MuRF1 and Nedd4 are differentially expressed in denervated rat fast- and slow-twitch muscles

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Denervation atrophy of skeletal muscle is predominantly mediated by ATP-dependent ubiquitination (Furuno *et al*. 1990), where target proteins are marked for degradation by the 26S proteasome. The last step of this process involves the transfer of ubiquitin from an E3 ubiquitin ligase to a specific group of target proteins. Three major families of E3 ligases have been identified in skeletal muscle, including the RING finger proteins, the F-box proteins (which form part of the Skp1–Cul1–F-box (SCF) protein complex) and the homologous to E6AP carboxyl terminus domain (HECT) proteins (Powell, 2006). However, very little is known about the regulation and temporal pattern of expression of these three families of E3 ligases in skeletal muscle. The aim of this study was to characterise the expression pattern of two of these E3 ligases during denervation-induced atrophy; a RING finger protein (Muscle RING finger, MuRF1) and a HECT protein (Nedd4). MuRF1 is known to target myofibrillar and related structural proteins, including titin, nebulin, myosin light chains and myosin heavy chains (Fielitz *et al.*, 2007), while Nedd4 has been found to target signalling proteins such as Notch1 (Koncarevic *et al.*, 2007). We tested the hypothesis that the expression of these two proteins would differ temporally and spatially in denervated *extensor digitorum longus* (EDL, fast-twitch) or *soleus* (slow-twitch) muscles.

Adult male Sprague-Dawley rats (300-350g, n = 5 per timepoint) were anaesthetised (100 mg/kg ketamine and 10 mg/kg xylazine) and the sciatic nerve severed, taking care not to damage adjacent muscles and ensuring the resected nerve ends were not in contact. The 'nerve intact' muscles of the contralateral limb served as controls. After 1, 3, 7 or 14 days denervation, rats were anaesthetised deeply with pentobarbital sodium (60 mg/kg) and the EDL and soleus muscles surgically excised, trimmed of their tendons and any adhering connective tissue, weighed on an analytical balance and frozen for western immunoblotting of MuRF1 and Nedd4.

EDL muscle mass was increased by 11% compared with contralateral control muscles after 1 day of denervation, indicative of oedema. However, after 7 and 14 days of denervation EDL muscle mass was decreased by 24% and 46%, respectively ($p < 0.05$). In contrast, soleus muscle mass was unchanged after 1 and 3 days of denervation, and reduced by 46% and 54% after 7 and 14 days of denervation, respectively, compared with contralateral control muscles ($p < 0.05$). EDL muscles exhibited a significant increase in MuRF1 protein after 3, 7 and 14 days of denervation, with a maximal 3-fold increase observed at 7 days ($p < 0.05$). Nedd4 protein levels were increased after 7 and 14 days, with a maximal 4-fold increase after 7 days. In denervated *soleus* muscles, MuRF1 protein levels were increased significantly after 1, 3, 7 and 14 days, with a maximal 3-fold increase after 7 days. Nedd4 protein levels were increased after 3, 7 and 14 days with a maximal 2-fold increase after 14 days ($p < 0.05$).

These results indicate that the E3 ligases MuRF1 and Nedd4 and differentially regulated in denervated fast- and slow-twitch skeletal muscles undergoing muscle atrophy. In addition, the temporal expression of these E3 ligases during the early stages of denervation has been identified. This study demonstrates a previously unidentified role for Nedd4 in denervated EDL muscles, and provides important information about the timing of structural (MuRF1) and signalling (Nedd4) protein ubiquitination during denervation atrophy.

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