Reviewer's report

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

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Reviewer: Debbie Trinder

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General
Developing experimental models of liver cell transformation has been proved difficult and previous studies have had variable success using non-biological forms of iron to promote liver cell tumors. The authors of this study have undertaken a very scientifically sound series of experiments that clearly illustrated for the first time in the presence of the tumor initiator N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) that NTBI in a biological form found in serum from humans with iron overload was able to promote transformation of liver epithelial cells in culture. They treated a liver epithelial cell line T51B with NTBI in the form of ferric ammonium citrate (FAC) for up to 20 weeks. Low FAC concentrations alone had little effect on proliferation or cell transformation. However, when the cells were incubated with both MNNG and FAC there was an increase in cell colony formation. This did not occur if FAC was replaced with ammonium citrate or desferoxamine was added indicating that iron was required for promotion of cell transformation. Changes in cyclin expression indicate that disregulation of the cell cycle may be mechanism responsible for the promotion of tumors.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Introduction p5 last sentence. “However, even in iron-overloaded livers, tumors do not contain high levels of iron, implicating non-transferrin mechanism in overload” Clarify this statement. It is not clear to me what this means.

Introduction p6 2nd paragraph, p7 1st paragraph and discussion page 14 paragraph 1 refers to FAC as a physiological form of NTBI which is misleading. Although ferric citrate is the form of NTBI found in human serum it is usually associated with iron overload indicating that it is a pathological rather than a physiological form of iron.

Discussion p14, paragraph 1, 2nd sentence
“We also found tumor-promoting concentrations of FAC had anti-proliferative effect on normal T51B cells.” This is not correct because 50µM and 200µM FAC concentration promoted tumor growth but it did not have significant anti-proliferative effects. While 500µM FAC had a slight effect on tumor
promotion which may not be significant but reduced proliferation. (Table 1 and Fig 2)

In Table 1 and 2 the results are the means from 4 values. Results should be expressed as mean ± SEM and p values stated.

Figure 2 and 3: p values need to be given.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Figure 1: Add units to Y-axis

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after discretionary revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests