Reviewer's report

Title: Suppression of thymosin beta10 increases cell migration and metastasis of cholangiocarcinoma

Version: 1 Date: 1 July 2013

Reviewer: Yeu Su

Reviewer's report:

1. They should discuss why the in vitro invasion ability of KKU-M214 cells was increased more strongly than the migration ability when endogenous Tb10 expression was silenced (Fig. 2B vs 2C).

2. It's very unusual to see that the endogenous Tb10 mRNA levels were decreased more than 50% in all the stable clones carrying only the vector (Fig. 3A, lower panel). Thereby, authors must make sure that they used the correct construct for transfection. In addition, they should switch the RT-PCR results with the IHC results because the clones chosen for IHC analysis were based on the RT-PCR results. In Fig. 3B, they need to mention the migration period (24 or 48 h) for vector-control clone and if the migration period was 24 h for this clone, then showing the result of shTbclone at 48 h is meaningless. Moreover, what's the advantage of using eGFP-tagged stable clones in migration assay unless the extent of migration was estimated by measuring the fluorescence intensity? But the authors didn't mention that.

3. What is the rationale for choosing KKU-M055, a very low Tb10-expressing cell line, for evaluating the effect of Tb10 knockdown on its migration ability? Did those two clones also express eGFP gene? If yes, they need to indicate that.

4. Why didn't the authors choose GFP C3 clone whose Tb10 mRNA levels were affected the least by vector transfection (Fig. 5A)? In addition, should the M055-Lenti-GFP cells migrate faster than (or at least equal to) the M213-Lenti-GFP cells if the assumption that the migration ability of those cells was inversely correlated with the expression levels of Tb10 by the authors was correct. Surprisingly, an opposite result was found in Fig. 5 (C vs F). Also, in their rescue experiment (P.14), they observed that transient transfection of a T#10-over- expressing plasmid could cause a 35-fold increase of T#10 mRNA level in CCA cells constitutively expressed Tb10 shRNAs (Fig. 5G). However, they didn't mention how they avoided shRNA-mediated degradation of Tb10 mRNAs generated from the transfected plasmid.

5. Were the data shown in Figure 7 repeated at least 3 times since no standard deviations were included? If they suspected that silencing Tb10 in CCA cells might activate Ras which in turn stimulates ERK1/2, leading to the increase of migration and invasion of these cells, the authors could examine whether the effects of Tb10 knockdown in CCA cells could be diminished by the addition of some commercially available pharmacological inhibitors such as manumycin A (for Ras) or U0126 (for ERK). Before validating this speculation, they should be
more conservative in drawing their conclusions by stating that “Suppression of Tb10 expression mediates cell migration possibly through the activation of ERK1/2 and upregulation of EGR1 and Snail”.

6. In the third paragraph of Discussion (P.17), authors tried to explain why Tb10 is downregulated in the metastatic CCA. However, no linkage between a constitutively active Ras and a loss of Tb10 expression was reported. Although VEGF, TSH, and 5-FU were shown to stimulate Tb10 expression, their roles in Tb10 downregulation in the metastatic CCA are far from being clear. In the fourth paragraph (P.17-18), the authors speculated that the activated ERK might enhance CCA cell migration by inducing focal adhesion disassembly via interfering with the interaction between FAK and paxillin. To quickly examine this possibility, they could estimate the extent of focal adhesion by immunostaining these cells with an anti-vinculin antibody.

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

**Quality of written English:** Not suitable for publication unless extensively edited

**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