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Research

Sonic hedgehog pathway inhibitor mitigates mouse hepatocellular carcinoma

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KEYWORDS:
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Smo;
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Shh;
Gli-1

Abstract

BACKGROUND: Hepatocellular carcinoma (HCC) is a leading cause of death in Asian countries. Sonic hedgehog (*Shh*) pathway plays a role in hepatocarcinogenesis. We investigated the treatment effect of mouse HCC with *Shh* inhibitor GDC-0449.

METHODS: Mouse hepatoma ML-1 cells were implanted in B6 mice. Fifteen days later, GDC-0449 (vismodegib), antagonist of smoothened, was used to treat HCC-bearing mice. The tumor size and liver histopathological features were analyzed, as well as gene expression in *Shh* pathways.

RESULTS: GDC-0449 treatment effectively reduced tumor size and cell infiltration of the HCC in mice. Gene expression of *Shh* pathway molecules was altered, including upregulated *Shh* expression and downregulated smoothened expression in tumor fractions after GDC-0449 treatment.

CONCLUSION: GDC-0449 could effectively mitigate HCC growth in vivo.

Hepatocellular carcinoma (HCC) is a leading cause of death in Asian countries.[1–6] In spite of the improvement in diagnostic tools and surgical techniques, prognosis is poor because of the rapid progression.[4–9] The effective chemotherapy or target therapy remains limited, so novel antitumor therapies are still necessary.

The sonic hedgehog (*Shh*) signaling pathway contributes to some human cancers.[10–22] Some authors suggested that abnormal activation of the *Shh* pathway is important in hepatocarcinogenesis.[23–29] The *Shh* signaling pathway plays a critical role in organizing cell growth and differentiation during embryonic tissue patterning.[10] Some authors support the hypothesis that activation of the hedgehog pathway is an important event in the development of HCC.[23–29] However, the correlation between the *Shh* signaling pathway and the invasiveness of HCC remains controversial.[23–28] In the *Shh* signaling pathway, *Shh*, patched homolog-1 (*Ptch-1*), glioma-associated oncogene homolog-1 (*Gli-1*), and smoothened (*Smo*) are important genes.[11] Activated *Shh* signaling pathway was associated with tumor size, capsular

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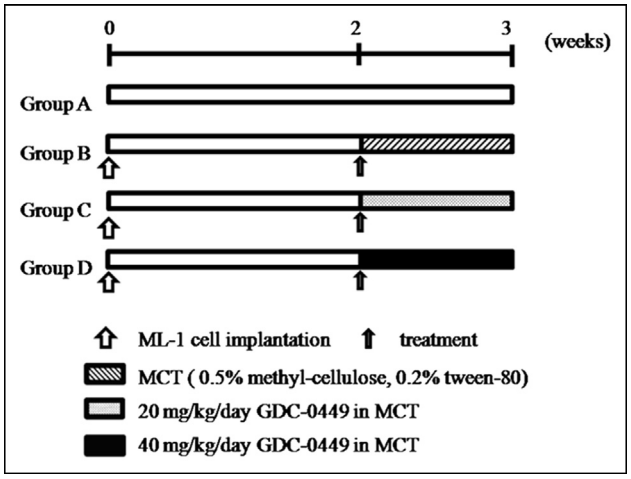

Figure 1 The schematic diagram of ML-1 cell implantation and GDC-0449 treatment for groups A, B, C, and D. Group A mice were for negative control without ML-1 cell implantation. Group B mice were for positive control with ML-1 cell implantation. Group C and group D mice with ML-1 cell implantation were treated with GDC-0449 20 and 40 mg/kg/day, respectively.

invasion, and vascular invasion.[27] In addition, *Shh*, *Ptch-1*, and *Gli-1* were all overexpressed in HCC tissues compared with paired adjacent noncancerous liver tissues. From our previous findings, activation of the *Shh* may also contribute to recurrence.[29] The blockage of the *Shh* pathway could inhibit tumor growth in vitro.[29–32]

GDC-0449 (vismodegib; 2-chloro-*N*-[4-chloro-3-pyridin-2-ylphenyl]-4-methanesulfonyl benzamide) is a potent and specific synthetic *Smo* inhibitor, with molecular weight 421.30 g/mol.[30,33–35] Some studies reported that GDC-0449 has an antitumor activity in a mouse model of medulloblastoma and in primary human tumor cell xenograft models of colorectal cancer and pancreatic carcinoma.[30,33–35] GDC-0449 treatment is effective in the advanced basal cell carcinoma animal.[36] GDC-0449 treatment in HCC remains unclear. We conducted this study to investigate the treatment effect of GDC-0449 upon mice HCC in vivo.

Methods

Animals and cell culture

Pathogen-free, 4-week-old male C57BL/6 mice were purchased from the National Science Council, Taiwan. Mice were housed at our hospital, and all animal work was performed in accordance with the guidelines from the Animal Ethics Committee of the hospitals. ML-1 cell line was established from hepatocytes of BALB/c mice by HBx DNA transfection.[25] It was constructed and kindly provided by Dr. Chen at National Yang-Ming University, Taiwan. ML-1 cells were cultured with Dulbecco's Modified Eagle Medium with 10% inactivated fetal bovine serum and 1% penicillin–streptomycin (Gibco, Grand Island, NY). All cells were incubated at 37 °C with 5% CO_2.

Cancer implantation and GDC-0449 treatment

Mice were randomly divided into 4 groups: group A, B, C, and D (Fig. 1). Group A was used as control. No treatment was given. To induce the growth of hepatoma in the liver, all mice (group B, C, and D) received implantation of mice hepatoma ML-1 cells (5×10^6/20 μL) by injection on the marginal site of parenchyma of the left lobe of the liver. Before treatment, the size of liver tumors in mice was examined after exploration at the 2nd week under general anesthesia. Treatment started after the 2nd week after implantation; oral methylcellulose with Tween 80 (MCT) solution (.5% methylcellulose and .2% Tween 80) as vehicle for 7 days for group B; oral GDC-0449 with a dose of 20 mg/kg/day for 7 days for group C; and a dose

Table 1 Sequences of primer pairs of *Shh* signaling pathway genes

Gene	Primers (5′–3′)	Polymer	Amplicon length (bp)
Gapdh			334
F	CACCACCAACTGCTTAG	17	
R	CTTCACCAC CTTCTTGATG	19	
Shh			102
F	AAAGCTGACCCCTTTAGCCTA	21	
R	TTCGGAGTTTCTTGTGATCTTCC	23	
Ptch			335
F	CCGTTCAGCTCCGCACAGA	19	
R	CTCACTCGGGTGGTCCCATAAA	22	
Gli			291
F	TGCCAGATATGCTTCAGCCA	19	
R	TGTGGCGAATAGACAGAGGT	20	
Smo			100
F	GAGCGTAGCTTCCGGGACTA	20	
R	CTGGGCCGATTCTTGATCTCA	21	

F = forward; *Gapdh* = glyceraldehyde-3-phosphate dehydrogenase; *Gli-1* = glioma-associated oncogene homolog-1; *Ptch-1* = patched homolog-1; R = reverse; *Shh* = sonic hedgehog.