Author’s response to reviews

Title: beta2-microglobulin induce epithelial-mesenchymal transition in human renal proximal tubule epithelial cells

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Author’s response to reviews: see over
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Version: 2 Date: 23 November 2004

Author's response to reviews: see over
Reviewer's report

Title: beta2-microglobulin induce epithelial-mesenchymal transition in human renal proximal tubule epithelial cells

Version: 2 Date: 14 October 2014

Reviewer: Mark Dockrell

Reviewer's report:

“#2-microglobulin induce epithelial-mesenchymal transition in human renal proximal tubule epithelial cells” by Zhang et al is an in vitro study of putative effects of #2-M using a transformed human PTEC cell line. The authors have clearly defined their question and used reasonable cell biology methods to answer it; existing published data might have led the authors to investigate mitochondrial activity in addition. On the whole the results are clear, although some modification is required- see below. The discussion of the paper does not take into account published data on the effect of iron on proximal tubule cells, the role of HIF 1 and require more balance – see below.

Major Compulsory Revisions

1. The authors should discuss the work of Josson et al in more detail as these authors demonstrate the #2-M and HFE combine to cause EMT characterised by E-cadherin loss and that down regulation of HFE by RNA interference resulted in an increase in E-cadherin expression. They also investigated the effect of HIF1 and Iron. Zhang et al need to explain
the critical differences between their work and that of Josson and colleagues.

Although Sajni Josson group have found that β2-M induced epithelial to mesenchymal transition and Confers Cancer Lethality and Bone Metastasis in Human Cancer Cells. However, the molecular biology mechanism of β2-M in damage of renal tubular epithelial cell is far from clear. For β2-M is also a major protein component of Proteinuria, to explore the effect of β2-M on renal tubular epithelial cell in the peritubular microenvironment is very significant for us to know the possible mechanism by which proteinuria caused kidney damage. The novelty and significance of this study is that: we firstly demonstrated that (1) β2-M also induced epithelial-mesenchymal transition in human renal proximal tubule epithelial cells; (2) β2-M/HFE complex as mediator also participated in β2-M induced EMT process in human renal proximal tubule epithelial cells. Overall, these results support that the effect of β2-M on EMT process and β2-M/HFE complex as mediator participated in β2-M induced EMT process is shared by renal proximal tubule epithelial cells and cancer cells.

The role of EMT in renal fibrosis, with the exclusion of post-transplant IF/TA, has become a controversial issue and the authors should not cite it as fact or even the prevailing theory. It has not been disproved as a phenomenon but elements such as epithelial cells becoming migratory are
increasingly questioned with little evidence from in vivo human studies. This does not nullify the authors’ work but they need to consider their results in the context of the current received wisdom. If cells in vivo lose E-cadherin they presumably lose polarity and a degree of function which may be important pathologically and it would have been interesting to know what happened to other functional markers of PTEC phenotype such as megalin expression. Hence; in my opinion, the authors need to justify the novelty of this work (compared to Josson et al) and its relevance to renal biology, in the light of the current view on PTEC EMT.

We absolutely agree with the reviewer’s comment. Although conclusive demonstration of EMT in vivo in the setting of kidney diseases still appears very challenging, evidence for EMT in vivo is emerging in various other animal models of CKD and clinical studies utilizing human kidney biopsies also suggest that EMT likely plays a role in the pathogenesis of human CKD [1]. The extent to which EMT contributes to renal fibrosis in vivo remains a matter of intense debate and is likely to be context-dependent. There are numerous reasons why EMT is often underestimated in injured kidney [2].

Many studies have demonstrated that serum or urine β2-M (a major protein component of Proteinuria) concentration is increased in a variety of diseases and animal models of protein overload support the putative role of proteinuria in the progression of tubulointerstitial fibrosis.
However, the effect of excessive β2-M on proximal tubular cells is relatively unknown. Although we did not find any new molecular mechanism by which β2-M induced EMT process, our results supported that β2-M/HFE complex as mediator participated in β2-M induced EMT process is shared by renal proximal tubule epithelial cells and cancer cells. Our findings suggested that excessive β2-M may potentially be responsible for renal fibrosis and this mechanism could be used to explain tumor associated nephropathy.

Minor Essential Revisions

3 There are a number of minor grammatical mistakes; e.g. “urinal” should be “urinary”.

   Done

4 The statement “Appearance of #2-M in the urine depends on its plasma level, when exceeding its renal reabsorptive threshold of 5 mg/l and/or from proximal tubular damage” requires a reference.

   Done

5 Figure 1 - y-axis not a percentage. Strictly speaking MTS is not the same as cell viability; it measures net metabolic activity of a population of cells, although this latter point is less important.

The reviewer is absolutely right. Firstly, Figure 1 - y-axis should be “Absorbance (490nm)”. Secondly, after taking the absorbance at 490nm in the control as 1, so Figure 1 - y-axis appears as a percentage to the
control. I am sorry for the confusing caused by us, because we did not mention this in our manuscript. We have added this point in our revised manuscript.

6 In the result described in figure 3b; what is the control medium? Does it contain protein or is it just vehicle?

   It is just vehicle.

7 In the results described in figure 4, the authors should include the effect of HFE siRNA in the absence of #2-M.

   The reviewer is absolutely right. We understand that knowing the effect of HFE siRNA in the absence of β2-M may better reveal the role of HFE in the EMT process of HK-2 cells. However, in the present study, we mainly focused on role of HFE in β2-M-induced EMT and we think that our result may not be optimal, but should be sufficient to draw a conclusion that HFE knockdown is sufficient to reversed β2-M mediated EMT in HK-2 cells.

8 The HIF 1 western blot in figure 5 is of too poor quality for publication, particularly as the authors have a much better blot of the same target protein in figure 6.

   We have replaced a new picture for Fig 5B in the revised manuscript.

9 It would be interesting for the authors to comment on the distribution of fibronectin in figure 3c; it looks to me to be cytoplasmic and not secreted.

   In our immunofluorescence staining experiments, HK-2 Cells were
fixed on coverslips. When we incubated overnight at 4°C with the primary antibodies and secondary antibodies, the slides were upside down on the glass slide. This repeated operations and washing with PBS (6 times, 3 for primary antibody and 3 for secondary antibody) probably cause some loss of secretory protein, including fibronectin. To exclude this possibility, we have redone this experiment. Unfortunately, the fibronectin primary antibody did not work, maybe due to our improper storage.

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

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

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:** I have no competing interests

**Reviewer's report**

**Title:** beta2-microglobulin induce epithelial-mesenchymal transition in human renal proximal tubule epithelial cells

**Version:** 2 Date: 27 October 2014

**Reviewer:** Mi-Kyoung K Kwak

**Reviewer's report:**

This manuscript demonstrates that #2-microglobulin (#2-M) induced
epithelial-mesenchymal transition (EMT) in human renal proximal
tubular HK-2 cells. In particular, the authors showed that #2-M reduced
cellular iron contents via interaction with hemochromatosis, and thereby
activated Hif-1# to promote EMT process. Several comments can be
suggested.

1) Fig. 6. Can DES treatment modulate cellular iron level to a similar
level of #2-M group? Iron level can be quantified following DES
treatment, which is used in Fig. 6.

   This is a really good question. However we just used DES as a
positive control and did not evaluate the cellular iron level after DES
treatment and compared the difference in cellular iron level between the
control and β2-M group.

2) To strengthen the linkage between Hif-1# and EMT, levels of EMT
genes can be determined in Hif-1#-knockdown HK-2 following #2-M or
DES treatment.

   The reviewer is absolutely right. We understand that evaluating the
levels of EMT genes in Hif-1α knockdown HK-2 cells in the presence or
absence of β2-M or DES will strengthen the linkage between Hif-1α and
EMT. However, HIF-1α has been widely proven to participate in EMT
process [3]. So we think that our result may not be optimal, but could
draw a conclusion that HFE knockdown reversed the increasing of
HIF-1α may be responsible for β2-M induced HK-2 EMT.
3) Fig. 3C. Nuclear staining and bright field images are required.

   The reviewer is absolutely right and we have redone this experiment.
   Unfortunately, the fibronectin primary antibody did not work, maybe due to our improper storage.

4) Fig. 5B. The quality of Hif-1# blot is too poor to be recognized

   We have replaced a new picture for Fig 5B in the revised manuscript.

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

**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:** I declare that I have no competing interests.

**References:**