2014-05-19

Manuscript Number 19723
Manuscript Title: Effect of fibulin-5 on the adhesion, migration and invasion of hepatocellular carcinoma cells via an integrin-dependent mechanism
Review Time: 2015-05-22 12:40

Dear Editor:
We are very grateful for this opportunity to revise our manuscript and submit it again. We would like to thank the reviewer for the highly useful comments on our manuscript. We have carefully taken the comments into consideration in preparing the revised version of our manuscript, and we hope that you find the new version suitable for publication in your esteemed journal. The following is a point-by-point response to the reviewer's comments. All the changes made have been underlined and italicized in the revised manuscript.

Thank you again!
Regards,

Prof. XiuJun Cai
Corresponding author

Revision — authors' response

We want to thank Referee for the constructive and insightful criticism and advice. We believe that the additional changes we have made in response to the comments have significantly improved the manuscript. We have addressed all the points raised by the reviewer, as summarized below.

All the changes made to the original manuscript are underlined and italicized in the revised manuscript.

The aim of this work was clear, and the authors performed plenty of experiments. It would be better to explain the reason replacement of Asp56 within the integrin-binding RGD motif with Glu. It was assumed that this mutation weakened of tumor suppression of FBNL-5.

Answer: We have added a brief explanation to clarify this in Line 105. Previous studies have confirmed that the site mutation D56E in the RGD motif prevents integrin binding (Smith LL et al., Cell Res 1998; Erb L et al., Cell Biol 2001); moreover, FBLN-5 with an RGE mutation has been found to have altered biological functions both in cells and mouse models lacking integrin-binding ability (Schluterman MK et al., Dis Model Mech 2010; Yang Z et al., J Cell Biol 2007; Albig AR et al., 2004 DNA Cell Biol).

"Site-directed mutagenesis of eukaryotically expressed FBLN-5 was performed, wherein Asp56 was substituted with Glu within the integrin-binding RGD motif, so as to prevent integrin binding [6, 7]. The KOD Hot start DNA polymerase kit (Millpore, USA) was used according to the manufacturer's recommendations."

Introduction. It would be better to introduce the relation FBLN-5 and cell migration. Based on the results of this study, down-regulation of FBLN-5 seemed to promote cell motility and proliferation.

Answer: We appreciate this suggestion; we have added this in Line 94.

"Experiments on FBLN-5-null mice have provided evidence for the role of FBLN-5 as an angiogenesis inhibitor and its roles in the proliferation, migration and invasion of certain tumors. The effect of FBLN-5 on tumorigenesis appears to be largely context-dependent, and may involve the inhibitory effect of fibuilin-5 on angiogenesis; however, the exact mechanisms are still under investigation [2]."

Figure 2. Magnification and scale bar should be presented.

Answer: We are sorry for not providing this before. The magnification and scale bar have been added in Fig. 2.

Discussion. "it seems FBLN-5 plays the role of tumor suppressor or oncogene in various cancer cells depending on a context-specific manner." was unclear. Did that mean FBLN-5 acted as a tumor suppressor or ongocene (promoting tumor promotion) depending on cancer? If so, were there any speculation to the reason regarding suppressor or oncogene?

Answer: Recent research has revealed that FBLN-5 regulates cancer cell proliferation, migration and invasion in a cell- and context-specific manner. FBLN-5 has reduced

expression in certain metastatic human tumors such as lung cancer, hepatocellular cancer and prostate cancer (Yue W et al., Cancer Res 2009; Tu K et al., BMC Cancer 2014; Wlazlinski A et al., Prostate 2007), but it is overexpressed in fibrosarcoma and nasopharyngeal carcinoma (Schiemann WP et al., J Biol Chem 2002; Hwang CF et al., PLoS One, 2013). Up to now, there have been no speculations about the mechanisms underlying these findings; however, it seems that FBLN-5 has diverse cellular and biological functions both in the matrix and neighboring cells. Interestingly, as a matricellular protein, the nuclear localization of FBLN-5 is found in TW01 and Hone1 nasopharyngeal tumor cells, which suggests that nuclear FBLN-5 may act as a transcriptional regulator in the modulation of downstream gene expression of FBLN-5 (Hwang CF et al., PLoS One, 2013). However, the underlying mechanism still needs to be fully elucidated.