Reviewer's report

Title: Expression of TGF-b1 and b3 but not Apoptosis Factors Relates to Flow-Induced Aortic Enlargement

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Reviewer: Dr Lucy Liaw

Level of interest: A paper of considerable general medical or scientific interest

Advice on publication: Accept after discretionary revisions

This manuscript describes a model of increased blood flow leading to arterial enlargement as a consequence of aortocaval fistula (ACF) in rats. Characterization of changes in arterial morphometry, wall shear stress, and cell proliferation and apoptosis was performed at various times up to 2 weeks following ACF. mRNA expression levels of TGFb1, TGFb3, and apoptosis related genes including Bcl-xS, were studied along this time course. The authors conclude that both TGFb1 and TGFb3 are upregulated following ACF, while most of the apoptosis genes examined did not change much following ACF. Areas that require revision, along with specific recommendations, are outlined below.

1. This is a descriptive study that is of interest, although the conclusions drawn by the authors are not entirely novel, as previous studies have examined a similar question. Negishi et al. studied rabbits with carotid arteriovenous shunt between the left carotid artery and the left external jugular vein, and demonstrated early increases in TGFb1 protein by immunostaining in endothelial cells (Arterioscler Thromb Vasc Biol 2001 (5):785-90 Upregulatory expression of furin and transforming growth factor-beta by fluid shear stress in vascular endothelial cells). This reference is quite relevant to the present study since it used a shunt model, and should be cited and discussed. In addition, increased blood flow and shear stress have been reported to enhance TGFb1 in vivo (Song et al., the authors do reference this work) and in vitro (Lum et al. Int J Mol Med 2000 Jun;5(6):635-41 Influence of different forms of fluid shear stress on vascular endothelial TGF-beta1 mRNA expression). Also, the authors did not reference previous vessel morphometry work in this model (Driss et al., Am J Physiol, 1997, H851-858), which should be included. A more complete discussion of the relevant publications and the importance of the results in this model as compared to other models of vascular injury (i.e. endarterectomy with altered blood flow, PA-subclavian fistula, carotid artery ligation) would be very helpful.

2. Fig. 3 suffers from poor resolution and the fact that the staining for TGFb1 is not at all convincing. It is difficult to appreciate the positive immunostaining in panels B and C in comparison to the control, whereas the immunostaining for TGFb3 in panels F and G can be clearly seen. An attempt to provide better quality photomicrographs should be made. In addition, for Fig. 2 and Fig. 4, the normalization
should be explained in the legend as well as the methods. For example, does relative mRNA level mean the transcript of interest was normalized to the GAPDH band, and how were the protein densitometry values normalized?

3. Fig. 5 and the accompanying legend are also problematic. On p. 15 in the legends it is mislabeled as Fig. 2. What is the color reaction substrate in the panels from control to 8W after ACF? There does not appear to be any significant differences between the control and any of the other panels, in terms of either blue or red levels, and similar to Fig. 3, the images appear slightly out of focus. Similarly, in the last two panels, BrdUA and B, it is difficult to appreciate the BrDU incorporated cells. I believe in BrdU B there might be 5 nuclei that are described in the legend as "blue" incorporation. Labeling with arrowheads would be helpful, as no blue nuclei are apparent in the panel. Again, an attempt to increase the quality of the photomicrographs should be made.

4. A more detailed description of the method used to obtain morphological data is needed. As vessel shape is often altered by tissue processing, lumen diameter generally cannot be directly measured. The calculations used to determine lumen diameter should be provided. Similarly, vessel wall thickness changes with dilation so as to maintain a constant vessel wall area. By only providing vessel wall thickness, it is not clear if the decrease in vessel wall thickness represents a change in overall vessel wall area. The calculations used to determine this should be provided. In addition, the methods should include more discussion about the segment of the vessel analyzed. Were controls done to determine that the two regions used for gene expression analysis and morphometry demonstrated a consistent response?

5. The manuscript should be re-scanned for clarity and typographical errors. Some statements are vague and/or misleading. Some examples follow:

"...(ECM) is involved in the vessel wall reconstruction..."

"Much has been known about TGFβ1."

More specific information should follow these two statements that will help the understanding of this particular study.

"Arterial wall stress is proven to be the single critical factor in regulation of vessel diameter" (no reference cited)

This is an over-simplification that is misleading. Several factors including injury, cytokines, serum proteins, etc. affect vessel diameter.

**Competing interests:**

None declared.