Reviewer's report

Title: Changes in the gene expression programs of renal mesangial cells during diabetic nephropathy

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Reviewer: Yuichi Makino

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The authors defined in vivo mesangial cell gene expression programs during normal and disease process under diabetic circumstances by isolation of Meis1-GFP transgenic mice-derived mesangial cells followed by microarray analyses and comparison of the gene profile of other renal cells. They demonstrated the exceptional nature of the normal mesangial cells which includes smooth muscle, phagocytic and neuronal traits. Moreover, the authors characterized diabetic mesangial cells by identification of genes involved in extracellular matrix posit, cell division, vasculogenesis, and growth factor modulation. The results may complement previous studies examining mesangial cells grown in culture to yield a deeper understanding of disease process of diabetic nephropathy. However, there are some concerns to be clarified before consideration for publication.

Major Compulsory Revisions

1. As the author stated in the text, they could not exclude the possibility of a contamination of the other cells than mesangial cells in their isolation systems. The purity of the cells is of a quite importance to conclude the results are specific to mesangial cells in vivo, otherwise it is difficult to make an advantage to in vitro culture experiments. Since some of the genes expressed in mesangial cells are at the same time expressed in the other cells in the glomeruli, simple comparison of raw signal levels of the gene expression may not conclusive to characterize the isolated cell population. To this end, further purification by isolating ‘double positive cells’, e.g. Meis1-GFP and Megsin or alpha SMA, or by ‘negative selection’ with the other cell marker such as podocin or CD31 might useful and rational.

2. It is appreciated the authors validated their microarray analyses by examination at protein expression level. However the methods they demonstrated is not necessarily convincing. First, immunohistochemical data they showed did not provide information about the types of the cells expressing those 28 gene products. It should be shown the majority of the cells are mesangial cells by e.g. co-immunofluorescence study with mesangial cell markers to complement the microarray data. Second, quantitative aspect is missing. If the authors emphasize dominant expression or upregulated expression of the protein, they need to demonstrate Western blot and semi-quantitative analysis of the bands.
3. Seven-months-age of db/db mice seems too late for analysis of diabetic nephropathy. Previous reports have shown the appearance of alubuminuria at 14-20 weeks and defined as diabetic nephropathy model. Why the authors employed the late stage model? Moreover, the disease course of diabetic manifestation such as blood glucose, body weight, kidney size, blood lipids is missing. Since the model in the present study is based on the cross with Meis1-GFP transgenic mice, nothing is guaranteed in terms of carrying diabetic character as authentic db/db mice.

Minor Essential Revisions

1. Especially in normal (healthy) mesangial cells, relevant control is absent. In the present study, only mesangial cells are analysed. How about expression profile in other cells such as podocytes or endothelial cells? The process isolating glomeruli and purification of mesangial cells by FACS may evoke variety of cellular events and expression of genes involved in inflammation, proliferation and apoptosis. Without comparison to the other cells from the glomeruli, how the authors discriminate the possibility that those gene expressions are global within glomeruli by experimental processes?

2. Immunostaining, especially presented as Fig 6, is not technically acceptable and should be more convincing. It is not easy to identify positive staining from the background.

3. Did the authors find expression of the genes formally identified in diabetic kidney disease processes such as TGF-beta, CTGF, PAI-1? In spite of their statement that TGF-beta as pathologic factors for diabetic nephropathy, they only found some related factors.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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