines expressing a mutant IDH1. The reactivation of Akt signaling by SC79 restored the inhibited growth of cell lines expressing a mutant IDH1 in IHCC.

Conclusions: Collectively, we demonstrated that mutant IDH1 correlates with a beneficial prognosis and inhibits tumor growth by suppressing Akt signaling in IHCC. We suggest that patients with IDH1 mutations could be considered for both less-aggressive therapy and therapy tailored to the presence of their mutant enzyme in the future.

Introduction

Intrahepatic cholangiocarcinoma (IHCC) is a liver malignancy originating at the intrahepatic biliary tree and is the second most common liver cancer.[1,2] The incidence and mortality of IHCC are increasing worldwide.[1,2] The prognosis of IHCC remains poor.[3] However, the molecular mechanism of the progression of IHCC remains largely unknown.[4]

Isocitrate dehydrogenase 1 (IDH1) mutations have been detected in many cancers, including glioma,[5-7] chondrosarcoma,[8,9] acute myeloid leukemia (AML),[10-12] T-cell lymphoma,[13,14] and IHCC[15-23] cases. IDH1 is an enzyme in tricarboxylic acid cycle that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate. Mutant IDH1 gains the function of conversion of α-ketoglutarate to 2-hydroxyglutarate.[21,24] In glioma, improved prognosis was

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observed in patients with a mutant IDH1.[5-7] In contrast with glioma, IDH1 mutations in AML patients either correlate with worse overall survival or have no impact on prognosis.[5-7] The prognosis of IDH1 mutations may vary with tumor types. Improved prognosis in glioma patients with mutant IDH1 has drawn widespread attention. Many functional studies have aimed to explain this interesting outcome. Some studies have demonstrated that IDH1 mutations confer an inhibitory effect on glioma growth, which could be a reasonable explanation for better prognosis.[25-28] Others have reported a growth-promoting effect,[29,30] and one group observed no impact of mutant IDH1 on tumor growth.[31]

In IHCC, Wang *et al.* reported that IDH1 and IDH2 (IDH1 + IDH2) mutations were associated with longer times to recurrence and longer overall survival.[19] Goyal *et al.*[22] found that the IDH mutation was not associated with prognosis in patients with advanced IHCC. We observed that IDH1 and IDH2 mutations might impact patient outcomes differently. For example, Boissel *et al.*[11] reported that IDH1 mutations had no significant impact on overall survival, and IDH2 mutations were associated with shorter overall survival in AML. Thus, it is necessary to examine the role of mutant IDH1 or IDH2 alone. Considering that IDH1 mutations account for more than 70% of total IDH mutations in IHCC,[19] the present study was focused on the IDH1 mutation. It is not known whether mutant IDH1 alone has a similar prognosis. Mutant IDH1 was found to block hepatocyte differentiation and to promote biliary cancer in $Kras^{G12D}$ mice.[32] IDH1 mutation in combination with loss of p53 and activated Notch signaling also promotes IHCC development in the mouse liver.[33] However, the effect of IDH1 mutation on IHCC tumor growth has not been addressed. Therefore, we investigated the clinical significance of mutant IDH1 in IHCC and further examined the role of mutant IDH1 in IHCC growth and characterized its potential mechanism.

Materials and methods

Patients and tumor biospecimens

Snap-frozen or paraffin-embedded tumor specimens were collected from 85 patients with IHCC at Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. The patients were followed up to July 11, 2016, with a median follow-up of 39 mo (range 1-77 mo). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of Tongji Hospital, Huazhong University of Science and Technology.

DNA extraction, amplification, and Sanger sequencing

Snap-frozen samples were ground in liquid nitrogen, and paraffin-embedded samples were deparaffinized with xylene. The remainder of the DNA extraction was performed using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. A DNA oligonucleotide containing codon 132 of IDH1 was amplified with primers (forward: TCACCAAATGGCACCATACGA and reverse: GCCAACATGACTTACTTGATCCC; 150-bp product) with a shorter product to avoid failure for DNA segmentation of the paraffin-embedded samples. Polymerase chain reaction (PCR) was performed in 20-μL reaction volumes containing 1.0 μL of DNA template (50-200 ng), 7 μL of nuclease-free water, 10 μL of 2× PCR mixture (TIANGEN, Beijing, China), 1 μL of forward primer (25 μmol/L), and 1 μL of reverse primer (25 μmol/L). The following PCR conditions were used: 95°C for 360 s, followed by 40 cycles at 95°C for 30 s, 61°C for 30 s, and 72°C for 45 s, with a final cycle at 72°C for 10 min. Pyrosequencing was performed at the Tsingke Company (Wuhan, China) using the forward amplification primer.

Cell lines and cell culture

Human cholangiocellular carcinoma T1 (HuCCT1) and OZ cells were purchased from Riken (Tokyo, Japan). The cells were maintained in Roswell Park Memorial Institute 1640 Medium (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (Gibco) in a humidified atmosphere of 5% carbon dioxide at 37°C.

Chemicals and antibodies

SC79 was purchased from Selleck (Shanghai, China). Antibodies were purchased from the following sources—(1) Abcam (Cambridge, UK): anti-IDH, anti-Cyclin D1, and anti-p27Kip1 and (2) Cell Signaling Technology (Beverly, MA): anti-flag, anti-actin, anti-Akt, anti-p-Akt (ser473), anti-ERK, anti-p-ERK, anti-JNK, anti-p-JNK, anti-p38 MAPK, anti-p38 MAPK, and anti-p21Cip1. The horseradish peroxidase—conjugated secondary antibodies were purchased from Jackson Immuno Research Laboratories (West Grove, PA).

Construction of mutant IDH1, retrovirus production, and cell infection

Plasmid pBABE-puro was a gift from Hartmut Land & Jay Morgenstern & Bob Weinberg (Addgene plasmid # 1764).[34] The plasmids pMD2.G and pRSV-Rev were gifts from Didier Trono (Addgene plasmid # 12,259 and # 12,253),[35] and gag/pol was a gift from Tannishtha Reya (Addgene plasmid # 14,887).[36] The open reading frame of human IDH1 was purchased from Vigene (Jinan, China). Overlap PCR was performed to introduce point mutations in codon 132 of IDH1 to obtain five mutant IDH1 open reading frames (R132C, R132G, R132L, R132S, and R132V). Wild-type (WT) and five mutant segments were cloned into pBABE-puro with BamHI and EcoRI NEB (Ipswich, Suffolk). The 293FT cells were transfected with WT and mutant IDH1 constructs together with the packaging plasmid (gag/pol, rev, and Pmd2.G) when cells were 30% confluent. The supernatant was harvested and filtered at 48 h. The infection of cholangiocarcinoma cells with the viral supernatant was conducted in the presence of 8 μg/mL of polybrene (Promotor, Wuhan, China). Puromycin (Promotor) was used to select the stable cell lines.

Western blotting

Total protein was extracted from the cell lines using RIPA lysis. Approximately 40 μg of total protein was separated by SDS-PAGE and transferred onto a polyvinylidene difluoride