Review of: "PDGFR-β signaling mediates HMGB1 release in mechanically stressed vascular smooth muscle cells"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

In this study, the authors have examined the effect of PDGFR-β signaling on the release of high mobility group box 1 (HMGB1) in mechanically stressed (MS) vascular smooth muscle cells (VSMC) isolated from the thoracic aorta of 7 weeks old male Sprague Dawley rats. They show that VSMC subjected to MS exhibited an increase in the release of HMGB1 in the culture media as judged by enzyme –linked immunosorbent assay. MS was shown to induce the translocation of HMGB1 from nucleus to cytosol in a time- dependent fashion and was associated with both phosphorylation and acetylation of this protein. By using both pharmacological inhibitors and small interfering RNA, the authors have provided strong evidence for a role of PDGF-β receptor in MS-induced HMGB1 secretion. However, as claimed in the title of the paper, the authors have not identified the specific PDGFR-β signaling pathways responsible for this process. There are other oversights in this manuscript some of these are described below:

1.The authors should have determined the basal change in the levels of HMGB1 secretion in VSMC that were not subjected to any MS in the results shown in Figure 1. This should have been the control in this experiment. Since this important control is missing, it is not possible to assess the real change in HMBG1 secretion due to MS. Moreover, if the data at 0 hr are used as ‘control’ (basal) value of around 20pg/ml the change in secretion after 12 hrs with 3% MS appears to be around 50pg/ml (Figure 1), thus the fold increase is about 2.5-fold and not 52.57- fold as stated in the results section on page 4 of the article.

2. The blots shown in Figure 2 A and B should have also been probed with β-actin and Lamin B respectively to check for the purity of the nuclear and cytosolic fractions. Methods for isolating the nuclear and cytosolic fractions should have been included.

3. The type and the source of antibodies used to detect changes in the levels of phosphorylation (p-Serine, p-Threonine or p-Tyrosine) and acetylation of HMGB1 in response to MS (Figure 3) should have been included in the ‘Materials and methods’ section. Moreover, the authors have not provided any evidence to suggest a role of PDGFR-β signaling in MS-induced phosphorylation and acetylation of HMGB1 in these studies (Figure 3). In addition, no results have been presented to directly support the suggestion that MS increased HMGB1 release via acetylation and phosphorylation of HMGB1 (page 11).

4.In Figure 4, it is not clear whether the control panel represents the data derived from VSMC that were not subjected to MS.

The use of proper controls and inclusion of the results from some critical experiments establishing a direct role of PDGFR- β and its downstream effectors in inducing the phosphorylation and acetylation of HMGB1 in MS -induced

release of HMGB1 should have strengthened this work.