Biochemical and Biophysical Research Communications 430 (2013) 7–13

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Metavinculin: New insights into functional properties of a muscle adhesion protein

Florian Thoss [a], Franziska Dietrich [a], Karla Punkt [b], Susanne Illenberger [c], Klemens Rottner [d,e], Mirko Himmel [a,*], Wolfgang H. Ziegler [a,f,*]

[a] Interdisciplinary Center for Clinical Research (IZKF) Leipzig, Faculty of Medicine, University of Leipzig, D-04103 Leipzig, Germany
[b] Institute of Anatomy, University of Leipzig, D-04103 Leipzig, Germany
[c] School of Engineering and Science, Jacobs University Bremen, D-28759 Bremen, Germany
[d] Institute of Genetics, University of Bonn, D-53115 Bonn, Germany
[e] Helmholtz Centre for Infection Research, D-38124 Braunschweig, Germany
[f] Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, D-30625 Hannover, Germany

ARTICLE INFO

Article history:
Received 29 October 2012
Available online 14 November 2012

Keywords:
Metavinculin
Vinculin
Costameres
Muscle fiber-type

ABSTRACT

Metavinculin is a muscle-specific splice variant of the ubiquitously expressed cytoskeletal adaptor protein vinculin. Both proteins are thought to be co-expressed in all muscle types where they co-localize to microfilament-associated adhesion sites. It has been shown that a metavinculin-specific insertion of 68 amino acids alters the biochemical properties of the five-helix bundle in the tail domain. Here, we demonstrate that the metavinculin-specific helix H1′ plays an important role for protein stability of the tail domain, since a point mutation in this helix, R975W, which is associated with the occurrence of dilated cardiomyopathy in man, further decreases thermal stability of the metavinculin tail domain. In striated muscle progenitor cells (myoblasts), both, metavinculin and the R975W mutant show significantly reduced, albeit distinctive residency and exchange rates in adhesion sites as compared to vinculin. In contrast to previous studies, we show that metavinculin is localized in a muscle fiber type-dependent fashion to the costameres of striated muscle, reflecting the individual metabolic and physiological status of a given muscle fiber. Metavinculin expression is highest in fast, glycolytic muscle fibers and virtually absent in *M. diaphragmaticus*, a skeletal muscle entirely lacking fast, glycolytic fibers. In summary, our data suggest that metavinculin enrichment in attachment sites of muscle cells leads to higher mechanical stability of adhesion complexes allowing for greater shear force resistance.

1. Introduction

Skeletal muscle is composed of muscle fibers, which derive from the fusion of muscle progenitor cells to form multi-nucleated syncytia. In most cases, skeletal muscle contains a mixture of different muscle fiber types which together define the functional characteristics of a given muscle. Depending on the level of exercise or immobilization, individual muscle fibers can undergo transition from fast to slow fiber type or vice versa, allowing adaption of the muscle to specific physiological needs [1]. In skeletal muscle, the cytoskeletal adaptor proteins vinculin and metavinculin were reported to co-localize in specialized microfilament-associated adhesion structures like myotendinous junctions and costameres. Costameres are characteristic rib-like protein complexes surrounding contractile myofibrils within the myofibers. They were suggested to act as sites of mechanical force transduction [2,3].

Vinculin is an ubiquitously expressed actin-binding protein found in cell-matrix and cell–cell adhesion sites [4], whereas its splice variant, metavinculin, is mostly restricted to muscle tissue [5,6]. Both proteins are synthesized from a single gene. Metavinculin expression is controlled by alternative splicing of exon 19, which encodes a 68 amino acid insertion in the tail domain [7,8] and correlates with contractility of differentiated muscle cells [9]. Several binding partners were described for vinculin and metavinculin, but to date no protein ligand, binding exclusively to either metavinculin or vinculin, has been described [4]. In solution, the vinculin molecule adopts an auto-inhibitory conformation, in which the vinculin tail domain is bound to the head domain. Release of this interaction is required for vinculin activation and ligand binding in cell adhesion sites [10,11]. The tail domain consists of a bundle of five alpha-helices (H1–H5), which are connected by loops of variable length [12]. X-ray crystallography of metavinculin revealed that a metavinculin-specific alpha-helix H1′ formed by the carboxy-terminal part of the insert displaces

* Corresponding authors. Present address: Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany. Fax: +49 40 7410 53250 (M. Himmel). Address: Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany. Fax: +49 511 532 8073 (W.H. Ziegler).

E-mail addresses: m.himmel@uke.uni-hamburg.de (M. Himmel), ziegler.wolfgang@mh-hannover.de (W.H. Ziegler).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.11.013

alpha-helix H1 of the vinculin tail domain [13]. Monomeric metavinculin tail decorates F-actin similar to vinculin tail monomers, but does not induce actin-bundling as vinculin tail, which dimerizes upon binding to F-actin [14].

Despite recent progress with respect to the metavinculin structure, the functional role of the muscle-specific splice variant is still not sufficiently understood. Its association with pathological conditions of the heart muscle suggests an essential function in muscle development and homeostasis. In patients with dilative or hypertrophic cardiomyopathies, reduced expression of the protein as well as several function-related point mutations in the metavinculin insert were identified [15–19]. In this study, we address functional properties of the metavinculin insert and determine the expression and the localization of metavinculin protein in skeletal muscle, in relation to fiber-type composition.

2. Materials and methods

2.1. Protein expression and purification

Cloning and protein expression of vinculin and metavinculin constructs were performed as described previously [16,20]. Full-length vinculin (amino acid residues 1–1066) and metavinculin (residues 1–1134) as well as tail domain constructs of both proteins (start: residue 858) were used. The cDNA fragments were cloned into pEGFP-C2 vector to observe transient expression of fluorescently labeled proteins in eukaryotic cells [21]. Expression of His-tagged recombinant proteins in *E. coli* strain M15 was performed as described earlier [20]. Affinity purification was followed by FPLC chromatography using a MonoQ anion exchange column. The purity of proteins prepared was verified by SDS–PAGE and protein concentrations were determined using a BCA assay kit (Pierce/ThermoFisher Scientific, Rockford, USA). CD spectroscopy and thermal denaturation analyses are described in the supplement.

2.2. Fluorescence Recovery After Photobleaching (FRAP)

FRAP experiments in C2C12 myoblasts were performed as described in [21]. For FRAP analysis, only peripheral focal adhesions of cells expressing medium levels of EGFP-tagged vinculin/metavinculin constructs and sufficient signal-to-noise ratio were selected.

2.3. Image processing and statistical analysis

FRAP movies were analyzed using ImageJ software (National Institutes of Health, http://rsb.info.nih.gov/ij, version 1.42) as described in [21]. Movies showing clear focus drifts or focal adhesion site growth or dissolution were discarded. A bi-exponential regression function describing an exponential rise to a maximum was used for the calculation of half-life times of recovery [21]. For statistical analysis a one-way ANOVA was performed using Sigma Plot.

2.4. Immunohistochemistry

Bovine muscle samples were obtained from slaughterhouse waste of freshly slaughtered cattle and processed for cryosectioning and immunohistochemistry (see Supplementary material). Serial sections were then used for immunofluorescence, immunohistochemical and enzyme histochemical analysis. Sections were incubated with metavinculin antibody 6E3 or with the mouse monoclonal hVin-1 antibody (Sigma Aldrich, Taufkirchen, Germany), detecting both, metavinculin and vinculin [20]. Staining of myosin heavy chain (MHC) isoforms by indirect immunoperoxidase technique was performed as described previously [22]. The metabolic physiologic fiber typing into slow oxidative (SO), fast oxidative glycolytic (FOG) and fast glycolytic (FG) was done according to [23].

3. Results

The sequence alignment in Fig. 1A illustrates the variability between the C-terminal regions of metavinculin and vinculin in different species. While conservation is high in the shared exon sequences (exons 18/20) the size and sequence of the metavinculin-specific insertion (exon 19) significantly varies among species. Common sequence elements in the metavinculin insert comprise (i) a rather variable amino acid stretch containing alanines, acidic residues, and prolines, followed by (ii) a conserved sequence of clustered acidic residues (DxEDDYEPELLL), and (iii) a highly conserved amino acid sequence (ILAAAQSLHREA). The latter sequence shows a striking degree of similarity to the first helix (H1) of the vinculin tail five-helix bundle [12]. Structural analyses revealed that this highly conserved sequence forms the metavinculin-specific alpha-helix H1′, which replaces the vinculin alpha-helix H1 in the five-helix bundle of the metavinculin tail domain [13]. A point mutation (R975W) of the human metavinculin helix H1′ was found associated with dilated cardiomyopathy and resulted in abnormal cross-linking of actin filaments [16].

Applying a combination of biophysical and cell biological methods we analyzed functional consequences of the metavinculin-specific insertion on biochemical properties of the tail domain. Secondary structure analysis using CD spectroscopy confirmed for both splice variants a predominately alpha-helical fold, which was also not disturbed by the cardiomyopathy-associated missense mutation R975W. Thermal denaturation experiments (Fig. 1B and C) revealed transition from a folded to a literally unfolded structure for the vinculin tail domain at a significantly higher temperature (77.0 ± 0.4 °C) as compared to the wild-type metavinculin tail domain (74.9 ± 0.4 °C) indicating a lower resistance of the latter domain fold towards thermal denaturation (Fig. 1D). In contrast, residual CD values at the end points of denaturation experiments were considerably higher for the metavinculin tail domain (Fig. 1C) suggesting that the metavinculin-specific insert more efficiently suppressed complete loss of the alpha-helical structure and/or allowed formation of alternative secondary structure elements, which were not observed for the vinculin tail domain. Analyzing the cardiomyopathy-associated R975W mutant, we determined a significantly lower thermal melting temperature of 70.5 ± 0.2 °C compared to the wild-type metavinculin tail domain (Fig. 1D), suggesting that the exchange of arginine-975 for tryptophan results in structural destabilization, which may contribute to defective metavinculin function in cardiac muscle [16].

Previously, we reported the average residency of EGFP-vinculin in adhesion sites to be 16.7 ± 2.0 s in C2C12 myoblasts [24]. Now, in the same cell model, a much longer average residency was observed for both, EGFP-metavinculin (48.1 ± 2.0 s) and the EGFP-metavinculin R975W mutant (59.8 ± 2.7 s). Using bi-exponential regression analysis [21], we analyzed the mobility distribution of labeled proteins in cell adhesion complexes by determining the immobile protein fractions (IF) as well as fractions of fast (I) and slow (II) exchange (Fig. 2 and Supplementary Table S1). Prolonged residency times of metavinculin and EGFP-metavinculin R975W in adhesion sites were mainly due to a prominent immobile fraction for both metavinculin proteins (54% and 52%, respectively) as compared to vinculin (36%). Furthermore, in metavinculin, the slow exchanging fraction (81% of the remaining 46%) clearly dominated the fast fraction further delaying protein release from adhesion sites (Fig. 2D). For the EGFP-metavinculin R975W mutant the mobility distribution between slow and fast exchanging proteins shifted moderately towards the fast pool for the mutant (69% fast; 31% slow). In summary, vinculin and metavinculin clearly differ in

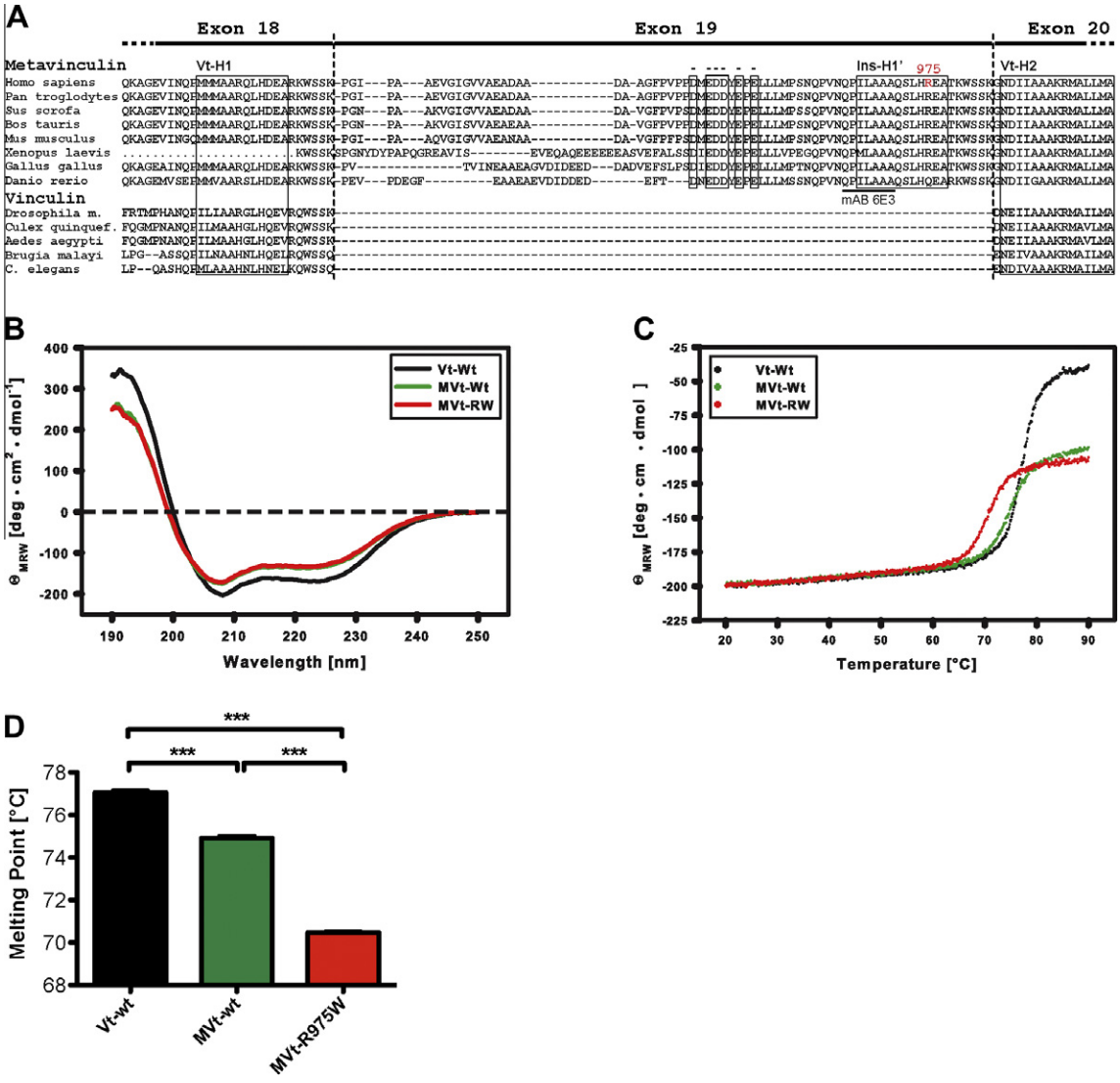


Fig. 1. Structure and thermal stability of vinculin and metavinculin tail. (A) Alignments of vinculin and metavinculin tail domain sequences flanking the vertebrate metavinculin insert. The sequence motif encompassing the conserved helix 1 of the vinculin tail domain is marked as Vt-H1. In the metavinculin insert, a similarly conserved sequence motif marks the position of helix H1′ (Ins-H1′). A point mutation (R975W, red) in helix H1′ is associated with cardiomyopathy in man. Epitope of the metavinculin-specific antibody 6E3 is indicated. (B) CD spectra of vinculin wild type (Vt-Wt) and metavinculin wild-type (MVt-Wt) and mutant (MVt-RW) tail domains confirming the alpha-helical structure of these domains. (C) Thermal denaturation experiments revealing reduced stability of MVt-Wt and MVt-RW constructs as compared to Vt-Wt and (D) Bar diagram showing calculated melting points of tail domain constructs indicated. Statistical significance of differences was confirmed (***$P < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

their turnover rates in cell-extracellular matrix adhesion complexes, which may indicate differential contribution to adhesion complex stability.

Since mechanical forces may differ according to skeletal muscle type we analyzed the distribution of metavinculin in cryosections of freshly isolated bovine muscle tissue using a metavinculin-specific monoclonal antibody [20]. Firstly, different muscle fiber types were classified based on a combination of enzyme activities and immunodetection of myosin heavy chain isoforms as described in [25]. In serial cross-sections of bovine skeletal muscle (*M. gastrocnemius*) we thereby identified slow oxidative (SO), fast oxidative glycolytic (FOG), and fast glycolytic (FG) muscle fibers. Based on expression of myosin 1, myosin 2a or myosin 2x heavy chain isoforms (MyHC) the following muscle fiber types were identified: SO-MyHC 1, FOG-MyHC 2a, FOG-MyHC 2a/2x, FG-MyHC 2a, FG-MyHC 2a/2x, and FG-MyHC 2x. In addition to fiber isotyping, costameres were visualized using a monoclonal antibody (h-Vin1) cross-reactive for vinculin and metavinculin [20].

While previous studies had noted only general differences in metavinculin expression levels, our approach allowed us a fiber type-selective staining of costameres for metavinculin: We observed that metavinculin expression was restricted to costameres of fast twitch skeletal muscle fibers, which express the fast myosin heavy chain isoforms MyHC 2a and/or 2x (Fig. 3A and B). In order to validate the selective expression pattern, we analyzed metavinculin distribution in 304 individual bovine skeletal muscle fibers of *M. gastrocnemius* (Fig. 3C). We confirmed a close correlation between the metabolic-physiological fiber type and costameric detection of metavinculin. All fast glycolytic (FG) muscle fibers that were analyzed and some of the fast oxidative glycolytic (FOG) muscle fibers stained positively for metavinculin. In contrast, slow oxidative (SO) muscle fibers were always found negative for metavinculin (Fig. 3B and C). Consistently, all MyHC 2x-positive fibers showed a fast glycolytic metabolism and contained metavinculin, again confirming the strong positive correlation between fast fibers and expression/localization of metavinculin.

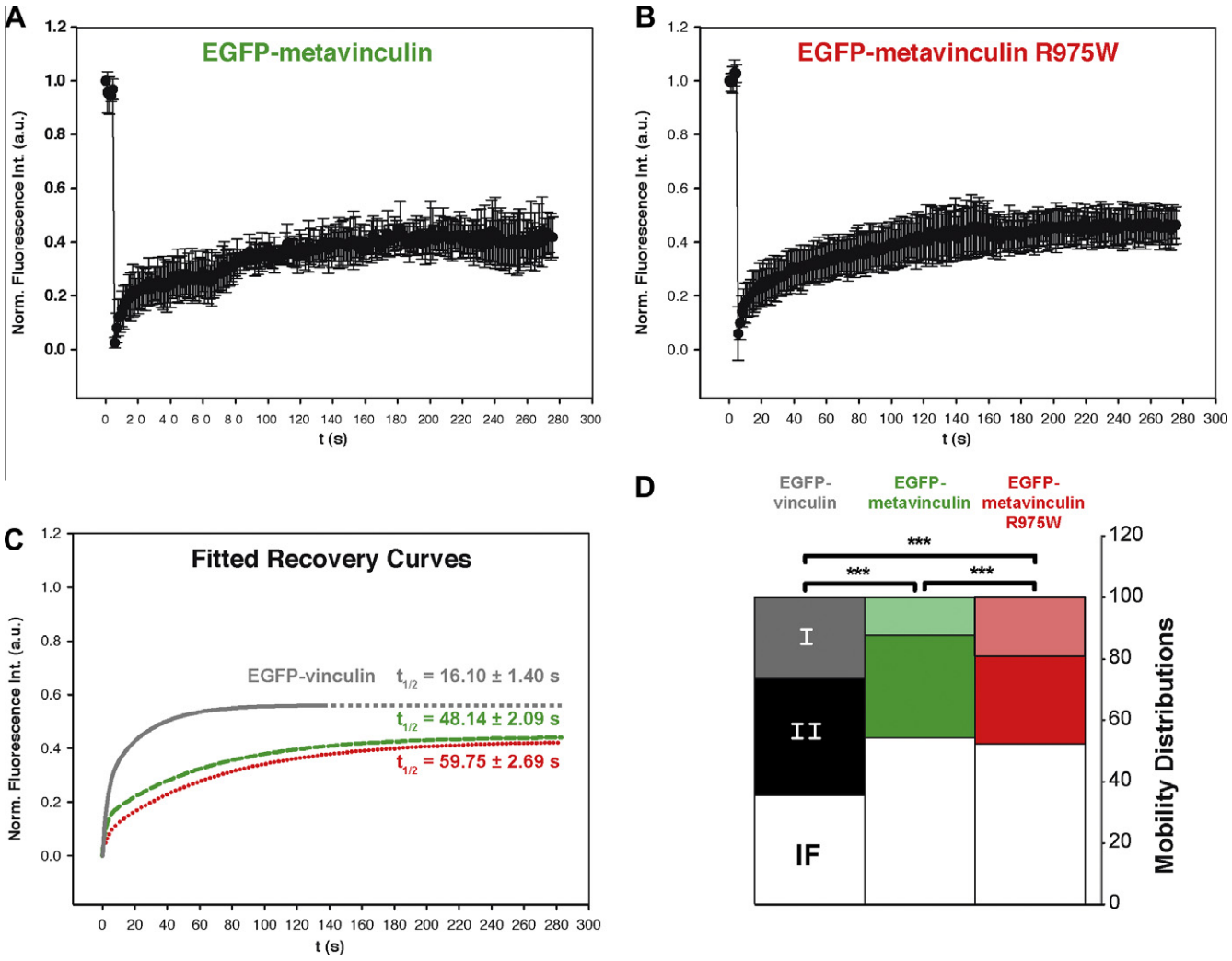


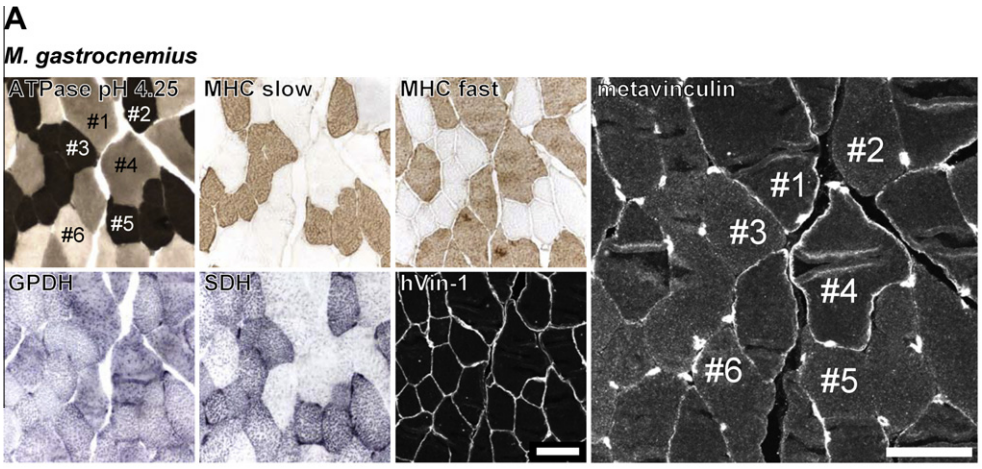
Fig. 2. Residency times of vinculin and metavinculin in focal adhesions. Residency times of EGFP-tagged metavinculin constructs in focal adhesion sites of C2C12 myoblasts were determined by FRAP. (A and B) Fluorescence recovery curves of metavinculin wild-type and the cardiomyopathy-associated metavinculin R975W mutant are shown ($n > 10$ adhesion sites). (C) Average residency times in focal adhesion sites as determined from fitted recovery curves for both metavinculin proteins reveal longer half-life times as compared to EGFP-vinculin (data previously obtained in the same cell model [24]). (D) Protein mobility distribution analysis shows an increased immobile fraction (IF) for both, metavinculin wild-type and R975W mutant, as compared to vinculin. The mobile fraction was further separated into fast (I) and slow (II) exchanging protein fractions with lowest mobility observed for metavinculin wild-type. Statistical significance of differences in the half-life times was confirmed (***$P < 0.01$).

Fiber type-selective metavinculin patterns were observed independent of the type of bovine skeletal muscles analyzed, whereas vinculin was evenly distributed in all skeletal muscle sections (Fig. 4). In *M. flexor digitorum brevis*, we observed mostly fast muscle fibers and a correspondingly high number of metavinculin-positive muscle fibers (Fig. 4A). For *M. diaphragmaticus* a skeletal muscle entirely lacking fast glycolytic muscle fibers, no costameric metavinculin was detected (Fig. 4B) which identifies this striated muscle as the first muscle with exclusive recruitment of vinculin into costameric adhesion complexes.

In summary, we demonstrate that metavinculin expression in skeletal muscle is much more selective than previously anticipated and clearly elevated in muscle fibers with higher mechanical shear forces. Increased residency times for metavinculin at microfilament-associated attachment sites further suggest metavinculin to increase costamere stability in these muscle fibers.

4. Discussion

The functional contribution of metavinculin to vinculin-containing cytoskeletal adhesion structures in different muscle types is still unknown, but the metavinculin-specific insertion has been shown to alter significantly biochemical properties of the tail domain *in vitro* [20]. While insert size and overall sequence vary among vertebrate species, sequence identity is very high in mammals (e.g. 94% in man and mouse, 93% in man and cattle). Interestingly, the C-terminal region of the insert containing the metavinculin helix H1′ is fully identical among all higher vertebrate species analyzed to date, pointing towards a unique role of this helix in the physiological function of the metavinculin tail. Structural analyses revealed a displacement of vinculin helix H1 by the metavinculin-specific helix H1′, the latter becoming an integral part of the five-helix bundle in the tail domain [13]. Our results show that this displacement leads to lower thermal stability in the metavinculin tail, which is further decreased in the cardiomyopathy-associated metavinculin mutant R975W. The tryptophan residue replacing arginine-975 of the highly conserved helix H1′ generates a large hydrophobic patch, which apparently results in lower affinity binding of helix H1′ to the remaining four-helical bundle formed by helices H2–H5, and leads to reduced stability of the mutant metavinculin tail [13] as observable in our results (Fig. 1D). Such altered stability may explain the association



B

Muscle Fiber No.	Myosin Type		GPDH	SDH	Metabolic Fiber Type	Vinculin/ Metavinculin staining (hVin-1)	Metavinculin staining (6E3)
	ATPase	Isoform					
1	IIX	fast	high	low	FG	positive	positive
2	I	slow	low	high	SO	positive	negative
3	I	slow	low	high	SO	positive	negative
4	IIX	fast	high	low	FG	positive	positive
5	I	slow	low	high	SO	positive	negative
6	IIA	fast	medium	medium	FOG	positive	negative

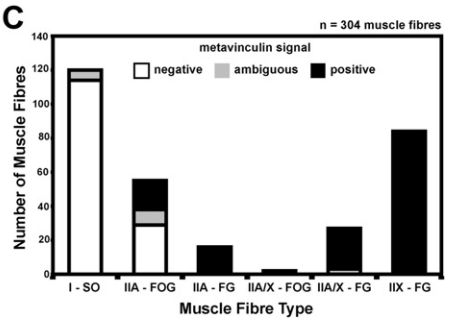


Fig. 3. Muscle fiber typing reveals selective recruitment of metavinculin into costameres. (A) Immunohistochemical analysis of bovine *M. gastrocnemius* (serial sections) allowing for exact muscle fiber typing with ATPase (at pH 4.25), MHC isoform (slow and fast), mitochondrial GPDH activity, SDH activity. The presence of costameres was shown by immunostaining with the cross-specific (meta-)vinculin antibody hVin-1 (Sigma). Immunofluorescence staining for costameric metavinculin using the metavinculin-specific monoclonal antibody 6E3 (right panel) revealed selective staining of muscle fibers #1 and #4 but not fibers #2, #3, #5 and #6. (B) Summary of muscle fiber typization and metavinculin detection fibers numbered in A. Fiber types I, IIA and IIX typed by acidic stability of the myosin ATPase correspond to the myosin heavy chain isoforms 1, 2a, and 2x. (C) Metavinculin distribution among different muscle fiber types in serial cryo-sections of bovine *M. gastrocnemius*. Costameric metavinculin staining was most prominent in MyHC IIX fast glycolytic muscle fibers (IIX-FG). MyHC I slow oxidative muscle fibers (I-SO) showed almost no metavinculin-specific signals at costameres. Scale bars = 50 μm.

of this mutant with dilated cardiomyopathy in man: In the force transmitting adhesion complexes like intercalated disks of the heart muscle, reduced stability of the mutant metavinculin tail could cause lower resistance to mechanical stress, which may finally facilitate disruption of the intercalated disks as detected in cardiomyopathy patients bearing the R975W point mutation [16].

In cell culture, analyses using vinculin mutants with altered/enhanced ligand binding provide evidence that residency times of vinculin influence the turnover of integrin receptors and other cytoskeletal adaptor proteins and lead to strongly enhanced size, distribution and lifetime of focal adhesions [24,26]. Our FRAP studies using vinculin and metavinculin constructs (Fig. 2) reveal that the dynamic properties of metavinculin significantly differ from those of vinculin. The enhanced residency times observed for metavinculin not only demonstrate a reduced turnover in muscular adhesion sites, but furthermore suggest an increased stabilization of microfilament anchorage in muscle-specific adhesion sites (including costameres and dense plaques). Surprisingly, the cardiomyopathy-associated EGFP-metavinculin R975W mutant showed a 1.2-fold prolonged average residency time as compared to wild-type metavinculin, even though the immobile fractions were similar and a slight shift towards fast exchange in the mobile fraction was noted for the mutant. These findings suggest that a regulated turnover at muscular adhesion sites is also essential for proper metavinculin function. At present, we cannot distinguish whether the decreased turnover observed for metavinculin R975W is due to impaired regulation of (ligand binding) activity in the mutant protein or directly reflects altered biochemical properties of tail domain, as observed in actin binding studies.

In contrast to previous assumptions, metavinculin expression levels are not homogeneous for a particular muscle but significantly differ according to muscle fiber type. We are the first to demonstrate that metavinculin is mainly found in all fast and glycolytic fibers (FG) of the type IIX and type IIA, as well as in some fast oxidative glycolytic fibers (FOG) of the type IIA but virtually absent from slow twitching and oxidative muscle fibers (SO). These findings are in good agreement with previous studies using quantitative immunoblotting, even though these studies did not allow correlation of metavinculin expression levels with fiber type composition: High protein levels of metavinculin were detected in rat fast twitch extensor digitorum longus (EDL) muscle indicating an association of metavinculin with fast EDL type II muscle fibers [27]. In contrast, no [27] or only low [20] metavinculin expression was observed in slow twitch *M. soleus*. Likewise the diaphragm muscle did not show any costameric metavinculin (this study) because of its unique composition based on oxidative muscle fibers.

Fast oxidative glycolytic fibers (FOG) have several subtypes depending on the proportion of glycolytic to oxidative activity. These subtypes can change and adapt to modified demands [23,25]. It is thus fair to assume that changes in metabolic demands are reflected by changes in metavinculin expression. Our hypothesis is corroborated by previous experiments demonstrating that *de novo* expression of metavinculin can be induced in *M. soleus* by hypokinesia resulting from hindlimb suspension in rats

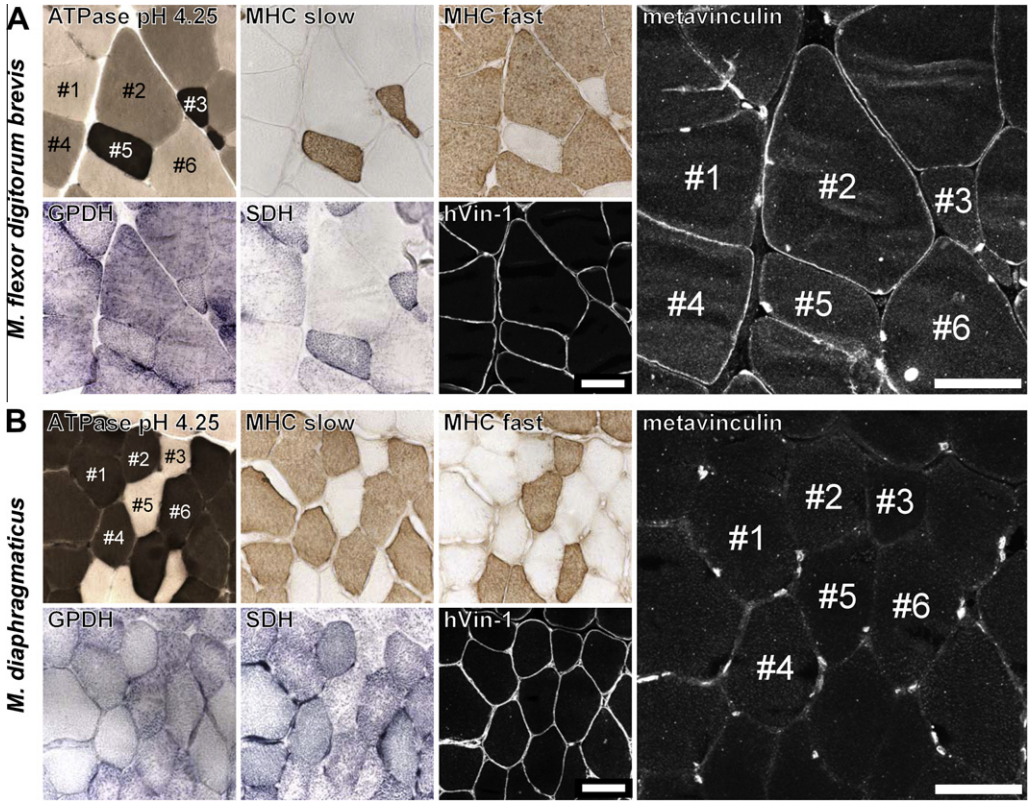


Fig. 4. Metavinculin distribution in different bovine skeletal muscles correlates with specific muscle fiber type. Serial cryo-sections of bovine muscle were analyzed and typed as described for Fig. 3. (A) Serial sections of bovine *M. flexor digitorum brevis* revealing predominantly fast fibers (#1, #2, #4 and #6; subtyping: #1 and #6 = type IIA FOG, fibers #2 and #4 = type IIX FG). Fibers #3 and #5 are slow fibers (type I, SO). Only the fast fibers #2, #4 and #6 are positive for metavinculin (right panel). (B) Serial sections of bovine *M. diaphragmaticus*, a muscle mainly composed of slow myosin 1 muscle fibers (#1, #2, #4 and #6, type I, SO) and some fast myosin 2a muscle fibers (#3 and #5, type IIA FOG) No costameric metavinculin was detected in this muscle whose fibers exert a certain fast oxidative glycolytic (FOG) metabolism only. Scale bars = 50 μm.

[27]. The fact that metavinculin (re-)expression was accompanied by expression of fast myosin heavy chain isoforms [28] further argues for a positive correlation between higher glycolytic activity and higher metavinculin expression.

Skeletal muscle costameres absorb and transduce shear forces between actomyosin fibrils and the cytoplasmic membrane [29,30]. These shear forces are thought to be higher in fast twitch muscle fibers due to (i) higher peak forces [31] and (ii) much faster contraction [32] in the order of I < IIA < IIX (<IIB). Considering that metavinculin is mainly expressed in fast, especially MHC IIX type muscle fiber costameres, we hypothesize that increased shear forces and mechanical stress in fast muscle fibers is accommodated by incorporation of metavinculin into costameres.

Acknowledgments

We are very grateful to Sabine Buchmeier (Technical University of Braunschweig, Germany) for the efficient cultivation of the mouse hybridoma cell clone 6E3. We thank Dr. Beck-Sickinger (Department of Biochemistry, University of Leipzig) for giving us access to the CD spectropolarimeter. This work was supported by IZKF Leipzig (MH, WHZ) and the Deutsche Forschungsgemeinschaft (FOR 471 to SI, FOR629 to KR, and Zi545/4-1 to WHZ).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.11.013.

References

[1] D. Pette, R.S. Staron, Cellular and molecular diversities of mammalian skeletal muscle fibers, Rev. Physiol. Biochem. Pharmacol. 116 (1990) 1–76.
[2] K.A. Clark, A.S. McElhinny, M.C. Beckerle, C.C. Gregorio, Striated muscle cytoarchitecture: an intricate web of form and function, Annu. Rev. Cell Dev. Biol. 18 (2002) 637–706.
[3] J.M. Ervasti, Costameres: the Achilles' heel of Herculean muscle, J. Biol. Chem. 278 (2003) 13591–13594.
[4] W.H. Ziegler, R.C. Liddington, D.R. Critchley, The structure and regulation of vinculin, Trends Cell Biol. 16 (2006) 453–460.
[5] J.R. Feramisco, J.E. Smart, K. Burridge, D.M. Helfman, G.P. Thomas, Co-existence of vinculin and a vinculin-like protein of higher molecular weight in smooth muscle, J. Biol. Chem. 257 (1982) 11024–11031.
[6] J.D. Siliciano, S.W. Craig, Meta-vinculin–a vinculin-related protein with solubility properties of a membrane protein, Nature 300 (1982) 533–535.
[7] B.J. Byrne, Y.J. Kaczorowski, M.D. Coutu, S.W. Craig, Chicken vinculin and meta-vinculin are derived from a single gene by alternative splicing of a 207-base pair exon unique to meta-vinculin, J. Biol. Chem. 267 (1992) 12845–12850.
[8] V.E. Koteliansky, E.P. Ogryzko, N.I. Zhidkova, P.A. Weller, D.R. Critchley, K. Vancompernolle, J. Vandekerckhove, P. Strasser, M. Way, M. Gimona, et al., An additional exon in the human vinculin gene specifically encodes meta-vinculin-specific difference peptide. Cross-species comparison reveals variable and conserved motifs in the meta-vinculin insert, Eur. J. Biochem. 205 (1992) 1218.
[9] S. Saga, M. Hamaguchi, M. Hoshino, K. Kojima, Expression of meta-vinculin associated with differentiation of chicken embryonal muscle cells, Exp. Cell Res. 156 (1985) 45–56.
[10] C. Bakolitsa, D.M. Cohen, L.A. Bankston, A.A. Bobkov, G.W. Cadwell, L. Jennings, D.R. Critchley, S.W. Craig, R.C. Liddington, Structural basis for vinculin activation at sites of cell adhesion, Nature 430 (2004) 583–586.
[11] T. Izard, G. Evans, R.A. Borgon, C.L. Rush, G. Bricogne, P.R. Bois, Vinculin activation by talin through helical bundle conversion, Nature 427 (2004) 171–175.
[12] C. Bakolitsa, J.M. de Pereda, C.R. Bagshaw, D.R. Critchley, R.C. Liddington, Crystal structure of the vinculin tail suggests a pathway for activation, Cell 99 (1999) 603–613.

[13] E.S. Rangarajan, J.H. Lee, S.D. Yogesha, T. Izard, A helix replacement mechanism directs metavinculin functions, PLoS One 5 (2010) e10679.
[14] M.E. Janssen, E. Kim, H. Liu, L.M. Fujimoto, A. Bobkov, N. Volkmann, D. Hanein, Three-dimensional structure of vinculin bound to actin filaments, Mol. Cell 21 (2006) 271–281.
[15] M. Maeda, E. Holder, B. Lowes, S. Valent, R.D. Bies, Dilated cardiomyopathy associated with deficiency of the cytoskeletal protein metavinculin, Circulation 95 (1997) 17–20.
[16] T.M. Olson, S. Illenberger, N.Y. Kishimoto, S. Huttelmaier, M.T. Keating, B.M. Jockusch, Metavinculin mutations alter actin interaction in dilated cardiomyopathy, Circulation 105 (2002) 431–437.
[17] V.C. Vasile, W.D. Edwards, S.R. Ommen, M.J. Ackerman, Obstructive hypertrophic cardiomyopathy is associated with reduced expression of vinculin in the intercalated disc, Biochem. Biophys. Res. Commun. 349 (2006) 709–715.
[18] V.C. Vasile, S.R. Ommen, W.D. Edwards, M.J. Ackerman, A missense mutation in a ubiquitously expressed protein, vinculin, confers susceptibility to hypertrophic cardiomyopathy, Biochem. Biophys. Res. Commun. 345 (2006) 998–1003.
[19] V.C. Vasile, M.L. Will, S.R. Ommen, W.D. Edwards, T.M. Olson, M.J. Ackerman, Identification of a metavinculin missense mutation, R975W, associated with both hypertrophic and dilated cardiomyopathy, Mol. Genet. Metab. 87 (2006) 169–174.
[20] S. Witt, A. Zieseniss, U. Fock, B.M. Jockusch, S. Illenberger, Comparative biochemical analysis suggests that vinculin and metavinculin cooperate in muscular adhesion sites, J. Biol. Chem. 279 (2004) 31533–31543.
[21] M. Himmel, A. Ritter, S. Rothemund, B.V. Pauling, K. Rottner, A.R. Gingras, W.H. Ziegler, Control of high affinity interactions in the talin C terminus: how talin domains coordinate protein dynamics in cell adhesions, J. Biol. Chem. 284 (2009) 13832–13842.
[22] C. Stockmar, H. Lill, A. Trapp, C. Josten, K. Punkt, Fibre type related changes in the metabolic profile and fibre diameter of human vastus medialis muscle after anterior cruciate ligament rupture, Acta Histochem. 108 (2006) 335–342.
[23] K. Punkt, Fibre types in skeletal muscles, Adv. Anat. Embryol. Cell Biol. 162 (2002) 1–109.
[24] S. Marg, U. Winkler, M. Sestu, M. Himmel, M. Schonherr, J. Bar, A. Mann, M. Moser, C.T. Mierke, K. Rottner, M. Blessing, J. Hirrlinger, W.H. Ziegler, The vinculin-DeltaIn20/21 mouse: characteristics of a constitutive, actin-binding deficient splice variant of vinculin, PLoS One 5 (2010) e11530.
[25] K. Punkt, M. Fritzsche, C. Stockmar, P. Hepp, C. Josten, M. Wellner, S. Schering, I.B. Buchwalow, Nitric oxide synthase in human skeletal muscles related to defined fibre types, Histochem. cell biol. 125 (2006) 567–573.
[26] J.D. Humphries, P. Wang, C. Streuli, B. Geiger, M.J. Humphries, C. Ballestrem, Vinculin controls focal adhesion formation by direct interactions with talin and actin, J. Cell Biol. 179 (2007) 1043–1057.
[27] A. Chopard, F. Pons, J.F. Marini, Vinculin and meta-vinculin in fast and slow rat skeletal muscle before and after hindlimb suspension, Pflugers Arch. 444 (2002) 627–633.
[28] A. Chopard, F. Pons, J.F. Marini, Cytoskeletal protein contents before and after hindlimb suspension in a fast and slow rat skeletal muscle, Am. J. Physiol. Regul. Integr. Comp. Physiol. 280 (2001) R323–330.
[29] B.A. Danowski, K. Imanaka-Yoshida, J.M. Sanger, J.W. Sanger, Costameres are sites of force transmission to the substratum in adult rat cardiomyocytes, J. Cell Biol. 118 (1992) 1411–1420.
[30] B. Russell, M.W. Curtis, Y.E. Koshman, A.M. Samarel, Mechanical stress-induced sarcomere assembly for cardiac muscle growth in length and width, J. Mol. Cell. Cardiol. 48 (2010) 817–823.
[31] M. Li, L. Larsson, Force-generating capacity of human myosin isoforms extracted from single muscle fibre segments, J. Physiol. 588 (2010) 5105–5114.
[32] K. Hilber, S. Galler, B. Gohlsch, D. Pette, Kinetic properties of myosin heavy chain isoforms in single fibers from human skeletal muscle, FEBS Lett. 455 (1999) 267–270.