Title: Nrf2 is a potential prognostic marker and promotes proliferation and invasion in human hepatocellular carcinoma

Reviewer: Yunpeng Hua

Reviewer’s report:

Nrf2, or nuclear factor E2-related factor 2, is a master transcriptional activator of genes encoding enzymes that protects cells from oxidative stress and xenobiotics by induction of a transcriptional regulation of several antioxidant enzymatic pathways and also various drug efflux pumps-members of multidrug resistance protein. However, some reports have shown that drugs that activate Nrf2 can promote cell growth and an increasing number of works point to a potential role for Nrf2 and its transcriptional target genes in tumorigenesis. Recently, a new paper, published in the magazine of Hepatology, indicated the onset of Nrf2 mutations is a very early event, likely essential for the clonal expansion of preneoplastic hepatocytes to HCC, and functional experiments demonstrate that Nrf2 is an oncogene, critical for HCC progression and development. But now there is no any report for the role of Nrf2 in the prognosis of HCC patients. I believe that the conclusion of this article, which Nrf2 is a potential prognostic marker and promotes proliferation and invasion in human hepatocellular carcinoma partly through regulating expression of Bcl-xL and MMP-9, is useful to our clinic and basic scientists.

Major Compulsory Revisions:

1. In this study, only 80 cases were involved in the study from January 2005 to December 2009. Why are there so few cases recruited during 5 years?
2. Today is April 4, 2015. Why were Patients only followed up closely until December 2011? I believe it is better if you follow up patients until now.
3. Can you tell me if the resection for HCC is curable in this study? It is well-known that tumor size, tumor number, tumor differentiation, vascular invasion, resection margin status, tumor recurrence, etc. are predictive for tumor recurrence after curative resection. Why didn’t the authors analyze the relationship between Nrf2 and the above factors?
4. In my clinical experience, the HCC cases with Lymph metastasis are few. Why is the proportion of Lymph metastasis close to half in the clinical study?
5. Is the survival disease free survival (DFS), or overall survival(OS)? I feel it is
better to analyze respectively the correlation of DFS and OS with Nrf2 in the HCC patients.

6. In page 6, raw 10-13, Nrf2 immunoreactivity was predominant in the nucleus. The number of positive-staining cells showing immunoreactivity on the membrane for L1CAM in ten representative microscopic fields was counted and the percentage of positive cells was calculated. I don’t understand what does mean. Is Nrf2 located in the nucleus or on the membrane? What is L1CAM?

7. How about the level of Nrf2 expression in the normal liver and liver cirrhosis? Can you show the data of the Nrf2 expression in Lymphatic metastatic tumor?

8. Why did you choose these three HCC cell lines, Hep3B, Bel-7402, and HepG2? You found that the level of Nrf2 are different in these cell lines. Can you tell me how about their abilities of proliferation and invasion , whether their abilities are associated with Nrf2 level?

**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.

**Declaration of competing interests:**

I declare that I have no competing interests