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# ANKRD1 modulates inflammatory responses in C2C12 myoblasts through feedback inhibition of NF-κB signaling activity

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ABSTRACT

Transcription factors of the nuclear factor-kappa B (NF-κB) family play a pivotal role in inflammation, immunity and cell survival responses. Recent studies revealed that NF-κB also regulates the processes of muscle atrophy. NF-κB activity is regulated by various factors, including ankyrin repeat domain 2 (AnkrD2), which belongs to the muscle ankyrin repeat protein family. Another member of this family, AnkrD1 is also a transcriptional effector. The expression levels of AnkrD1 are highly upregulated in denervated skeletal muscle, suggesting an involvement of AnkrD1 in NF-κB mediated cellular responses to paralysis. However, the molecular mechanism underlying the interactive role of AnkrD1 in NF-κB mediated cellular responses is not well understood. In the current study, we examined the effect of AnkrD1 on NF-κB activity and determined the interactions between AnkrD1 expression and NF-κB signaling induced by TNFα in differentiating C2C12 myoblasts. TNFα upregulated AnkrD1 mRNA and protein levels. AnkrD1-siRNA significantly increased TNFα-induced transcriptional activation of NF-κB, whereas overexpression of AnkrD1 inhibited TNFα-induced NF-κB activity. Co-immunoprecipitation studies demonstrated that AnkrD1 was able to bind p50 subunit of NF-κB and vice versa. Finally, CHIP assays revealed that AnkrD1 bound chromatin at a NF-κB binding site in the AnrkD2 promoter and required NF-κB to do so. These results provide evidence of signaling integration between AnkrD1 and NF-κB pathways, and suggest a novel anti-inflammatory role of AnkrD1 through feedback inhibition of NF-κB transcriptional activity by which AnkrD1 modulates the balance between physiological and pathological inflammatory responses in skeletal muscle.

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## 1. Introduction

Nuclear factor-kappa B (NF-κB), composed of homo- and heterodimers, is a transcription factor that regulates the expression of more than 150 genes related to inflammation and cell survival responses [1,2]. Recent studies revealed that NF-κB also plays important roles in regulating muscle atrophy or wasting found in aging, disuse, denervation, muscular dystrophy, and cachexia due to illness, such as cancer [3]. NF-κB consists of five members, including p105/p50 (NF-κB1) and p100/p52 (NF-κB2), and is present in cells in an inactive state [4,5]. NF-κB belongs to the category of rapid-acting primary transcription factors [5], which allows it to be a first responder to harmful cellular stimuli, such as stress, free radicals, ultraviolet irritation, and inflammation [6]. Upon activation, the p50 and p52 products are proteolytically processed by proteasomes from p105 and p100, respectively, and complexes with RelA (p65), which are able to bind DNA and regulate transcription [7].

The muscle ankyrin repeat protein (MARP) family, include AnkrD1, AnkrD2, and diabetes-associated ankyrin repeat protein (DARP), has been identified as a family of titin filament-based stress response molecules [8], and shown to be involved in muscle stress response pathways, such as that occurring after acute resistance exercise or spinal muscle atrophy, or in those with Duchenne muscular dystrophy [9–12]. AnkrD1 was first identified in endothelial cells [13], but subsequently identified in skeletal muscle, heart, ovarian, cancers [14], renal podocytes [15], healing wounds [16], and mouse mammary epithelial cells [17]. AnkrD1 is localized

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in the nucleus in myoblasts, and is redistributed with myogenic differentiation to the cytoplasm [18] where, in skeletal and cardiac muscle, it and other MARPs bind preferentially to titin at the I band of the sarcomere [19]. AnkrD1 is preferentially expressed in type 1 skeletal muscle fibers and is markedly induced by denervation [20]. AnkrD2 expression has also been reported to be elevated in denervated skeletal muscle, though less so, and is also upregulated in stretched skeletal muscle [21]. AnkrD2 protein has been shown to migrate from myofibrils to the nucleus of myofibers after muscle injury by injection of cardiotoxin [22]. Both AnkrD1 and AnkrD2 are proposed to be able to transmit signals from the sarcomere to the nucleus, particularly, in response to muscle stress [22]. Moreover, AnkrD1 down-regulates gene expression in the heart [23] and binds to and regulates the transcriptional activity of p53 [24]. Silencing of AnkrD2 alters the expression of genes for multiple pathways that included TGFß, Wnt signaling and p53 [24,25]. AnkrD2 has also been shown to bind proteins with PDZ and SH3 domains, and to multiple transcription factors including YB-1, p53, PAX6, LHX2, NHIL3 and MECP2 [25,26].

Recent reports revealed that NF-κB activity is regulated by several additional checkpoints, such as MARPs [9,27]. For example, AnkrD2 protein is able to modulate NF-κB transcriptional activity through direct interaction with the p50 subunit in human airway smooth muscle cells [27]. Since the p50 subunit of NF-κB lacks a transactivation domain, its transcriptional activity is dependent on dimerization with other members of the NF-κB family, or on other co-regulators [28]. We have previously reported that AnkrD1, but not AnkrD2, serves as a transcriptional repressor for the androgen receptor (AR), and the expression of AnkrD1 and AnkrD2 is differentially regulated by androgens [29], suggesting that AnkrD1 and AnkrD2 may play different roles as transcriptional co-regulators. To determine whether AnkrD1 influence p50 subunit transcriptional activity, we examined the interactions between AnkrD1 and NF-κB signaling induced by TNFα in differentiating C2C12 myoblasts.

## 2. Materials and methods

### 2.1. Cell line and cell culture

C2C12 cells were maintained in DMEM containing 10% FBS supplemented with 1% penicillin/streptomycin at 37 °C. All experiments were performed with C2C12 cells that had been incubated for 48 h in DMEM containing 2% horse serum (HS) to initiate differentiation.

### 2.2. Preparation of cell lysates, immunoprecipitation (IP) and Western blotting (WB)

Cells were rinsed twice with ice-cold PBS and scraped with 1.5 ml of PBS containing 4 mM iodoacetate. After centrifugation, the pellets were resuspended in CHAPS extraction solution (10 mM CHAPS, 2 mM EDTA, pH 8.0, and 4 mM iodoacetate in PBS) with protease inhibitors. The samples were incubated for 30 min on ice and centrifuged at 15,000× g for 10 min. The supernatants were collected and stored at −70 °C. Proteins from the cytosolic and nuclear fractions were isolated using a commercial kit from Pierce (Rockford, IL), according to the manufacturer's instructions. IP was performed using a kit from Thermo-Sci. Inc. (Rockford, IL). For immunoblotting, cell lysates were electrophoresed on SDS-polyacrylamide gels, electrophoretically transferred to a PVDF membrane, and incubated with targeting primary antibodies overnight at 4 °C. β-tubulin and histone antibodies were used as loading controls. Antibody against AnkrD1, AnkrD2, histone were purchased from Santa Cruz (Santa Cruz, CA); Anti-NF-κB-p50 and β-tubulin antibodies were obtained from Cell Signaling (Danvers, MA).

### 2.3. Transient transfection and luciferase reporter assay

Transient transfection was done using Lipofectamine 2000 reagent according to the manufacturer's instructions (Life Technologies, Grand Island, NY). The AnkrD1 expression plasmid and control vector were purchased from Origene (Rockville, MD). The NF-κB luciferase reporter vector (QIAGEN, Germantown, MD) was premixed with a plasmid constitutively expressing Renilla luciferase which served as an internal control for normalizing transfection efficiencies. Cells were cultured in 12-well cluster plates and co-transfected with either 1 μg of the reporter plasmid or control vector (as mock controls) and 1 μg of AnkrD1 expression plasmid or control vector for 24 h. The transfected cells were then lysed by scraping into reporter buffer (Promega, Madison, WI). Luciferase activity was assayed and quantitated using a luminometer. The results were normalized to Renilla activity.

### 2.4. Quantitative real-time (Rt) PCR

*q*Rt-PCR was performed as described previously [29] using a thermocycler (model 7500; Applied Biosystems). For each sample, the determinations were performed in triplicate, and the means for the crossing points of triplicates were used in subsequent calculations. Relative mRNA levels were expressed as fold-change using the $2^{-\Delta\Delta Ct}$ method. Data were normalized relative to 18s RNA.

### 2.5. Small interfering RNA (siRNA) transfection

siRNA against AnkrD1 and non-silencing random siRNA (negative control) was purchased from Applied Biosystems. Cells were cultured in 6-well plates and transfected with either non-silencing random siRNA or 20 nM AnkrD1-siRNA in 100 μl of PepMute-Plus siRNA transfection reagent (SignaGen Lab., Ijamsville, MD), following the manufacturer's recommended procedures. Cells were then treated with either vehicle or TNFα (5 ng/ml, Millipore, Temecula, CA) for 24 h under differentiating conditions.

### 2.6. Chromatin immunoprecipitation (CHIP) assay

CHIP assays were conducted as previously described [30] using a kit purchased from USB-Affymetrix Co. (Santa Clara, CA) Briefly, cells were washed once with PBS and then cross-linked with 1.5% formaldehyde at 37 °C for 10 min. After washing twice with ice-cold PBS, the cells were collected in lysis buffer and incubated for 30 min on ice. Cell lysates were sonicated using a Sonicator 3000 and then diluted 5-fold with dilution buffer. Diluted cell lysates were precleared with salmon sperm DNA/protein A-agarose for 2 h at 4 °C. Monoclonal anti-AnkrD1 antibody (5 μg) or normal mouse IgG (control) was used to immunoprecipitate protein-DNA complexes from precleared supernatants containing 500 μg of protein. Immunoprecipitated DNA was amplified by PCR (35 cycles) using Taq polymerase (New England Biolabs, Ipswich, MA), resolved by 1% agarose gel electrophoresis, and visualized with ethidium bromide staining. The following primer set was used: forward, 5′-GTT-TCT-GCA-AGC-CAC-AGG-GC-3′; and reverse, 5′-AAC-AGA-TGG-ACA-GGT-TCT-GT-3′. The sequence of the DNA pulled down was verified by DNA sequencing (DNA Sequencing Facility, Albert Einstein College of Medicine, Bronx, NY).

### 2.7. Statistics

Data are expressed as means ± SEM. The significance of differences between pairs of means was determined with an unpaired two-tailed Student's *t*-test. Statistical calculations were performed with Prism 6.0 (GraphPad).

## 3. Results

### 3.1. TNFα upregulated AnkrD1 expression in C2C12 myoblasts

To explore the possible links between signaling through NF-κB and expression and function of AnkrD1, we initially investigated the effect of TNFα on AnkrD1 mRNA and protein expression in differentiating C2C12 cells. As shown in Fig. 1A, qRt-PCR revealed that treatment of cells with TNFα induced a significant increase in AnkrD1 mRNA within 24 h. The effect of TNFα on AnkrD1 protein expression was also evaluated. Western blot analysis revealed that TNFα induced a moderate increase in AnkrD1 protein levels in total cell lysates. In contrast, AnkrD1 protein levels were greatly increased in the nuclear fraction in response to TNFα (Fig. 1B and C). These results confirmed an upregulation of AnkrD1, both at mRNA and protein levels, in response to inflammatory stimulation.

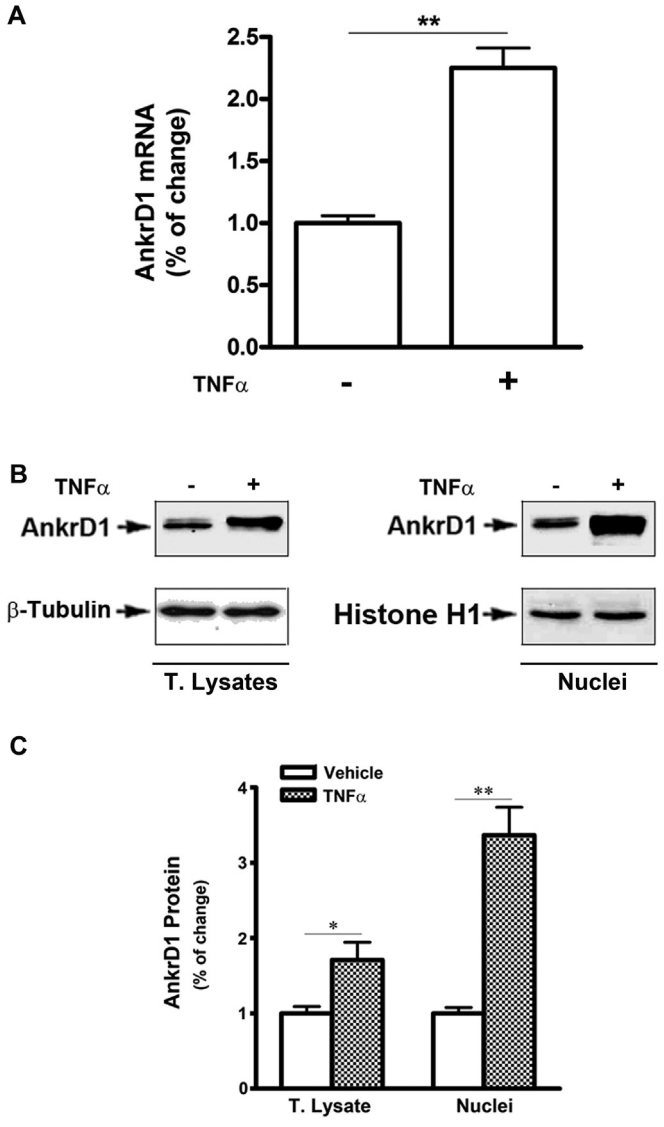


**Fig. 1.** *TNFα upregulates AnkrD1 expression.* (A) C2C12 cells were cultured in DMEM containing 2% HS and treated with either vehicle or TNFα (5 ng/μl) for 24 h. Total RNA was isolated and subjected to qRT-PCR analysis. (B) Total cell lysates and nuclear protein were isolated and subjected to Western blotting. (C) Blots in *B* were quantified by scanning densitometry and normalized relative to either β-tubulin (total cell lysates) or histone (nuclear protein). Data shown in *B* are representative Western blot analysis; Data shown in *C* are mean values ±SEM for 3 separate determinations; $^{*}p < 0.05$, and $^{**}p < 0.01$.

### 3.2. AnkrD1 protein formed complexes with p50 subunit of NF-κB in C2C12 cells

We next determined whether AnkrD1 protein bound to p50 subunit of NF-κB in cytosolic and nuclear compartments of the cells in response to TNFα stimulation using co-immunoprecipitation. As shown in Fig. 2, in pull-downs of the protein complexes formed by p50 with AnkrD1 using antibody against p50, Western blotting confirmed the presence in immunoprecipitated protein of AnkrD1 (upper panel). Similarly, when pull-down conducted with anti-AnkrD1 antibody, presence of p50 protein was evidenced (lower panel). Thus, AnkrD1 was associated with p50, and the binding was enhanced in presence of TNFα.

### 3.3. AnkrD1 modulated NF-κB luciferase activity in C2C12 cells

We further test whether the complexes formed by AnkrD1 and p50 subunit were functional, we examined how manipulating AnkrD1 levels influenced TNFα-induced activity of an NF-κB reporter gene in C2C12 myoblasts. We co-transfected C2C12 cells with an AnkrD1 expression vector to overexpress AnkrD1 gene and together with an NF-κB luciferase reporter vector. Overexpression of AnkrD1 did not impact luciferase activity, but significantly blunted TNFα-induced increases of reporter activity (Fig. 3A). To extend this observation, AnkrD1-siRNA was employed to inhibit the expression of AnkrD1 followed by NF-κB luciferase activity assays. As shown in Fig. 3B, AnkrD1 gene knockdown with siRNA significantly increased NF-κB transcriptional activity induced by TNFα. These findings suggested a novel effect of AnkrD1 on modulating the NF-κB activity in response to inflammatory stimulation.

### 3.4. TNFα promoted DNA binding of AnkrD1 to a NF-κB binding site

Finally, we performed CHIP assays to determine whether exposure of C2C12 cells to TNFα induced recruitment of AnkrD1 to an NF-κB binding site previously reported in the AnkrD2 promoter [27]. PCR amplification of DNA fragments immunoprecipitated with AnkrD1 antibody gave a single band of 500 bp as expected [27].

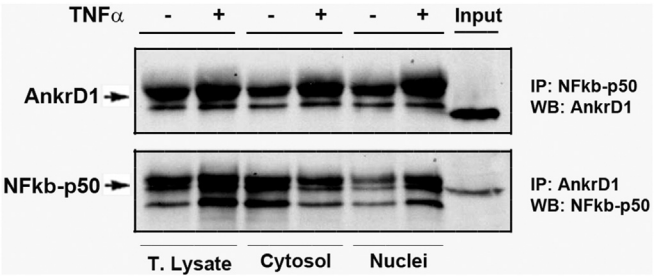


**Fig. 2.** *AnkrD1 protein forms complexes with p50 subunit of NF-κB.* C2C12 cells were cultured in DMEM containing 2% HS and treated with either vehicle or TNFα (5 ng/ml) for 24 h. Total protein lysates as well as cytosolic and nuclear proteins were isolated and subjected to immunoprecipitation with antibodies against either p50 (upper panel) or AnkrD1 (Low panel), followed by Western blotting, as indicated. Images are representative of Western blot analysis for two separate experiments.

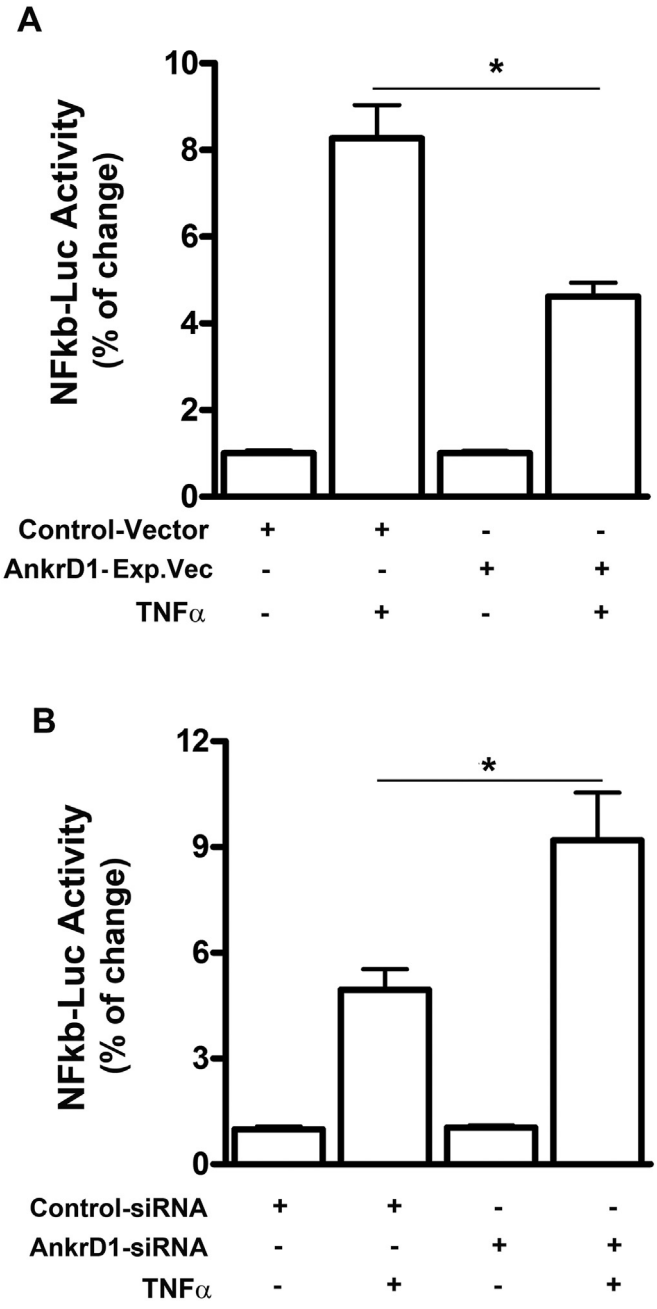


**Fig. 3.** *AnkrD1 modulates NF-κB transcriptional activity.* (A) Cells were cultured in 12-well cluster plates and co-transfected with AnkrD1 expression plasmid (1 μg) or control vector together with an NF-κB reporter plasmid or control vector (mock controls) in the presence or absence of 5 ng/ml TNFα for 24 h. (B) Cells were co-transfected with either AnkrD1 siRNA (20 nM) or non-silencing random siRNA (negative control) together with the NF-κB reporter plasmid or control vector, and treated with TNFα for 24 h. The results were normalized to Renilla activity. $^{*}p < 0.05$, and $^{**}p < 0.01$.

Cells treated with TNFα resulted in an increase in density of the band suggesting enhanced binding of AnkrD1 to the region of chromatin in response to TNFα (Fig. 4A). To confirm a functional interaction of AnkrD1 with NF-κB bound to the AnkrD2 promoter, we treated cells with siRNA against p50 subunit of NF-κB prior to CHIP assay. As shown in Fig. 4B, knockdown of p50 gene led to a decreased intensity of the band suggesting that binding of AnkrD1 to chromatin involved the formation of AnkrD1/p50 complexes. These data support the notion that AnkrD1 forms complexes with DNA-bound NF-κB.

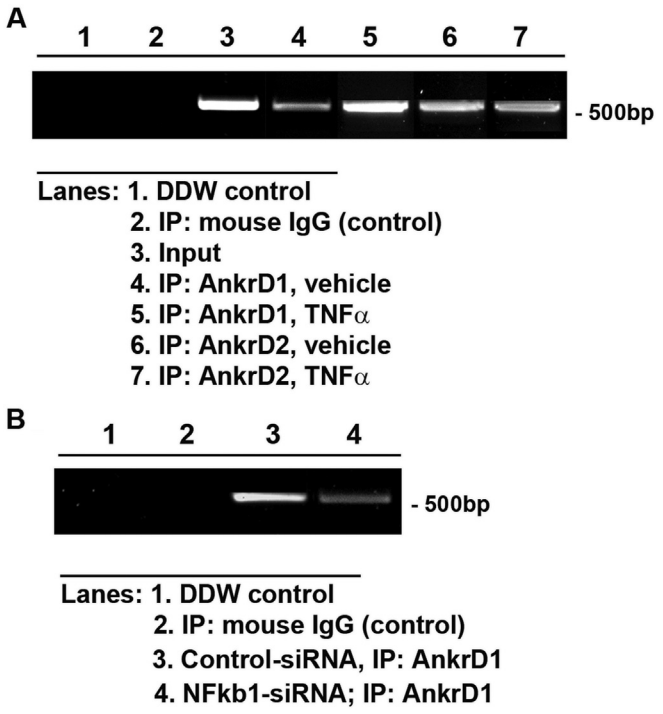


**Fig. 4.** *TNFα promotes DNA binding of AnkrD1-NF-κB complexes.* (A) Cells were treated with or without TNFα, as indicated, for 24 h. (B) Cells were transfected with either NF-κB siRNA (20 nM) or non-silencing random siRNA (negative control). CHIP assays were then performed with anti-AnkrD1 antibody and PCR was used to detect AnkrD2 promoter fragments. Data shown in *A* & *B* are representative photographs from 3 independent assays.

## 4. Discussion

The above findings indicate that TNFα stimulates the upregulation of AnkrD1 expression, that AnkrD1 binds the p50 subunit of NF-κB and that AnkrD1 is recruited to NF-κB bound to chromatin at a proven NF-κB binding site in the AnkrD2 promoter. Furthermore, these data reveal AnkrD1 as a transcriptional repressor of signaling induced by TNFα through NF-κB. When taken together, the findings support the existence of a negative feedback loop in skeletal muscle wherein activation of NF-κB, such as might occur due to upregulation of TNFα, increased levels of reactive oxygen species, or cellular stress, elicits expression of AnkrD1 and thereby represses continued NF-κB actions in gene expression. It is attractive to propose the possibility that one function of AnkrD1 in paralyzed muscle is to act as a break on continued deterioration of skeletal muscle mass and function by inhibiting ongoing NF-κB signaling. This interpretation of the findings is supported by observations that TNFα expression is upregulated after nerve transection [20,31], and that signaling by TWEAK, another TNF family member [32] which signals through NF-κB, is also activated during denervation atrophy.

Signaling through NF-κB is upregulated in skeletal muscle during a variety of conditions that predispose to muscle atrophy including paralysis, sarcopenia related to aging, and cancer cachexia [33]. AnkrD1 expression has been shown to be increased after nerve transection, muscle stretch, resistance exercise, or Duchenne's muscular dystrophy; the full spectrum of conditions which upregulate AnkrD1 expression is unknown. Data from several reports reveals that upregulation of AnkrD1 and activation of NF-κB occurs concurrently in diseased or paralyzed muscle. Therefore it is likely that interactions between NF-κB signaling and AnkrD1 observed in C2C12 myoblasts in the above studies is also participating in NF-κB signaling in skeletal muscle *in-vivo*, although

further studies are needed to confirm this prediction. Further elucidation of the roles of AnkrD1 and AnkrD2 in regulating muscle gene expression programs in response to exercise, injury or disease will be an interesting area to additional investigation.

How AnkrD1 contributes to muscle homeostasis, whether in health, disease states or after immobilization or paralysis, is unknown and is an area that requires further study. Mice with germline deletions of AnkrD1 and DARP develop normally but their muscles demonstrate altered elastic properties and impaired responses to bouts of eccentric resistance exercise [34]. One might presume that AnkrD1 aids in muscle responses to stretch, paralysis, overloading or disease by appropriately modulating gene expression programs triggered by pro-inflammatory cytokines, reactive oxygen species, or tissue responses to damaged muscle fibers. The above findings suggest that AnkrD1 might direct NF-κB signaling in a way that facilitates optimal tissue adaptations or repair. Interesting and unanswered questions include which effects of AnkrD1 on muscle gene expression and adaptation to stress and disease are distinct from those of AnkrD2, which is expressed at much higher levels in normal, healthy muscle.

AnkrD1 and other MARPs act as transcriptional regulators that are released from binding sites on Titin during periods of stress after which they migrate to the nucleus to regulate gene expression. How AnkrD1, or other MARPs participate in regulating such gene expression programs has not been elucidated. Our findings are, to our knowledge, the first to relate AnkrD1 function in skeletal muscle to a specific transcriptional circuit and function. Further studies are required to better define the functional consequences of repression of NF-κB signaling in skeletal muscle.

## Disclosure summary

The authors have nothing to disclose.

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## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.118.

## References

[1] P.A. Baeuerle, T. Henkle, Function and activation of NF-κB in the immune system, Annu. Rev. Immunol. 12 (1994) 141–179.
[2] P.A. Baeuerle, D. Baltimore, NF-κB: ten years after, Cell 87 (1996) 13–20.
[3] C.L. Wu, S.C. Kandarian, R.W. Jackman, Identification of genes that elicit disuse muscle atrophy via the transcription factor p50 and Bcl-3, PLoS ONE 6 (2011) 1–9.
[4] T.D. Gilmore, The Rel/NF-κB signal transduction pathway: introduction, Oncogene 18 (1999) 6842–6844.
[5] S. Haskill, A.A. Beg, S.M. Tompkins, J.S. Morris, A.D. Yurochko, A. Sampson-Johannes, K. Mondal, P. Palph, A.S. Baldwin, Characterization of an immediate-early gene induced in adherent monocytes that encodes I kappa B-like activity, Cell 65 (1991) 1281–1289.
[6] N.S. Chandel, W.C. Trzyna, D.S. McClintock, P.T. Schumacker, Role of oxidants in NF-kappa B activation and TNF-alpha gene transcription induced by hypoxia and endotoxin, J. Immunol. 165 (2000) 1013–1021.
[7] A.R. Brasier, The NF-κB regulatory network, Cardiovasc. Toxicol. 6 (2006) 111–130.
[8] M.K. Miller, M.L. Bang, C.C. Witt, D. Labeit, C. Trombitas, K. Watanabe, H. Granzier, A.S. McElhinny, C.C. Gregorio, S. Labeit, The muscle ankyrin repeat proteins: CARP, ankyrin2/Arpp and DARP as a family of titin filament-based stress response molecules, J. Mol. Biol. 333 (2003) 951–964.
[9] J.S. Mohamed, A.M. Boriek, Loss of desmin triggers mechanosensitivity andup-regulation of Ankrd1 expression through Akt-NF-κB signaling pathway in smooth muscle cells, FASEB J. 26 (2011) 1–9.
[10] C.T.Y. Nakada, A. Oka, I. Nonaka, K. Sato, S. Mori, H. Ito, M. Moriyama, Cardiac ankyrin repeat protein is preferentially induced in atrophic myofibers of congenital myopathy and spinal muscular atrophy, Pathol. Int. 53 (2003) 653–658.
[11] C. Nakada, Y. Tsukamoto, A. Oka, I. Nonaka, K. Sato, S. Mori, H. Ito, M. Moriyama, Altered expression of ARPP protein in skeletal muscles of patients with muscular dystrophy, congenital myopathy and spinal muscular atrophy, Pathobiology 71 (2004) 43–51.
[12] Y.W. Chen, G.A. Nader, K.R. Baar, M.J. Fedele, E.P. Hoffman, K.A. Esser, Response of rat muscle to acute resistance exercise defined by transcriptional and translational profiling, J. Physiol. 545 (2002) 27–41.
[13] W. Chu, D.K. Burns, R.A. Swerlick, D.H. Presky, Identification and characterization of a novel cytokine-inducible nuclear protein from human endothelial cells, J. Biol. Chem. 270 (1995) 10236–10245.
[14] L.L. Scurr, A.D. Guminski, Y.E. Chiew, R.L. Balleine, R. Sharma, Y. Lei, K. Pryor, G.V. Wain, A. Brand, K. Byth, C. Kennedy, H. Rizos, P.R. Harnett, A. DeFazio, Ankyrin repeat domain 1, ANKRD1, a novel determinant of cisplatin sensitivity expressed in ovarian cancer, Clin. Cancer Res. 14 (2008) 6924–6932.
[15] K. Matsuura, N. Uesugi, N. Hijiya, T. Uchida, M. Moriyama, Upregulated expression of cardiac ankyrin-repeated protein in renal podocytes is associated with protein uria severity in lupus nephritis, Hum. Pathol. 38 (2007) 410–419.
[16] Y. Shi, B. Reitmaier, J. Regenbogen, R.M. Slowey, S.R. Opalenik, E. Wolf, A. Goppelt, J.M. Davidson, CARP, a cardiac ankyrin repeat protein, is up-regulated during wound healing and induces angiogenesis in experimental granulation tissue, Am. J. Pathol. 166 (2005) 303–312.
[17] E. Labbe, L. Lock, A. Letamendia, A.E. Gorska, R. Gryfe, S. Gallinger, H.L. Moses, L. Attisano, Transcriptional cooperation between the transforming growth factor-beta and Wnt pathways in mammary and intestinal tumorigenesis, Cancer Res. 67 (2007) 75–84.
[18] A. Baumeister, S. Arber, P. Caroni, Accumulation of muscle ankyrin repeat protein transcript reveals local activation of primary myotube endcompartments during muscle morphogenesis, J. Cell Biol. 139 (1997) 1231–1242.
[19] M.L. Bang, R.E. Mudry, A.S. McElhinny, K. Trombitas, A.J. Geach, R. Yamasaki, H. Sorimachi, H. Granzier, C.C. Gregorio, S. Labeit, Myopalladin, a novel 145-kilodalton sarcomeric protein with multiple roles in Z-disc and I-band protein assemblies, J. Cell Biol. 153 (2001) 413–427.
[20] Y. Tsukamoto, T. Senda, T. Nakano, C. Nakada, T. Hida, N. Ishiguro, Arpp, a new homology of CARP, is preferentially expressed in type 1 skeletonal muscle fibers and is markedly induced by denervation, Lab. Invest. 82 (2002) 645–655.
[21] T.J. Kemp, T.J. Sadusky, F. Saltisi, N. Carey, J. Moss, S.Y. Yang, D.A. Sassoon, G. Goldspink, G.R. Coulton, Identification of Ankrd2, a novel skeletal muscle gene coding for a stretch-responsive ankyrin-repeat protein, Genomics 66 (2000) 229–241.
[22] Y. Tsukamoto, N. Hijiya, S. Yano, S. Yokoyama, C. Nakada, T. Uchida, K. Matsuura, M. Moriyama, Arpp/Ankrd2, a member of the muscle ankyrin repeat proteins (MARPs), translocates from the I-band to the nucleus after muscle injury, Histochem. Cell Biol. 129 (2008) 55–64.
[23] O. Zolk, M. Frohme, A. Maurer, F.W. Kluxen, B. Hentsch, D. Zubakov, J.D. Hoheisel, I.H. Zucker, S. Pepe, T. Eschenhagen, Cardiac ankyrin repeat protein, a negative regulator of cardiac gene expression, is augmented in human heart failure, Biochem. Biophys. Res. Commun. 293 (2002) 1377–1382.
[24] S. Kojic, A. Nestorovic, L. Rakicevic, A. Belgrano, M. Stankovic, A. Divac, G. Faulkner, A novel role for cardiac ankyrin repeat protein AnkrD1/CARP as a co-activator of the p53 tumor suppressor protein, Arch. Biochem. Biophys. 502 (2010) 60–67.
[25] A. Belgrano, L. Rakicevic, S. Mittempergher, V.C. Campanaro, V. Martinelli, G. Mouly, S. Valle, G. Kojic, G. Faulkner, Multi-tasking role of the mechanosensing protein Ankrd2 in the signaling network of striated muscle, PLoS One 6 (2012) e25519.
[26] S. Kojic, E. Medeot, E. Guccione, H. Krmac, I. Zara, V. Martinelli, G. Valle, G. Faulkner, The Ankrd2 protein, a link between the sarcomere and the nucleus in skeletal muscle, J. Mol. Biol. 339 (2004) 313–325.
[27] C. Bean, N.K. Verma, D.L. Yamamoto, F. Chemello, V. Cenni, M.C. Filomena, J. Chen, M.L. Bang, G. Lanfranchi, AnkrD2 is a modulator of NF-κB-mediated inflammatory responses during muscle differentiation, Cell Death Dis. 5 (2014) e1002.
[28] A.M. Elsharkawy, F. Oakley, F. Lin, G. Packham, D.A. Mann, J. Mann, The NF-κB p50:p50;HDAC-1 repressor complex orchestrates transcriptional inhibition of multiple pro-inflammatory genes, J. Hepatol. 53 (2010) 519–527.
[29] Y. Wu, C.L. Ruggiero, W.A. Bauman, C.P. Cardozo, AnkrD1 is a transcriptional repressor for the androgen receptor that is downregulated by testosterone, Biochem. Biophys. Res. Commun. 437 (2013) 355–360.
[30] X.H. Liu, Y. Wu, S. Yao, A.C. Levine, A. Kirschenbaum, L. Collier, W.A. Bauman, C.P. Cardozo, Androgens up-regulate transcription of the Notch inhibitor Numb in C2C12 myoblasts via Wnt/β-catenin signaling to T cell factor elements in the Numb promoter, J. Biol. Chem. 288 (2013) 17990–17998.
[31] L.A. Cea, B.A. Cistema, C. Puebla, M. Frank, X.F. Figueroa, C. Cardozo, K. Willecke, R. Latorre, J.C. Saez, De novo expression of connexin hemichannels in denervated fast skeletal muscles leads to atrophy, Proc. Natl. Acad. Sci. 110 (2013) 16229–16234.

[32] S. Sato, Y. Ogura, A. Kumar, TWEAK/Fn14 signaling axis mediates skeletal muscle atrophy and metabolic dysfunction, Front. Immunol. 5 (2014) 1–9.
[33] R.W. Jackman, E.W. Cornwell, C.-L. Wu, S.C. Kandarian, NF-keppaB signaling and transcriptional regulation in skeletal muscle atrophy, Exp. Physiol. 98 (2013) 19–24.
[34] I.A. Barash, M.-L. Bang, L. Mathew, M.L. Greaser, J. Chen, R.L. Lieber, Structural and regulatory roles of muscle ankyrin repeat protein family in skeletal muscle, Am. J. Physiol. Cell Physiol. 293 (2007) C218–C227.