Reviewer’s report

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

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Reviewer: Mark Dockrell

Reviewer’s report:

“beta2 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.

2 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.

Minor Essential Revisions

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

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.

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.

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

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

Discretionary Revisions

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.

**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