Author's response to reviews

Title: Norcantharidin inhibits tumor growth and vasculogenic mimicry of human gallbladder carcinomas by suppression of the PI3-K/MMPs/Ln-5gamma2 signaling pathway

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Author's response to reviews: see over
Dear Ms Roselyn Remoto  
BMC Cancer-BioMed Central Editorial

We have revised our manuscript in light of the reviewers' comments and provided a covering letter with a point-by-point description of the changes carried out in below.

**Referee 1:**

**Major compulsory revisions:**

Western blot data including housekeeping gene $\beta$-actin and Figure 10 have been revised.

So far, the most effective treatment for gallbladder carcinoma (GBC) is still surgical resection although this resection rate is lower. Norcantharidin (NCTD) is used in our studies in order to observe whether NCTD can be served as an adjuvant therapy for preventing tumor local recurrence and metastasis after operation. Therefore, we believe that 40% inhibition of tumor growth by NCTD as an adjuvant agent is satisfactory in this experiment. And, at the end of the experiment (by eighth weeks) and by 180 days, the xenografts volume was decreased significantly, with increased tumor inhibition (Figure 5A, all $P<0.001$) and prolonged survival time in NCTD group when compared with control group (unpublished data). It was reported that NCTD had higher drug concentration in liver, stomach, intestine and carcinoma after absorption, reached the peak concentration in the liver cancer tissue 15 min after administration, decreased significantly after 6 hours; and most were excreted by the kidneys after 24 hours; that NCTD is clinically treated by oral, intravenous injection or drip for liver, esophageal, gastric cancers, also is used as a premedication or used in combination with chemotherapy drugs for improving efficacy and reducing side effects. It is possible that NCTD may be more effective if used in combination of other anticancer drugs, as McNamara MG et al have suggested, that the future therapeutic spectrum for GBC will likely encompass novel combinations of targeted therapies with cytotatics in scientifically and molecularly directed schedules, thus permitting fewer mechanisms of escape for tumor cells.

**References**


Minor essential revisions:
An image showing comparative sizes of dissected tumors has been included in Figure 5.

Referee 2:

GBC is a highly aggressive malignant tumor with a poor prognosis. Surgical resection, chemotherapy and radiotherapy for the disease are disappointing. Clearly, novel adjuvant therapies or anticancer agents are needed in order to treat gallbladder carcinoma in vivo. Like some aggressive malignant tumors such as melanoma, growth and metastasis of GBCs are dependent on not only microcirculation via the traditionally recognized mechanisms of angiogenesis, but the recently found vasculogenic mimicry (VM). We found that the formation of VM in GBCs through the activation of the PI3K/MMPs/Ln-5γ2 signaling pathway in the 3-D matrix of GBC-SD cells in vitro and GBC-SD nude mouse xenografts in vivo. Because differential endothelial cells involved in angiogenesis and VM, and their different molecular regulation mechanisms, it should be considered to develop new anti-vascular therapeutic agents that target both angiogenesis and VM, in especial, an anti-VM therapy for VM when in antitumor treatment of highly aggressive tumors, because simple application of angiogenic inhibitors have been confirmed no effect on VM.

Evidence has shown that NCTD has multiple antitumor activities against GBCs in vitro and in vivo. However, the exact antitumor mechanism is not thoroughly elucidated. In this study, we further investigated anti-VM activity of NCTD as a VM inhibitor, showed firstly that NCTD inhibits tumor growth and VM of GBCs by suppression of the PI3-K/MMPs/Ln-5γ2 signaling pathway in vitro and in vivo, so may serve as a potential anti-VM agent for human GBCs.

Thus, we believe that our work makes a significant contribution to the field.

References

Referee 3:
Abstract: 1, 2, 3, have been revised in light of the reviewers’ comments.
Background: 1, 2, have added current citation reviewing GBC and a several sentences describing the rationale behind choosing NCTD in light of the reviewers’ comments.
Methods:
1, 2, has been revised in light of the reviewers' comments.
3, invasion assay, The ability of tumor cells to invade normal surrounding tissue contributes in large part to the morbidity and mortality of cancers. Cell invasion requires several distinct cellular functions including adhesion, motility, detachment, and extracellular matrix proteolysis. Invasion assay is a useful method for assaying the invasive properties of tumor cells. Our invasion assay utilizes Transwell membranes to assay the invasive properties of GBC-SD cells. Cells were untreated (control group) and treated with TIMP-2 or NCTD. After 24-hr, invasive cells invade through the matrix-coated membrane to the lower wells of the chamber. Non-invasive cells are then removed from the upper chamber, and invasive cells were stained with H&E and counted using a light microscope. Thus, we are able to assay comparatively different invasive ability of GBC-SD cells in control group, TIMP-2 group or NCTD group; regardless of the fact that no correction for proliferation or invasive capacity may be influenced by the low proliferation rate.
4, have been checked the wording throughout the methods section.
Results: at the end of the experiment (by 8th weeks) and by 180th days, the xenografts volume was decreased significantly, with increased tumor inhibition (Figure 5A, all \( P<0.001 \)) and prolonged survival time in NCTD group when compared with control group (unpublished data). It was clinically reported that NCTD is used to treat liver cancers, or used in combination with chemotherapy drugs for improving efficacy and reducing side effects, because of its higher drug concentration in liver and carcinoma tissue after absorption. So, tumor and VM inhibition may be sustained after NCTD withdrawal. Of course, it is still necessary to further study the efficacy and action time of NCTD for human GBCs in vitro and in vivo.
Discussion:
1, has shortened the introduction in the discussion section in light of the reviewers' comments.
2, has clarified the last sentence in the first paragraph.
3, has been revised in light of the reviewers’ comments.

We appreciate your consideration of our manuscript, and we look forward to receiving comments from the reviewers.

Sincerely yours

Yue-Zu Fan