Reviewer's report

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

**Version:** 1  **Date:** 17 March 2011

**Reviewer:** Fanyin Meng

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In the manuscript submitted by Wang et al, the authors investigated the expression of ZBTB20 in HCC tissues and its potential association with clinicopathological features and post-resectional survival. They observed that increased expression of ZBTB20 in HCC was positively correlated with tumor vein invasion, recurrence and metastasis. Moreover, patients with high intratumoral ZBTB20 expression had significantly worse OS and DFS when compared with patients with low expression of ZBTB20. Multivariate analysis demonstrated that among the factors analyzed, intratumoral ZBTB20 expression was an independent prognostic factor for OS and DFS in patients with HCC. Meanwhile, tumor size, serum AFP, TNM stage, tumor number and vein invasion were all independent prognostic factors for OS; while tumor size, HBsAg positive, TNM stage, tumor number, vein invasion and peritumoral ZBTB20 expression were independent prognostic factors for DFS. These results clearly demonstrated that high ZBTB20 expression is associated with poor progression and unfavorable clinical outcome of HCC. It is revealed that ZBTB20 is overexpressed in a large proportion of patients with HCC and high ZBTB20 expression correlated with the disease progression and poor clinical outcome in HCC. The manuscript is well written and the study is interesting; however, some issues should be addressed before it is eligible for publication.

**Major points:**

1. Although the provided starting results in vitro appear to be consistent, the Western blot images seem like to be manipulated. The ZBTB20 antibody staining has several bands in their gel image. The whole gel images should be displayed plus the positive and negative controls instead of just cut the bands as they wish. The molecular weight should also be marked. This fact precludes the possibility to the specificity of their ZBTB20 analysis in HCC and therefore represents a limitation to the overall significance of authors' findings.

2. Since the author made the conclusion that “ZBTB20 proved to be a risk factor for tumor recurrence and independent molecular marker of prognosis in HCC and may become a novel molecular target in the strategies for the prediction of tumor recurrence and prognosis or treatment of HCC.” The related mechanistic studies should be performed. The expressions of P450 family members, plus MMP-2 and MMP-9, should be detected in tumor samples.

3. The ZBTB20 expression between normal and fibrotic/cirrhotic livers should
also be analyzed to clarify its dynamic changes during liver injury.

Minor points:
1. Since the information of “Ethics Committee of the Eastern Hepatobiliary Surgery Hospital” could not be obtained on the internet, the approval notice for the current human studies should be provided as the supplementary information.
2. All the normal and HCC cells were cultured in DMEM medium, which is not consistent with the recommendations from ATCC for PLC/PRF/5, HepG2, SK-Hep-1 and Hep3B. The explanation should be included.
3. The origin of primary anti-ZBTB20 polyclonal antibody should be described in Material and Methods section.
4. AFP expression should be included in Fig. 2.
5. The legends of Fig. 1, Fig. 3 and Fig. 5 should be enriched.
6. The manuscript is too ordinary to have three 1st authors and 2 correspondence authors. The rational for the unusual strategy should be explained in Authors’ contributions section.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

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