INCREASED STRESS PRODUCTION AND RESPONSE TO INJURY IN DESMIN KNOCKOUT MUSCLES RESCUED BY PLASMID TRANSFECTION

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INTRODUCTION

The muscle intermediate filament protein desmin provides structural support and integrates muscle force throughout the cell (Lazarides, 1980). In the absence of desmin, muscles have disorganized myofibrils (Shah et al. 2002) and are injured less compared to their wild-type counterparts during eccentric contraction-induced injury (Sam et al. 2000). These changes could result directly from lack of desmin or could be due to secondary changes that result from development in the absence of desmin. To determine whether desmin plays a direct role in stress generation and response to injury, desmin knockout muscles were transfected with a GFP-desmin DNA plasmid and their mechanical function was probed using a high stress eccentric contraction model.

METHODS

All procedures were performed on mice in accordance with the NIH Guide for the Use and Care of Laboratory Animals. For transfection, GFP-desmin plasmids were injected into the tibialis anterior (TA) muscles of desmin-null mice and DNA incorporation facilitated by electroporation (Ichor Med. Systems. Inc., San Diego, CA). Seven days later, the fifth toe extensor digitorum longus (EDL), which was also transfected, was excised and mounted in a chamber bathed in Ringer's solution from one of five groups (n=7-12/group): (1) wild-type (WT), (2) desmin knockout (KO), (3) KO transfected with a plasmid encoding GFP-desmin, (4) KO transfected with GFP plasmid alone, and (5) KO transfected with PBS (EP only). For mechanical testing, muscle sarcomere length was set to 2.91 µm by laser diffraction and maximum isometric tension was measured twice at 3 min intervals.

Muscles were then eccentrically-activated by stimulating isometrically for 200 ms, stretching the muscles by 15% of the fiber length ($L_f$) and holding this length for an additional 500 ms. This procedure was repeated for 10 eccentric contractions every 3 min as previously described (Sam et al. 2000) to produce muscle injury. Maximum isometric tension was measured twice after the exercise bout. Percent stress decrease (change in post-eccentric maximum isometric stress relative to pre-eccentric maximum isometric stress) was compared across groups by one-way ANOVA as the

![Figure 1: Percent stress decrease of five experimental groups. KOs (open bar) have about the same stress decrease of either KO transfected with GFP or PBS controls (hatched bars). KOs transfected with GFP-desmin (grey bar) were injured almost to the level of WT muscles (black bar).](image-url)
index of muscle injury. Paired comparisons were made between groups with post hoc Tukey tests. All results are presented as mean±standard error. Significance level (α) was set to 0.05. To quantify GFP-desmin expression, western blots were obtained using monoclonal antibodies against desmin (DER11, Novacastra Laboratories) for endogenous GFP-desmin using an exogenous desmin standard for calibration. All bands were quantified using enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech).

RESULTS AND DISCUSSION

Injury due to eccentric contraction is represented by percent decrease in maximum isometric stress. As previously demonstrated (Sam et al. 2000) WT muscles had the largest drop in stress (Fig. 1, black bar), decreasing by 67.5%±7.7, which was significantly greater than KO controls which decreased by only 33.7%±5.2 (Fig. 1, open bar; p<0.05). KO muscles transfected with GFP or PBS were not significantly different from KO controls (Fig. 1, hatched bars 36.5%±4.1 and 34.9%±2.9, p>0.05, respectively). In contrast to all other KO muscle groups, KO muscles that were successfully transfected with GFP-desmin showed a significantly greater stress decrease of 54.8%±4.3 (Fig. 1, grey bar) compared to other KO muscle groups (p<0.05). A nonlinear relationship between desmin expression levels and injury level was observed with higher desmin levels corresponding to greater amounts of injury (Fig. 2). This may imply some type of biomechanical cooperativity across the fiber.

SUMMARY/CONCLUSIONS

Here we provide direct evidence that the desmin protein increases the efficiency of stress production by the muscle and increases injury resulting from eccentric contraction. Thus, GFP-desmin not only correctly localizes at the Z-disk, but also incorporates and plays a functional role in skeletal muscle after expression.

Figure 2: Percent stress decrease as a function of desmin concentration. Wildtype muscles have the greatest stress injury (black bars) while desmin null muscles have the least (green bars). Individual transfected muscles are shown as blue circles and have intermediate injury levels.

To date there are over 20 known different mutations in the desmin gene that result in an accumulation of desmin into clumps scattered throughout the cytoplasm (Goldfarb et al. 2004). This study has important implications in the pursuit of gene therapy models for rehabilitation and in understanding the apparently biomechanical etiology of desmin related muscle diseases.

REFERENCES

3. Sam et al. (2000). Am. J. Physiol. 279:C1116-C1122

ACKNOWLEDGEMENTS

We acknowledge NIH grant AR40050 and the Department of Veterans Affairs.