Reviewer's report

Title: Zinc finger protein ZBTB20 expression is increased in hepatocellular carcinoma and associated with poor prognosis

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Reviewer: C. Bart Rountree

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Zinc finger protein ZBTB20 expression is increased in HCC and associated with poor prognosis.

By Wang et al.

This is a very interesting manuscript, and a follow-up work to a 2008 PNAS paper, in which some of the authors described a novel interaction between AFP and ZBTB20. In the prior work, the authors created a ZBTB20 knock-out using a cre-lox system, and demonstrated that loss of ZBTB20 correlated with high levels of post-natal AFP, and describe a role of ZBTB20 as a repressor of AFP transcription. In this current manuscript, the authors seek to correlate the expression of ZBTB20 to HCC disease progression. Given that AFP is a well defined clinical marker of HCC, this work is a natural extension of the previous investigation.

The authors utilize a tissue bank of 152 samples from surgical HCC resections. ZBTB20 expression was correlated to clinical data such as disease progression and more importantly, survival.

Although this work is extremely interesting and well conducted, several key questions should be addressed (in addition, questions posed by the BMC reviewer site are also detailed below):

Major compulsory revisions:

1. Are the data sound? Yes, with the exception of figure 4. The correlation of AFP staining to ZBTB20 staining in the inverse is not well demonstrated with the figure presented. Figure 4 c/d does not appear to be different in overall intensity compared to Figure 4 a/b, where less ZBTB20 appears to correlate with more AFP staining. If this is the best example, it calls into question the methodology of staining quantification.

2. Are the discussion and conclusions well balanced and adequately supported by the data? Yes, but limited based on the fact that this is a completely descriptive study. Although a clear correlation between ZBTB20 expression and clinical parameters appears to be established, there is a lack of any related mechanism, in terms of role of ZBTB20 in disease progression or why it results in
more rapid death of HCC patients. The authors have at their disposal the ZBTB20 knock-out mouse line. This would beg the question, for example, of the response of this ZBTB20 KO to DEN induced HCC. Do these mice have more tumors, more rapidly progressing, and larger, with more metastatic disease? What then might be the relationship between a potential AFP repressor and tumor progression? Without further investigation, this manuscript is very limited is relevance.

3. Can the authors explain the lack of correlation between serum AFP level and ZBTB20 level? This seems to go against their prior publication of ZBTB20 regulating AFP, and should be addressed more clearly.

4. Is the writing acceptable? The writing needs attention.

Minor essential revision:
1. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes, but this could be highlighted better. Xie et al. PNAS. 105(31) 10859-10864, described a ZBTB20 knock-out mouse which demonstrated increased AFP expression.

Discretionary reviews:
None

Addition questions:
1. Is the question posed by the authors well defined? yes
2. Are the methods appropriate and well described? yes
3. Does the manuscript adhere to the relevant standards for reporting and data deposition? yes
4. Are limitations of the work clearly stated? No.
5. Do the title and abstract accurately convey what has been found? Yes.

Overall the design, methods, and examples presented are fine, and the clinical analysis is thorough.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future?
I have received grant support for clinical research from Bayer.

Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future?
No

Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?
I do not personally hold any patents nor am I applying for them. I have received grant support for clinical research from Bayer. I am not aware of any pending patents from Bayer, but they do hold current patents in cancer treatment drugs.

Do you have any other financial competing interests?
No.

Do you have any non-financial competing interests in relation to this paper?
No.

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