

Manuscript 25317: Tenascin C upregulates IL-6 expression in Human Cardiac Myofibroblasts via Toll-Like Receptor 4. Basic Study - The World Journal of Cardiology.

Response to Reviewers:

We would like to thank all the Reviewers for their useful comments. A revised manuscript has been submitted and each comment raised by the Reviewers is addressed individually below and in the manuscript:

1. Reviewer 00607640

Comments to Authors: This manuscript reports the effects of tenascin-C on the expression of the pro-inflammatory cytokines in human cardiac myofibroblasts. The findings are of interest and suitable for the publication by the journal.

Author response: No comments to be addressed. Thank you

2. Reviewer 00291404

Comments to Authors: The authors have investigated if and how tenascin C (TNC) upregulate IL-6 expression in human myofibroblasts. The main finding is that TNC upregulate IL-6 expression in human CMF and that this effect is mediated through its FBD domain and the TLR4 receptor. The experiments were well designed and performed, and supported the conclusions. The manuscript is well written. It should be accepted upon minor revision. Minor points: 1. Legend to figure 1. Lines 4-5. The phrase “(solid arrows)” in front of “TLR4 staining” should be relocated afterwards. 2. Page 10 and Figure 2. In the Results (page 10), the authors stated that only IL-6 was enhanced while neither IL-1 β nor any other MMP showed significant changes. However, upon inspection of the data presented on Figure 2, it seems that both IL-1 β and MMP10 were enhanced quite significantly. If this is the case, please change the statements in the Results (on page 10). If not, leave it as it is.

Minor Point 1: Legend to figure 1. Lines 4-5. The phrase “(solid arrows)” in front of “TLR4 staining” should be relocated afterwards.

Author response: As suggested by the Reviewer the phrase “solid arrow” in the Legend to Figure 1 has been relocated after the words “TLR4 staining”. This has been highlighted in the manuscript. Thank you.

Minor Point 2: Page 10 and Figure 2. In the Results (page 10), the authors stated that only IL-6 was enhanced while neither IL-1 β nor any other MMP showed significant changes. However, upon inspection of the data presented on Figure 2, it seems that both IL-1 β and MMP10 were enhanced quite significantly. If this is the case, please change the statements in the Results (on page 10). If not, leave it as it is.

Author response: Whilst there were measurable increases in Tenascin C-stimulated IL-1 β and MMP10 mRNA, these were not statistically significant. Importantly, basal (unstimulated) levels were on the lower limit of detection and hence any level of increase is unlikely to be of functional significance. We have therefore retained the statement in the Results section (on page 10) –as suggested by the Reviewer. Thank you.

3. Reviewer 00503405

Comments to Authors: In the original article of Maqbool et al. the authors found that tenascin in vitro increased both IL-6 and MMP3 gene expressions in cultured human cardiac myofibroblasts. IL-6 protein expression was also elevated. They also found that incubation of cells with TLR4 neutralising antisera attenuated the effect of tenascin on IL-6 mRNA and protein expression. They demonstrated that tenascin upregulates MMP3 and the pro-inflammatory cytokine IL-6 in human cardiac myofibroblasts, moreover, the latter effect of tenascin was found to be mediated via TLR4 and FBG domain of tenascin. Their results are of clinical importance as targeting the FBG domain of tenascin may provide a future therapeutic strategies to counteract aberrant inflammation and maladaptive cardiac remodelling after infarction. This is an excellent study, which is well designed and well presented. Accept as it is. Congratulation.

Author response: No comments to be addressed. Thank you.

Response to Editorial Comments:

We would like to thank the Editors for their useful comments. Each comment raised by the Editor is addressed individually below:

Editorial Comments (1-5): Inclusion in title page and provision of supportive information and where appropriate signed pdf's for:

- Supportive foundation acknowledgement
- Institutional animal care and use committee statement
- Conflict of interest statement
- Data sharing statement
- Biostatistics statement
- Institutional review board statement
- Animal care and use statement.

Author action: All statements have been included in the title page (page 2) in addition all statements have been signed by the corresponding author on behalf of the rest of the authors. Where appropriate necessary documentation has been included. These have been uploaded as separate files. Thank you.

Editorial Comment 6: Please offer more details of address such as street or avenue (in the Correspondence to section):

Author action: The Institutions address has been modified to include "Clarendon Way" in the "Correspondence to section" of the title page. This has been highlighted in the manuscript. Thank you.

Editorial Comment 7: Please offer the audio core tip:

Author action: Audio core tip recording has been made and uploaded as an mp3 file as requested. Thank you

Editorial Comment 8: All figure number should be in order. Please correct:

Author action: To ensure that all the Figure numbers appeared in order we have remove the following sentence (from page 10 of the Results section) “The observed changes in mRNA expression were mirrored by changes in secreted IL-6 protein levels (Figure 4B). ” as this sentence was referring to a result that is also described later on in the Results section (page 11 second paragraph). This not only avoids repetition but also ensures the Figures appear in order in the text. The changes are highlighted in the manuscript. Thank you.