Berberine suppresses metastasis and recurrence of hepatocellular carcinoma by targeting circulating tumour cells: abridged secondary publication

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KEY MESSAGES

1. In mice with hepatocellular carcinoma with and without surgical resection, a combination of berberine and sorafenib potently improved the therapeutic efficacy and outcome without major adverse effects.
2. In our mouse model, berberine suppressed the in vitro viability and presentation of CD44+EpCAM+ circulating tumour cells (CTCs), as berberine is associated with inhibition of the invasiveness and re-attachment of CTCs and induction of cell apoptosis.
3. The inhibitory effect of berberine on CTCs was due to suppressed expression of CD44 and epithelial-mesenchymal transition pathways in CTCs.

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Introduction

The mechanism of haematogenous invasion and metastasis of hepatocellular carcinoma (HCC) is multi-factorial. Primary cancer–derived circulating tumour cells (CTCs) are the active source of HCC metastasis and recurrence.[1] CTCs disseminating from the primary lesion to the blood circulation is the intermediate stage of tumour metastasis.[2] In a meta-analysis of 23 papers, the presence of CTCs in HCC patients were strongly correlated with the relapse-free survival (relative risk=3.03, P<0.00001), overall survival (relative risk=2.45, P<0.00001), tumour, node, and metastasis stage (relative risk=1.30, P=0.03), tumour size (relative risk=1.36, P=0.006), vascular invasion (relative risk=1.99, P<0.0001), portal vein tumour thrombus (relative risk=1.73, P=0.0001), and serum alpha-fetoprotein level (relative risk=2.05, P=0.01).[3] CTCs are of clinical significance in HCC progression, especially metastasis and recurrence.

Berberine is a natural isoquinoline alkaloid from various medicinal plants such as *Coptis chinensis*. Berberine has anti-tumour effects on HCC.[4] Berberine is a potent blocker of intrahepatic and distant metastasis of HCC.[5] We postulate that berberine may inhibit metastasis and recurrence of HCC by suppressing CTCs.

Methods

In a mouse HCC model, we studied the efficacy and safety of a combination of berberine and sorafenib in improving treatment outcomes and preventing recurrence after surgical resection. In vitro inhibition of berberine on the cell viability, invasion, and re-attachment of CTCs were measured by relevant assays. Pathways related to the inhibitory effect of berberine were studied by a gain-of-function assay using CRISPR activation plasmid.

Results

In mice with sorafenib treatment alone, a marginal response was observed. However, co-treatment with berberine significantly improved the efficacy of sorafenib in a dose-dependent manner. Berberine treatment with or without sorafenib had minimal effect on the body weight of mice. The inhibitory effect on HCC progression was supported by the reduced plasma alpha-fetoprotein level and end-point tumour size. To determine whether sorafenib or berberine could suppress the CD44+EpCAM+ CTCs, CTC population was measured by flow cytometry. Sorafenib treatment alone did not affect the presentation of CTCs, but berberine treatment with or without sorafenib potently reduced CTCs levels in circulation. This suggested that co-treatment with berberine improved the sorafenib efficacy in reducing CTCs level. For safety, histological analysis of the liver, lung, and kidney showed no major tissue damage after berberine treatment with or without sorafenib. These observations indicated that berberine was effective and safe to improve sorafenib efficacy in HCC.

In postsurgical HCC mice with berberine treatment, plasma alpha-fetoprotein level and hepatic tumour burden were potently reduced, without body weight gain or loss. This suggested an inhibitory effect on tumour recurrence. Although a higher abundance of CTCs was observed in post-surgical mice, berberine treatment decreased the CTCs in the circulating system. For safety, histological analysis of the liver, lung, and kidney showed no major tissue damage after berberine treatment. This suggested that berberine was effective and safe to be an adjuvant therapy for HCC after surgical resection.

Berberine significantly reduced CD44 expression, which is the marker on the cell surface of CTCs. Expression of epithelial-mesenchymal transition, including Snail and Vimentin, was potently suppressed by berberine treatment. To determine the mechanism of action, we overexpressed CD44 in the parent HCC cells using CRISPR activation plasmid, which significantly recovered the expression of CD44 in the isolated CTCs treated with berberine. Recovery of CD44 expression potently nullified the inhibitory effect of berberine on Snail and Vimentin expression. This suggested that CD44 was the critical mediator involved in the action of berberine. Recovery of CD44 expression increased viability of CTCs upon berberine exposure, restored the invasion and re-attachment of CTCs, and inhibited cell apoptosis. This suggested that berberine regulated CTCs through CD44-mediated epithelial-mesenchymal transition pathways.

Discussion

In our mouse model, berberine suppressed the in vitro viability and presentation of CD44+EpCAM+ CTCs, as berberine inhibited the invasiveness and re-attachment of CTCs and induced cell apoptosis. Recovery of CD44 expression nullified the inhibitory effect of berberine on epithelial-mesenchymal transition expression in CTCs, restored invasion and re-attachment of CTCs, and inhibited cell viability. In HCC mice with and without surgical revision, combined treatment with berberine and sorafenib potently improved the therapeutic efficacy and outcomes without major adverse effects.

In patients with HCC not suitable for surgery, recurrence of HCC is common. Treatment option for advanced and/or recurrent HCC is limited, and distant metastasis is a risk factor for poor survival. Berberine is an affordable and safe natural compound; it provides an alternative treatment for HCC metastasis and recurrence by targeting CTCs.

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Disclosure

The results of this research have been previously published in:

1. Lu Y, Chan YT, Tan HY, Li S, Wang N, Feng Y. Epigenetic regulation in human cancer: the potential role of epi-drug in cancer therapy. Mol Cancer 2020;19:79.
2. Chen F, Zhong Z, Tan HY, Wang N, Feng Y. The significance of circulating tumor cells in patients with hepatocellular carcinoma: real-time monitoring and moving targets for cancer therapy. Cancers (Basel) 2020;12:1734.
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