Author's response to reviews

Title: Beta-synemin expression in cardiotoxin-injected rat skeletal muscle

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Author's response to reviews: see over
Dear Editor,

Thank you for giving us the opportunity to resubmit our revised manuscript (MS: 1119106637132653) entitled “Beta-synemin expression in cardiotoxin-injected rat skeletal muscle” for consideration in the journal *BMC Musculoskeletal Disorders*. We greatly appreciate the time you and the reviewers took to evaluate our work and have provided the following responses to each of the suggestions:

**Reviewer (Dr. Takeda)**

**Major compulsory revisions:**

**Comment:** The reviewer failed to point out previously, but they did not have the internal control, when they tried to quantitatively analyze the expression of beta-synemin, or alpha-dystrobrevin-1, -2 by Western blotting. They tried to normalize the data by doing different experiments, but only showed representative one, as described in the legend for Figure 4. They are asked to show the bands of Myosin heavy chain by CBB staining of the gel, or compare the bands with the internal control such as alpha-tubulin or alpha-actin in Figure 4 and 6.

**Our response:** Since alpha-tubulin, alpha-actin, and myosin heavy chain are all muscle proteins whose expression could change as a result of the experimental conditions being tested, these proteins were not used as controls; rather, we used the non-specific background bands visible in the western blots as controls. We believe this approach best controls for loading variability since this uses multiple random proteins directly visible on the western blot being quantitated. We have updated the Methods to reflect this.

**Minor essential revisions:**
As suggested, both of the reviewer’s minor essential revisions have been made.

**Discretionary revisions:**
As suggested, we replaced the panel of the muscle at 14 days following cardiotoxin injection (Figure 2) with a clearer photograph.

**Reviewer (Dr. Li)**

**Minor essential revisions:**
Comment (1): It is confusing to set the relative protein levels in days 1 and 28 at 0% and
100%. In addition, it is not applicable to alpha-dystrobrevin-2. If you put the relative protein level of alpha-dystrobrevin-2 on day 1 as 0%, the pattern of this protein is similar to that of b-synemin except the days 28. It is more logical to set only the relative protein level on days 28 at 100%.

**Our response:** Previous reports (references #10-13) have evaluated the relative expression levels for proteins such as dystrophin and its associated protein by setting days 1 and 28 at 0% and 100%. Therefore, we would like to use the same evaluation technique so that our data can be compared with previously published data.

**Comment (2):** There are mistakes in the reference citation. Reference of Xue et al is omitted. It is suggestive that beta-synemin is also associated with signaling (24) should be (23). Xue et al should be 24, the present reference 24 and 25 should be 25 and 26.

**Our response:** We have corrected these references.

**Comment (3):** Figure 4 seems have an extra picture in the alpha-dystrobrevin-1 western blot.

**Our response:** We have updated the blot of alpha-dystrobrevin-1 of Fig. 4.

Thank you for considering our resubmission and if there are any questions concerning our response, please feel free to contact me at the address below or by e-mail at: mizunoy@med.gunma-u.ac.jp.

Sincerely,

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