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

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LETTER TO THE EDITOR

TO THE EDITOR: In their recent paper, Wette et al. (4) describe the distribution of muscle ankyrin repeat proteins, i.e., Ankrd 1, Ankrd 2, and Ankrd 3, in muscle cells (4). One of the aims of the authors is to understand if Ankrd 2 and its phosphorylated form at serine 99 (pAnkrd 2-Ser99) redistribute from titin to the nucleus following a prolonged stretch stress. Although the authors have tried to deepen an interesting issue on Ankrd 2 distribution in muscle cells stressed by stretch, the article presents multiple limitations in the experimental plan and text, rendering some of the results uncertain. A detailed list of comments is here provided.

By Western blot analysis performed on subcellular fractions of single muscle fibers, the authors reach the 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, because the majority of these forms of Ankrd 2 are already free in the cytosol.” Unfortunately, the study does not present any type of stretch treatment on muscle cells able to support the above conclusion. In fact, there is no evidence in the text on the subcellular localization of any form of Ankrd 2 upon stretch stimulation. This could be examined by immunohistochemistry or by Western blot analysis, as shown, for example, for resting cells (Fig. 6B of the paper). The authors missed commenting on the results of Miller et al. (3) and Hayashi et al. (2) that point to Ankrd 2 as a titin-associated protein (2, 3), localized in the central I-band in resting conditions, that upon stretch stimulation of skeletal myofibrils and adult bovine atrial muscle becomes nuclear located (3).

A further critical point comes from an assumption of the authors according to which: “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.” This statement is true only in part. Our paper [Cenni et al. (1), to which the authors refer] indeed reports that in differentiating C2C12 muscle cells, Ankrd 2 phosphorylation at serine 99 is induced following cellular exposure to H2O2, regulating the cellular distribution of Ankrd 2 in these conditions. However, in our study (1), we neither demonstrated nor suggested that stretch, inflammation, muscle differentiation, or any other type of stimulus might induce the phosphorylation of Ankrd 2 at serine 99.

Therefore, starting from an assumption that every signal propagates in the cells (of different type and of different species) through the same pathway, and without providing any evidence regarding the amount, the localization, or the phosphorylation of Ankrd 2 under stretch conditions, the results presented by Wette et al. seem to be inadequate to support another conclusion according to which: “It is therefore difficult to see how phosphorylation at Ser99 of any further bound (or unbound) Ankrd 2 could be the stretch-sensitive signal in adult human muscle, because the phosphorylated Ankrd 2 does not appear to relocate to the nucleus, as it reportedly does in developing cultured mouse C2C12 cells.” This conclusion refers to Fig. 8, A and B, which shows phospho-Ankrd 2 serine 99 levels. Figure 8, however, has two macroscopic limitations that make impossible to reach the conclusion reported in the article. First of all, the blots of Fig. 8A are performed on extracts from resting and not stimulated samples. Second, Fig. 8 does not include the stoichiometry of phosphorylation of Ankrd 2, which is mandatory when discussing percentage of phosphorylation of a given protein. Finally, the cited “developing cultured mouse C2C12 cells” to which the authors refer, are differentiating C2C12 muscle cells also subjected to H2O2 exposure [see Cenni et al. (1)], which is clearly not the same thing.

All in all, the lack of experiments performed in stimulated conditions, and these limitations in the experiments and the text, leave some of the results presented in this article as of questionable reliability.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

V.C. drafted manuscript, edited and revised manuscript, approved final version of manuscript.

REFERENCES


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