Author’s response to reviews

Title: The synergistic antitumor effect of Huaier combined with 5-florouracil in human cholangiocarcinoma cells

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Dear Reviewer 1

Thank you for your sincere advice, and we tried our best to make revisions.

1. We got fingerprint of Huaier extract. Referenced from an article in Chinese, called “Changes in Chemical Component Content of Fungal Substance Trametes roobiniophila /Isatidis Radix Co-Fermentation System”

2. To examine whether agents-reduced STAT-3 activation is the reason involved in the cellular proliferation, cell cycle, apoptosis as well as cellular migration and invasion, a series of experiments were conducted to verify the effect of Huaier combined with 5-FU on Huh28 cells cultured under the
induction of IL-6. The results showed that combination of two drugs could reduce the proliferating effects caused by IL-6, similar results also appeared in Flow cytometry and Transwell cell migration and invasion assays. Please check our Supplement Figure.

Supplement Figure Effects of treatment with combination of Huaier and 5-FU in Huh28 cells cultured under the induction of IL-6.

The combination of Huaier and 5-FU on colony formation with Huh28 cells induced by IL-6 were shown, and the colony numbers were calculated (A). Flow cytometry histograms of Huh28 cells after different treatments. The percentage of apoptotic cells was calculated (B), and the percentage of cells in different phases was detected (C). Transwell cell migration (D) and invasion (E) assays were performed to further identify the motility inhibiting effect induced by different treatments. All results are expressed as mean ± SD of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

3. We are sorry that we only have one kind of cholangiocarcinoma cell line, so it is difficult for us to detect agents in other human cholangiocarcinoma cell lines.