

JOURNAL CLUB

Skeletal muscle stem cell defects in burn-induced cachexiaJean Farup¹, Alessio Torcinaro^{2,3} and Luca Madaro^{2,3}¹Research Laboratory for Biochemical Pathology, Department for Clinical Medicine, Aarhus University, Aarhus, Denmark²IRCCS Fondazione Santa Lucia, Rome, Italy³Institute of Cell Biology and Neurology (IBCN), National Council of Research (CNR), Rome, Italy

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Satellite cells (SCs), the skeletal muscle stem cells, are essential for muscle regeneration in genetic or autoimmune muscle diseases as well as after ischaemic, chemical or mechanical trauma to the myofibres. Furthermore, SCs are the primary source to supply new myonuclei to growing myofibres during non-traumatic mechanical overload. Thus, when SCs are conditionally ablated using a tamoxifen inducible Cre-LoxP system, the addition of myonuclei during overload is abrogated (McCarthy *et al.* 2011). However, despite the lack of myonuclei addition, substantial hypertrophy could be induced in SC-ablated mice (McCarthy *et al.* 2011). More recent evidence, however, indicates that the ability of SC-depleted muscles to hypertrophy could be compromised during the later stages of muscle hypertrophy (Fry *et al.* 2014). Thus, while myonuclei addition, due to the fusion of muscle progenitors, may not be a prerequisite for initial muscle hypertrophy, a functional pool of SCs still play a key role during muscle hypertrophy. In contrast, less is known concerning the role of SCs and myonuclei turnover (e.g. myonuclei loss through apoptosis) during myofibre atrophy. Previous studies indicate that myofibre atrophy, for instance during immobilization or disease (cachexia), is generally not associated with a loss of myonuclei or an increase of DNA damage and apoptosis (Bruusgaard *et al.* 2012; Suetta *et al.* 2012).

In an intriguing recent paper published in *The Journal of Physiology*, Fry and colleagues (Fry *et al.* 2016) explore the potential involvement of SCs and

myonuclei apoptosis in young burn patients, a condition characterized by hyper-metabolism and extreme muscle wasting. Biopsies of vastus lateralis muscle were collected from patients (children between 8 and 18 years), with burns encompassing more than 30% of their total body area, during the flow phase of their recovery (characterized by hyper-metabolic demand). Biopsies were also collected from vastus lateralis muscles in a healthy control group, including males between 18 and 29 years of age, for comparison purposes. Through extensive immunohistochemical analyses, the authors collected information on myonuclei apoptosis (deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) and caspase-3 positive myonuclei), SC content and activity Ki67/Myogenic differentiation 1 (MyoD) expression, SC apoptosis (TUNEL positive satellite cells), muscle regeneration by analysing embryonic myosin heavy chain (embMHC) expression and levels of connective tissue content (by wheat germ agglutinin staining). The authors hypothesized that severe burn trauma could induce myonuclear apoptosis along with increased SC activation, in order to counteract the loss of myonuclei. In brief, the major findings by Fry and colleagues were (summarized in Table 1): (1) burn trauma induces myonuclear and SC apoptosis, (2) SC content is decreased although the content of active SCs is increased in burn patients, and (3) the latter two are associated with a generalized regenerative response (increased central nuclei and embMHC positive fibres) in burn patients.

Previous studies have reported myonuclear apoptosis to occur in the case of myofibre atrophy, potentially in order to maintain a relatively constant myonuclear domain size (myofibre cross-sectional area/myonuclei), although more recent studies have challenged this notion (Bruusgaard *et al.* 2012). Thus, the data concerning myonuclei and SC apoptosis reported by Fry *et al.* (2016) and colleagues suggest that the circumstances under which muscle atrophy is induced are different between immobilization and burn induced cachexia. In order to substantiate the findings on TUNEL⁺ myonuclei, Fry *et al.* (2016) confirmed these results with

a caspase-3/dystrophin staining, indeed indicating that apoptosis of myonuclei is induced in severe-burn patients. Overall, the results from Fry *et al.* (2016) suggest that different models of muscle atrophy are inherently different and although myonuclear apoptosis is not induced in one model of atrophy (e.g. immobilization) it could be part of the atrophy process in other models (e.g. burn-induced cachexia). The mechanisms underlying these differences have not yet been investigated in either of the referenced studies; however, such knowledge is highly relevant in understanding intrinsic differences among different conditions of muscle atrophy.

In addition to cellular apoptosis, Fry *et al.* (2016) observed a regenerative response characterized by an increase of centrally nucleated myofibres, SC activity and embMHC expression, correlated to the extent of burn-induced injury. Interestingly, the total SC content was lower in severe-burn patients compared to the healthy control group. This may be related to the increase of SC apoptosis, as discussed above, or, alternatively, may be due to an increased number of differentiating SCs, where paired box transcription factor 7 (Pax7) is down regulated. The latter may thereby compensate for the loss of myonuclei due to apoptosis.

The lowered SC content may be related to a reduced SC self-renewal and instead SC activation seems to be boosted, leading these cells to enter the myogenic programme and differentiate. Intriguingly, interleukin-6 (IL-6) increase and downstream activation of signal transducer and activator of transcription 3 (STAT3) signalling has recently shown to increase MyoD expression and SC differentiation (Tierney *et al.* 2014). In support of this speculation Fry *et al.* (2016) observed a marked number of MyoD-positive cells beneath the basal lamina (i.e. presumably activated SCs), which could be due to the activation of an IL-6–STAT3 pathway. While IL-6 could be secreted from mature myofibres, tissue-infiltrating macrophages or resident fibro-adipogenic progenitors (FAPs) may also contribute to the production of this cytokine. Therefore, dissecting the role of inflammatory or interstitial cells (i.e. FAPs) in skeletal muscle during atrophy conditions (such as burn-induced atrophy) could reveal

Table 1. Schematic representation of the evidence and experimental procedures shown by Fry *et al.* (2016)

Severely burned muscles		
	Hallmark	Experimental evidence
↑	Muscle regeneration	<ul style="list-style-type: none"> • Increase of embMHC positive fibres • Increase of centrally nucleated myofibres
↑	Expansion of extracellular matrix	<ul style="list-style-type: none"> • Increase of <i>N</i>-acetylglucosaminyl positive residues
↓	SC content	<ul style="list-style-type: none"> • Decrease of Pax7⁺ cells • Increase of Pax7⁺ TUNEL⁺ cells
↑	Myonuclear apoptosis	<ul style="list-style-type: none"> • Increase of TUNEL⁺ myonuclei • Increase of cleaved caspase-3⁺ myonuclei
↑	SC activation	<ul style="list-style-type: none"> • Increase of MyoD⁺ cells • Increase of Pax7⁺ Ki67⁺ cells

significant information in understanding SC regulation (activation) observed by Fry *et al.* (2016). Moreover, accumulation of extracellular matrix proteins in the burn-induced atrophic muscle can be related to a persistent inflammation and FAP activation.

It should be stressed, as noted by the authors (Fry *et al.* 2016), that the control group was young adults, whereas the burn patients were children under the age of 18. This limitation may in particular influence fibre area and SC quantity data, whereas the apoptosis, SC activation and embMHC data are expected to be less influenced by this constraint. The latter is based on the fact that very few apoptotic myonuclei/SCs, active SCs and embMHC⁺ fibres are present in non-perturbed skeletal muscle. With reference to previous literature, Fry *et al.* (2016) can, however, argue that the burn patients are indeed cachexic compared to subjects of the same age.

In conclusion, Fry *et al.* (2016) revealed significant information regarding the role of myonuclear and SC apoptosis during burn-induced injury. Moreover, they suggested that SCs might be an important cell target for the reduction of cachexia and the enhancement of recovery following burn-induced atrophy.

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Additional information

Competing interests

None declared.

Funding

J.F. was supported by a grant from The Danish Council For Independent Research (5053-00195). L.M. is partially supported by the Italian Ministry of Health (GR-2013-02356592).

Acknowledgements

The authors wish to thank Pier Lorenzo Puri and Niels Jessen for the helpful and constructive inputs.