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MuRF1 is a muscle fiber-type II associated factor and together with MuRF2 regulates type-II fiber trophicity and maintenance

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ABSTRACT

MuRF1 is a member of the RBCC (RING, B-box, coiled-coil) superfamily that has been proposed to act as an atrogin during muscle wasting. Here, we show that MuRF1 is preferentially induced in type-II muscle fibers after denervation. Fourteen days after denervation, MuRF1 protein was further elevated but remained preferentially expressed in type-II muscle fibers. Consistent with a fiber-type dependent function of MuRF1, the tibialis anterior muscle (rich in type-II muscle fibers) was considerably more protected in MuRF1-KO mice from muscle wasting when compared to soleus muscle with mixed fiber-types. We also determined fiber-type distributions in MuRF1/MuRF2 double-deficient KO (dKO) mice, because MuRF2 is a close homolog of MuRF1. MuRF1/MuRF2 dKO mice showed a profound loss of type-II fibers in soleus muscle. As a potential mechanism we identified the interaction of MuRF1/MuRF2 with myozenin-1, a calcineurin/NFAT regulator and a factor required for maintenance of type-II muscle fibers. MuRF1/MuRF2 dKO mice had lost myozenin-1 expression in tibialis anterior muscle, implicating MuRF1/MuRF2 as regulators of the calcineurin/NFAT pathway. In summary, our data suggest that expression of MuRF1 is required for remodeling of type-II fibers under pathophysiological stress states, whereas MuRF1 and MuRF2 together are required for maintenance of type-II fibers, possibly via the regulation of myozenin-1.

1. Introduction

Skeletal muscles represent a tissue type that can be functionally remodeled in remarkable ways. For example, upon increasing load, as occurs during exercise, skeletal muscle respond with a large increase in fiber-diameter (hypertrophy); upon unloading, as occurs for example in bedridden patients, muscles rapidly reduce diameter (atrophy). The mechanisms for hypertrophy/atrophy include an up-regulation of the calcineurin-NFAT pathway (stimulated by the calcium influx into the sarcoplasm), activation of protein synthesis via the mTOR/p70S6 K pathway (caused by myofibrillar stretch), and activation of intracellular stress/strain-response signaling (including the p38/ERK/MEK kinase pathways), for review see (Potthoff et al., 2007). These adaptations to exercise include not only positive feeback regulation of fiber trophicity but also include metabolic adaptations, such as increase in mitochondrial content (Hood, 2009), and changes in fiber-type (Lee-Young et al., 2009). For example, enhanced neuro-muscular activity as induced by chronic low-frequency stimulation, which is experimentally used to mimic exercise, evokes transitions in myofibrillar protein expression, ultimately leading to a conversion of fast into a slower skeletal muscle fiber type (Putman et al., 2004). Although the characterization of factors determining fiber-type specification are still ongoing, calcineurin, MEF2, CaMK and PGC-1 (Chin et al., 1998; Lin et al., 2002) have been identified as muscle fiber-type I promoting factors, whereas thyroid hormone acts as a type-II driving factor (Simonides and van Hardeveld, 2008). One recent factor emerging for the maintenance of fast fiber-types is myozenin-1 (also called calsarcin-2 or FATZ). Functionally, myozenin-1 belongs to the calsarcin protein family whose members inhibit the protein phosphatase calcineurin (Frey et al., 2008) which in turn activates the transcription factor NFAT by dephosphorylation. Among the calsarcin family, myozenin-1 has a specialized function for muscle fiber-type specification. Recent studies on the myozenin-1 gene-deficient mouse model demonstrated that normal expression of myozenin-1 is required for the maintenance of fast fibers (Frey et al., 2008). Consistent with having almost pure type-I muscle fiber skeletal muscles; myozenin-1 KO mice have a marked capacity for long distance running (Frey et al., 2008).

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The chronic loss of sarcomeres, particularly of type-II fibers with increasing age (Snijders et al., 2009) is receiving growing attention because of its clinical importance. During chronic muscle wasting, loss of sarcomeres is accompanied by numerous alterations in the proteome, metabolome and transcriptome, including down-regulation of mitochondrial metabolism, and of muscle specific genes. Genome-wide comparisons of healthy and wasting muscle tissues identified a specific set of genes that become up-regulated during diverse states of muscle wasting (Lecker et al., 1999, 2004). These transcriptional changes are not merely a down-regulation of the exercise-stimulated genes and include genes that have been postulated to be causally linked to the development of muscle atrophy, the so-called atrogins (Lecker et al., 1999; Bodine et al., 2001). In particular, atrogin-1 (also known as MAFBx) and MuRF1 (muscle ring finger-1) might play key roles in the regulation of muscle remodeling (Bodine et al., 2001). While gastrocnemius mouse leg muscle loses as much as ~50% mass loss during 2 weeks after denervation, gastrocnemius muscles in knock-out mice deficient for atrogin-1 or for MuRF1 are significantly spared from mass loss (only 20% of control) (Bodine et al., 2001). In conclusion, these knock-out studies suggest that the upregulation of MafBx/atrogin-1 and MuRF1 in wild-type mice after denervation is required for the development of atrophy. MuRF1 has been implicated in amino acids metabolism control (Koyama et al., 2008) and might also be a target of certain amino acids such as leucine, since it has been shown that MuRF1 up regulation during skeletal muscle atrophy is minimized by leucine supplementation (Baptista et al., 2010). MuRF1 might also be an important player in skeletal muscle longitudinal growth (Soares et al., 2007).

A direct mechanistic link explaining why up-regulation of atrogin-1 and MuRF1 might trigger the degradation of muscle tissues during atrophy is that both proteins have ubiquitin ligase activities. Atrogin-1 and MuRF1 can potentially catalyze as E3 ubiquitin ligases the specific multi-ubiquitination of target protein, thereby initiating their proteasome-dependent degradation by the ubiquitin protesome system (UPS) Pickart and Eddins, 2004. Here, we focus on MuRF1 and MuRF2 (muscle ring finger-2) that share an N-terminal RING finger domain (that contains the ubiquitin E3 ligase activity), followed by a B-box and a central coiled-coil domain. MuRFs are also members of the RBCC superfamily (RING + B-box + coiled-coil) as are TRIM (Tripartite Motif-containing) (Mrosek et al., 2008). With regards to its E3 ligase activity, MuRF1 for example was shown in vitro to multi-ubiquitinate troponin-I (Kedar et al., 2004), myosin (Fielitz et al., 2007; Clarke et al., 2007) and actin (Cohen et al., 2009). However, in vivo, the mechanisms leading to the targeting of certain muscle proteins are more complex, because atrogin-1 and MuRF1 might have differential accessibility to different proteins within the myocyte. To address this issue, Cohen and colleagues (Cohen et al., 2009) used in a well-designed set of studies an inducible cre-lox mouse model that upon Cre-induction deletes MuRF1's RING finger domain, thus abolishing its ubiquitination-activity while leaving the remainder of MuRF1 and its potential structural functions for M-line assembly intact (McElhinny et al., 2002; Mrosek et al., 2007). In this model, myosin was not protected from multi-ubiquitination and degradation after denervation, contrasting its previous identification as an in vitro target of MuRF1 (Clarke et al., 2007). Rather, two other components of the thick filament, myosin light chain-2 (MLC2) and myosin-binding protein-C were protected from degradation, suggesting that their ubiquitination is MuRF1-mediated (Cohen et al., 2009). Therefore, current research is focused on the identification of the in vivo targets of atrogin-1 and MuRF1 using for example transgenic mouse models and adenoviral gene transfer of selected E3 ubiquitin ligase activities. Accordingly, a recent study used adenoviral based approaches suggested that atrogin-1 and MuRF1 are likely to recognize different targets (Mearini et al., 2009). Hirner et al. demonstrated by using a transgenic mouse model that the skeletal muscle specific overexpression of MuRF1 was not sufficient to cause muscle wasting and instead resulted in metabolic defects, thereby implicating MuRF1 in metabolism re-routing during muscle atrophy (Hirner et al., 2008).

The above discussed denervation studies were focused on the protection of muscle tissue mass from atrophy following long term denervation (>10 days) and less on other physiological parameters such as force or fiber-type composition. Here, we characterized the MuRF1-deficient muscles after denervation with regards to fiber-type composition in an attempt to clarify whether MuRF1-deficient muscles are physiologically normal and thus whether removal of MuRF1 activity could indeed present a muscle protective strategy. Surprisingly we found that MuRF1 is not evenly distributed in normal muscle tissue, and is preferentially expressed in type-II fibers. In addition, MuRF1is preferentially induced in type-II fibers after denervation. Consistent with a role of MuRF1 in fast fiber-type remodeling we found that in MuRF1-KO mice fast fiber-rich muscles are considerably more protected than slow fiber-type muscles. The direct physical interaction of MuRF1 with myozenin-1 may provide a mechanistic basis of how MuRF1 is involved in type-II fiber specification, thereby implicating MuRF1 in chronic pathophysiological fiber-type remodeling conditions such as occurs during denervation and ageing.

2. Materials and methods

This study was conducted with animal care guidelines issued by the German National Research Foundation. All protocols were approved by the Animal Care and Use Committee of *Universitätsmedizin Mannheim*.

2.1. Animals

All mice were bred in-house and genotyped by standard methods. MuRF1 and MuRF2 null mice were on a C57/BL6 background and were made as previously described (Witt et al., 2001, 2008). MuRF1/2 double null mice (5–6 months) were obtained by breeding double heterozygous mice to avoid any selection of potential modifier genes. The MuRF1-overexpressing transgenic line (MuRF1 TG) was described recently (Hirner et al., 2008). Animals were housed in standard plastic cages in an animal room with controlled environmental conditions and maintained on standard food and water *ad libitum*.

2.2. Experimental design

MuRF1 mice had their sciatic nerve sectioning as described elsewhere (Stockholm et al., 2001). Briefly, the dorsal skin of the thigh was cut and the posterior muscles divided to show the sciatic nerve. A chronic denervation was obtained by carefully cutting a 10-mm section of the sciatic nerve. After 5 and 14 days tibialis anterior and soleus muscles were excised. The effectiveness of denervation was confirmed by observing the locomotion of the life mouse and also by verifying the discontinuity of the sciatic nerve at the thigh level after the animal was sacrificed.

2.3. Immunohistochemistry

The primary antibodies used for immunostaining were: (1) monoclonal mouse anti-myosin heavy chain (MHC) II, clone MY-32 (1:1000; cat# M4276, Sigma) and (2) monoclonal mouse anti-skeletal myosin MHC I, clone NOQ7.5.4D (1:4000; cat# M8421, Sigma), (3) monoclonal multi-ubiquitin (1:1000; cat#