Author's response to reviews

Title: Neoplastic transformation of rat liver epithelial cells is enhanced by non-transferrin-bound iron.

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Author's response to reviews: see over
Dr. John Kerr  
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RE: MS 4448342141553270-Neoplastic transformation of rat liver epithelial cells is enhanced by non-transferrin-bound iron.

Dear Dr. Kerr,

Thank you for the favorable disposition of our manuscript and for forwarding the reviewers comments to us. As you suggested, we have made further modifications in response to the suggestions of Reviewer 4.

**In response to reviewer 1:**  
No changes requested.

**In response to reviewer 4:**  
At last submission, our study made use of cell counts to determine the effects of FAC on T51B cell proliferation and the MTT assay to investigate FAC toxicity. These were done primarily to identify suitable FAC concentrations for the tumor promotion experiments. They are widely used and generally accepted assays. However, the reviewer suggested additional measures of toxicity may elucidate the mechanism of tumor promotion by FAC. In response, we examined surface membrane integrity using Trypan Blue. We found that nearly all of the cells (>95%) recovered after FAC treatment for up to 7 days excluded Trypan Blue. A note to this effect was added to the methods section (p 7).

Tumor promoting concentrations of FAC slowed cell growth (Figure 2A), reduced cellular mitochondrial activity (Figure 2B), and disrupted cell cycling (Figure 4), apparently with minimal cell death (Trypan Blue results). We do not know the fraction of cells killed by FAC treatment and lost prior to these analyses, but relevant toxic effects of FAC need not include death. To clarify this we added the phrase "anti-proliferative or other toxic effects" to our discussion of tumor promotion by FAC (p15). Importantly, FAC did not increase the rate of cell proliferation, one NTBI tumor promotion mechanism proposed by others.

**Major (0) points:**  
None.

**Minor (1) points:**  
1. The sentence in question was changed (p16). The reviewer correctly objected to the implication that iron overload causes widespread liver necrosis. The primary
point is that the concentration of iron citrate in a cirrhotic, iron overloaded liver (the setting of highest HCC in humans) is unknown, but may in fact be higher than in blood.

In addition to these requested changes, we also added a new reference that links liver iron to increased HCC in liver diseases other than hemochromatosis (first paragraph of the background, p4). Finally, we reviewed the manuscript formatting according to the template and authors’ checklist for BMC Gastroenterology, and made some necessary formatting changes.

Thank you once again for your assistance. Please confirm the acceptance of our manuscript at your earliest opportunity.

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