Detyrosinated microtubule arrays drive myofibrillar malformations in mdx muscle fibers.

Anicca Harriot[1], Tessa Altair-Morris[2], Camilo Vanegas[3], Jacob Kallenbach[3], Kaylie Pinto[4], Humberto C. Joca[1], Marie-Jo Moutin[5], Guoli Shi[3], Jeanine A. Ursitti[3], Anna Grosberg[2], Christopher W. Ward[1,3] (see publication for affiliations)*

Abstract

Altered myofibrillar structure is a consequence of dystrophic pathology that impairs skeletal muscle contractile function and increases susceptibility to contraction injury[1–3]. In murine Duchenne muscular dystrophy (*mdx*), myofibrillar alterations are abundant in advanced pathology (>4 months) [1–3], an age where we formerly established densified microtubule (MT) arrays enriched in detyrosinated (deTyr) tubulin as negative disease modifiers impacting cell mechanics and mechanotransduction[4,5]. *Given the essential role of deTyr-enriched MT's in myofibrillar growth, maintenance, and repair, we examined the increased abundance of these arrays as a potential mechanism for these myofibrillar alterations.*

Here (see[6]) we find an increase in deTyr-tubulin as an early event in dystrophic pathology (4 weeks) with no evidence myofibrillar alterations. At 16 weeks, we show deTyr-enriched MT arrays significantly densified and co-localized to areas of myofibrillar malformation. Profiling the enzyme complexes responsible for deTyr-tubulin, we identify vasohibin 2 (VASH2) and small vasohibin binding protein (SVBP) significantly elevated in the *mdx* muscle at 4 wks. Using the genetic increase in VASH2/SVBP expression in 4 wk wild-type mice we find densified deTyr-enriched MT arrays that co-segregate with myofibrillar malformations similar to those in the 16 wk *mdx*. Given that no changes were identified in fibers expressing sfGFP as a control, we conclude that disease-dependent densification of deTyr-enriched MT arrays underscores the altered myofibrillar structure in dystrophic skeletal muscle fibers.

Results

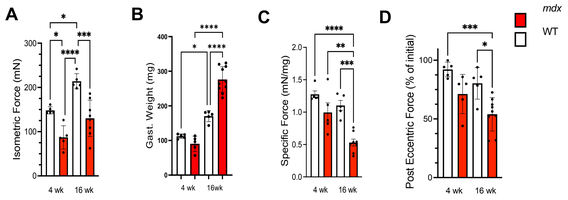

Fig 1. Force production decreases with age in *mdx* while injury susceptibility increases **(A)** *In vivo* isometric force produced at 150Hz in wild-type (white) and *mdx* (red) gastrocnemius muscles at 4 and 16 wks, respectively. **(B)** Weights of the gastrocnemius muscles from WT and *mdx* mice used to normalize force production to measure **(C)** specific force production in age-matched WT and *mdx* mice at 150 Hz. **(D)** Percent force production in gastrocnemius muscles from 4 and 16 wk WT and *mdx* mice after 25 eccentric contractions. *Mdx* mice experience increased force loss when compared to wild-type. Values are means ± SEM. All analysis are one-way ANOVA with Šídák's multiple comparisons test (* P < 0.05; ** P < 0.01; ***P<0.001; ****P <0.0001)

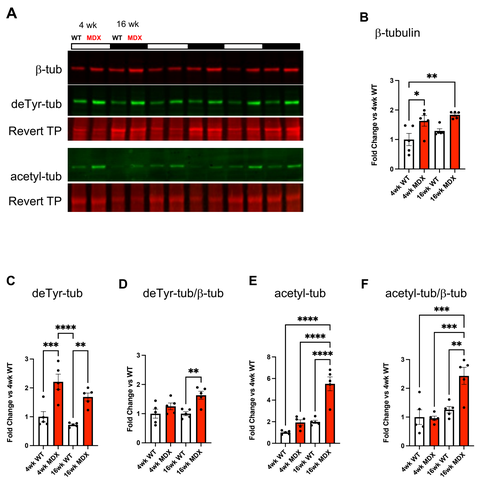

Fig 2. Detyrosination increases with dystrophic disease progression. **(A)** Western blots of the gastrocnemius muscle from wild-type and *mdx* mice at 4 wks (n=5) and 16 wks (n=5) **(B)** increased tubulin abundance and post-translational modification by **(C)** detyrosination and **(D)** acetylation. **(E)** We also see a significant increase in the NOX2 subunit $GP91_{phox}$. Values are means ± SEM. All analysis are one-way ANOVA with Šídák's multiple comparisons test (* P < 0.05; ** P < 0.01)

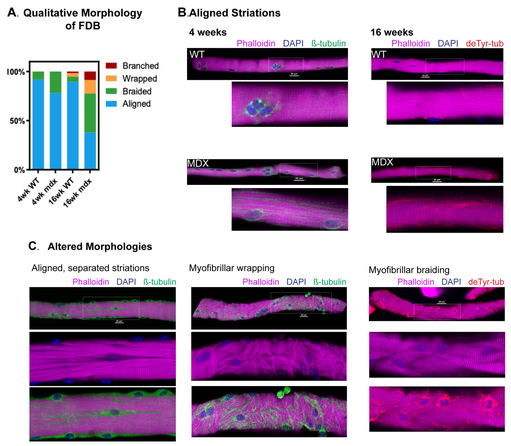

Fig 3. Qualitative survey finds altered myofibrillar morphology in WT and *mdx FBD* muscle fibers **(A)** The percent distribution of each morphology was determined based on a survey of approx. of 150 fibers (n = 5, ~30 fibers/animal) from each condition. **(B)** Representative images showing aligned striations at 4 and 16 wks with canonical rectilinear MT structure. MT bundling is apparent in *mdx* as early as 4wks. Detyrosinated tubulin appears increased in 16 wk *mdx*. **(C)** Fibers from 16 wk *mdx* representative of altered morphologies, showing areas of MT bundling appearing coincident with alterations in striation continuity, in fibers with myofibrillar malformation as well as in fibers with otherwise aligned striations.

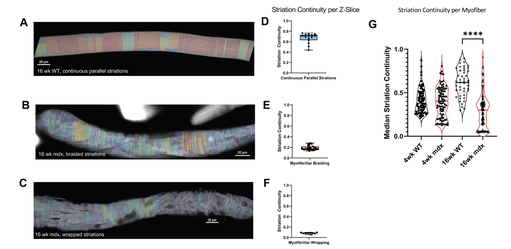

Fig 6. Median Striation Continuity captures myofibrillar changes and decreases with altered morphology. Striation detection of phalloidin for **(A)** 16wk wild-type fiber **(B)** 16wk mdx fiber with braided myofibrils, and **(C)** 16wk mdx fiber with wrapped myofibrils. Their respective continuity scores per z-slice are illustrated with min, max, median scores displayed in boxplots (E-F). (G) Average striation continuity scores for each fiber within each experimental group (n=50-125 fibers per condition). One-way ANOVA revealed significant difference between groups with Šídák's multiple comparisons test elucidating a significant difference between 16wk WT and MDX ($p < 0.0001$).

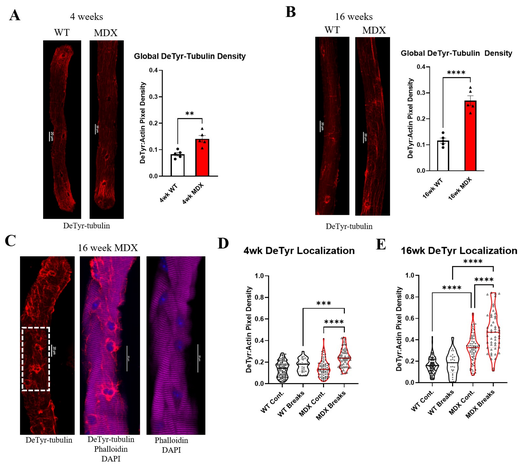

Fig 7. Detyrosination is enriched in dystrophic fibers and at the site of myofibrillar malformation. **(A-B)** We show the average deTyr density normalized to fiber size via the actin stain in fibers from wild-type and *mdx* mice 16 wks (n = 5 mice). Values are means ± SEM. Statistical significance determined using t-tests. **(C)** Representative images showing co-localization of detyrosinated tubulin with myofibrillar break sites. **(D-E)** Quantification of deTyr-tubulin density in regions with continuous striations across myofibrils vs. within break sites. Analysis was completed using one-way ANOVA with Šídák's multiple comparisons test (**P < 0.01; ***P<0.001; ****P <0.0001).

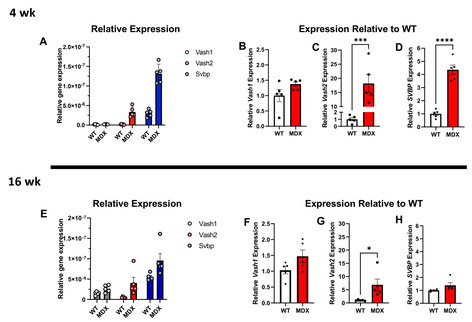

Fig 8. VASH2 and SVBP transcripts are increased in *mdx*. Detyrosination is by the enzyme complex of vasohibin 1 or 2 (VASH1/2) and small vasohibin binding protein (SVBP). **(A-D)** At 4 weeks, VASH1 expression is not significantly increased in the *mdx* whereas VASH2 is increased approximately 18-fold. **(D)** and SVBP is increased approximately 4-fold. **(F)** Relative gene expression of both tubulin carboxypeptidases, and especially VASH1, appears increased in wild-type 16 wk compared to 4 wk and *mdx* expression relative to wild-type remains increased at 16 wks. **(G)** VASH1 shows a trend toward increased expression in *mdx*, only **(H)** VASH2 expression remains significantly increased, while its binding partner **(I)** SVBP is not significantly increased in *mdx* at 16 wks. All analysis was performed using one-way ANOVA with Šídák's multiple comparisons test (*P < 0.05; ***P<0.001; ****P <0.0001).

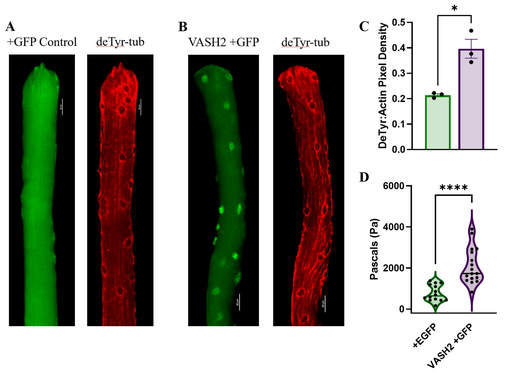

Fig 9. VASH2 overexpression in young WT increases the density of deTyr-MTs and the passive stiffness of the muscle fiber **(A)** FDB overexpressing EGFP(+) as a control finds deTyr-tubulin enriched MTs dispersed throughout the fiber. **(B)** VASH2+GFP expressing muscle fiber exhibits a denser and more bundled network of deTyr-tubulin enriched MTs. **(C)** Quantification of the density of deTyr-MTs finds a significant elevation with VASH2 overexpression (n=3 mice per condition; 10-15 fibers per mouse). **(D)** Nano-indentation (5μm/sec) measure of viscoelastic resistance finds increased stiffness in VASH2-GFP muscle fibers. (t-test, * P < 0.05; ****P < 0.0001).

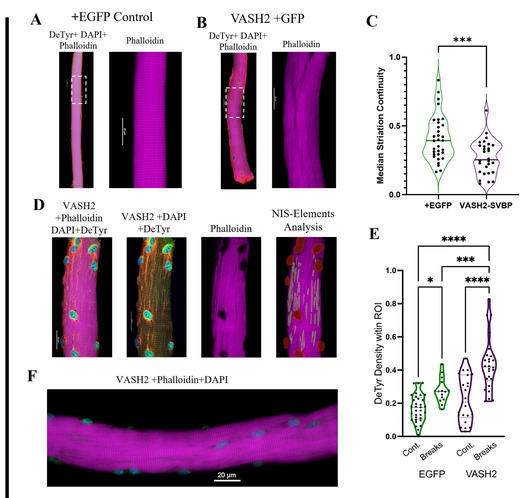

Fig 10. VASH2 overexpression in WT models myofibrillar alterations seen in the *mdx* **(A-B)** Representative images of FDB fibers isolated from EGFP and VASH-SVBP electroporated FDB muscles respectively, with insets showing the disparate morphology. A closer look at the inset **(B)** reveals extensive veneering of striations, perhaps indicative of the onset of myofibrillar "braiding". **(C)** Quantification of striation continuity using Z-line detection. There was a statistically significant difference between groups as determined by t-test **(D)** Representative image of EGFP(+)VASH2 myofiber showing myofibrillar break sites identified as regions-of-interest (ROI) within NIS-Elements. **(E)** The density of the deTyr-MTs within each ROI was calculated and normalized to the respective area. There was a statistically significant difference between groups as determined by one-way ANOVA with Šídák's multiple comparisons test ($F(3,92) = 25.12$). **(F)** Representative image of VASH2-SVBP electroporated FDB fiber with evidence of altered myofibrillar directionality (i.e., braiding). (* P < 0.05; ***P < 0.001; ****P < 0.0001).

Citations

1. Head SI. Branched fibres in old dystrophic mdx muscle are associated with mechanical weakening of the sarcolemma, abnormal Ca2+ transients and a breakdown of Ca2+ homeostasis during fatigue. Exp Physiol. 2010 May;95(5):641–56.
2. Head S. Does skeletal muscle fiber branching play a role in the inability of old EDL dystrophic muscle to resist large passive stretches? J Appl Physiol (1985). 2012 Jan;112(2):331; author reply 332.
3. Friedrich O, Both M, Weber C, Schurmann S, Teichmann MD, von Wegner F, Fink RH, Vogel M, Chamberlain JS, Garbe C. Microarchitecture is severely compromised but motor protein function is preserved in dystrophic mdx skeletal muscle. Biophys J. 2010 Feb 17;98(4):606–16. PMCID: PMC2820646
4. Khairallah RJ, Shi G, Sbrana F, Prosser BL, Borroto C, Mazaitis MJ, Hoffman EP, Mahurkar A, Sachs F, Sun Y, Chen YW, Raiteri R, Lederer WJ, Dorsey SG, Ward CW. Microtubules underlie dysfunction in duchenne muscular dystrophy. Sci Signal. 2012 Aug 7;5(236):ra56. PMCID: PMC3835660
5. Kerr JP, Robison P, Shi G, Bogush AI, Kempema AM, Hexum JK, Becerra N, Harki DA, Martin SS, Raiteri R, Prosser BL, Ward CW. Detyrosinated microtubules modulate mechanotransduction in heart and skeletal muscle. Nat Commun. 2015;6:8526. PMCID: PMC4633818
6. Harriot AD, Altair Morris T, Vanegas C, Kallenbach J, Pinto K, Joca HC, Moutin MJ, Shi G, Ursitti JA, Grosberg A, Ward CW. Detyrosinated microtubule arrays drive myofibrillar malformations in mdx muscle fibers. Front Cell Dev Biol. 2023 Aug 25;11:1209542.

Summary

- Our former work in murine DMD showed densified microtubule (MT) arrays enriched in deTyr-tubulin contribute to contraction injury by increasing both cell mechanics (i.e., stiffness) and mechanotransduction though Nox2-ROS and Ca^{2+} [4,5]
- Here we show that these deTyr-enriched MT arrays also underscore the altered myofibrillar structure linked to decreased muscle specific force and injury susceptibility [6]
- We propose deTyr-enriched MT arrays as a therapeutic target to improve muscle force and reduce contraction injury in DMD.

University of Maryland School Of Medicine: Department of Orthopedics