LETTER TO THE EDITOR

Reply to “Letter to the editor: Comments on Wette et al. (2017): ‘Characterization of muscle ankyrin repeat proteins in human skeletal muscle’”

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WE APPRECIATE THE INTEREST in our recently published study (6) shown by the Letter to the editor by Dr. Cenni (2), but we are compelled to point out that the main criticisms made are entirely misplaced and that the Letter includes some major misstatements about findings in the literature and the text and rationale of our paper.

First and foremost, our conclusion that “these data are not consistent with the proposal that Ankrd 2, per se, or pAnkrd 2-Ser99 mediates stretch-induced signaling in skeletal muscle, dissociating from titin and translocating to the nucleus” is fully appropriate. Our data forced us to this conclusion even without directly examining the effects of muscle stretch, because our experiments unequivocally demonstrated that even in an unstretched muscle fiber, >70% of the total cellular Ankrd 2 [Figs. 4–6 of our paper (6)], and >90% of the pAnkrd 2-Ser99 (Fig. 8), are continuously present and freely diffusible in the cytosol and yet do not translocate into the nucleus (Fig. 7). Consequently, it is untenable to maintain that stretch could be signaled simply by causing some further amount of these same types of molecules to dissociate off titin into the cytoplasm so that they could then enter the nucleus, as clearly those molecules are not doing this. As we concluded in the Abstract, “It will be necessary to show that the stretch-sensitive signaling molecule.”

Second, Dr. Cenni is mistaken in saying that we did not comment on the results of Miller et al. (Ref. 5 here) and Hayashi et al. (3); in the Introduction we explicitly state “Using immunohistochemical (IHC) techniques, Miller et al. (30) performed experiments in fetal rat cardiomyocytes showing the accumulation of CARP and DARP in the nucleus following prolonged passive stretch,” and in the DISCUSSION we state that Hayashi et al. (3) provided “quantitative data indicating that the majority of Ankrd 2 is present in the cytosol in mouse skeletal muscle.” We also wish to note that, contrary to Dr. Cenni’s claim, neither of these studies examined the effects of stretch on Ankrd 2 distribution in skeletal muscle.

Furthermore, we reiterate our comments in the Introduction about some inherent limitations of IHC data, in particular that the fixation process does not properly identify, and can even lead to misplacement of, readily diffusible cytosolic constituents. This is highlighted by the findings of the diffusion and fractionation experiments in our study showing that >70% of the Ankrd 2 is indeed freely diffusible in the cytoplasm, which was not apparent in the IHC data (Fig. 7); the latter instead showed only the location of the remaining Ankrd 2, which was present almost entirely at the N2A region in the I-band with very little or none associated with the nuclei (Fig. 7). Clearly, IHC data by itself does not provide all the necessary information to fully understand the distribution and possible redistribution of Ankrd 2 in different circumstances.

Finally, we stated in the Introduction that “Although several putative phosphorylation sites have been identified for MARPs (25), only the serine 99 site of Ankrd 2 has been examined in vitro and in vivo and appears to regulate Ankrd 2 redistribution in mouse C2C12 cells (2)” (the latter reference being Cenni et al., Ref. 1 here). This was simply meant to convey that the latter study proposed that phosphorylation of Ankrd 2 at Ser99 induces its translocation to the nucleus [see legend of Fig. 7 in Cenni et al. (1)]. We did not claim, nor mean to imply, that Dr. Cenni’s study (1) suggested “that stretch, inflammation, muscle differentiation or any other type of stimulus might induce the phosphorylation of Ankrd 2 at serine 99.” Instead, we attributed the proposal that “the stretch-induced redistribution of MARPs to the nucleus is triggered by phosphorylation at a hidden phosphorylation site (25)” to Lun et al. (Ref. 4 here), not to the work of Cenni et al.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.D.L. drafted the manuscript; S.G.W., H.K.S., G.D.L., and R.M.M. edited, revised, and approved final version of manuscript.

REFERENCES


