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Extraocular muscle satellite cells are high performance myo-engines retaining efficient regenerative capacity in dystrophin deficiency

Pascal Stuelsatz [a], Andrew Shearer [a], Yunfei Li [a], Lindsey A Muir [b], Nicholas Ieronimakis [c], Qingwu W Shen [a], Irina Kirillova [a], Zipora Yablonka-Reuveni [a,*]

[a] Department of Biological Structure, University of Washington School of Medicine, Seattle, WA, USA
[b] Program in Molecular and Cellular Biology and Department of Neurology, University of Washington School of Medicine, Seattle, WA, USA
[c] Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

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ABSTRACT

Extraocular muscles (EOMs) are highly specialized skeletal muscles that originate from the head mesoderm and control eye movements. EOMs are uniquely spared in Duchenne muscular dystrophy and animal models of dystrophin deficiency. Specific traits of myogenic progenitors may be determinants of this preferential sparing, but very little is known about the myogenic cells in this muscle group. While satellite cells (SCs) have long been recognized as the main source of myogenic cells in adult muscle, most of the knowledge about these cells comes from the prototypic limb muscles. In this study, we show that EOMs, regardless of their distinctive Pax3-negative lineage origin, harbor SCs that share a common signature (Pax7^{+}, Ki67^{-}, Nestin-GFP^{+}, Myf5^{nLacZ+}, MyoD-positive lineage origin) with their limb and diaphragm somite-derived counterparts, but are remarkably endowed with a high proliferative potential as revealed in cell culture assays. Specifically, we demonstrate that in adult as well as in aging mice, EOM SCs possess a superior expansion capacity, contributing significantly more proliferating, differentiating and renewal progeny than their limb and diaphragm counterparts. These robust growth and renewal properties are maintained by EOM SCs isolated from dystrophin-null (mdx) mice, while SCs from muscles affected by dystrophin deficiency (i.e., limb and diaphragm) expand poorly in vitro. EOM SCs also retain higher performance in cell transplantation assays in which donor cells were engrafted into host mdx limb muscle. Collectively, our study provides a comprehensive picture of EOM myogenic progenitors, showing that while these cells share common hallmarks with the prototypic SCs in somite-derived muscles, they distinctively feature robust growth and renewal capacities that warrant the title of high performance myo-engines and promote consideration of their properties for developing new approaches in cell-based therapy to combat skeletal muscle wasting.

Introduction

Extraocular muscles (EOMs) comprise a group of highly specialized skeletal muscles controlling eye movements (Demer, 2007). The EOMs represent a unique skeletal muscle phenotype based on a range of properties, including specialized patterns of innervation and diversity of expressed sarcomeric myosin isoforms (Spencer and Porter, 2006). The developmental origin of EOMs adds another distinct facet to this muscle group. While body and limb muscles develop from the somites, EOMs are descended from prechordal and paraxial head mesoderm (Couly et al., 1992; Noden and Francis-West, 2006). Accordingly, the progenitors establishing the EOM primordia are of Pax3-negative origin, in contrast to the Pax3-positive lineage origin of limb and body muscles (Goulding et al., 1994; Horst et al., 2006; Tajbakhsh et al., 1997). Nevertheless, EOM development is orchestrated by the same members of the bHLH transcription factor family (MyoD, Myf5, MRF4, myogenin) that are involved in the specification and differentiation of body and limb muscles (Kassar-Duchossoy et al., 2004; Noden and Francis-West, 2006; Sambasivan et al., 2009).

The EOMs are also distinct from other skeletal muscles in their differential response to disease, being preferentially involved or spared in a variety of metabolic, mitochondrial and neuromuscular disorders (Kaminski et al., 2002; Schoser and Pongratz, 2006; Valdez et al., 2012; Yu Wai Man et al., 2005). Especially intriguing for muscular dystrophy research is the sparing of this muscle group in Duchenne muscular dystrophy. EOMs remain anatomically and functionally spared even at the late stages of the disease despite the severe pathology observed in other skeletal muscles (Kaminski et al., 1992; Khurana et al., 1995). Likewise, EOMs are

* Correspondence to: Department of Biological Structure, Health Sciences Building, Room G520, Box 35740, University of Washington School of Medicine, 1959 NE Pacific Street, Seattle, WA 98195, USA. Fax: +1 206 543 1524.
E-mail address: reuveni@uw.edu (Z. Yablonka-Reuveni).

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spared in animal models of muscular dystrophy resulting from the absence of dystrophin or other dystroglycan complex-related proteins (Khurana et al., 1995; Porter and Karathanasis, 1998; Porter et al., 2001). The mechanism behind EOM sparing has remained unclear (Pacheco-Pinedo et al., 2009; Porter, 1998; Zeiger et al., 2010), but specific properties of EOM myogenic progenitors have been proposed as possible contributory factors (Kallestad et al., 2011; Porter et al., 2006).

Satellite cells (SCs), Pax7^{+} myogenic progenitors situated between the basal lamina and sarcolemma of the myofibers, have long been recognized as the major source of myonuclei during muscle growth and repair (Mauro, 1961; Seale et al., 2000; Yablonka-Reuveni, 2011). SCs are proliferative during the postnatal growth phase, adding nuclei to the enlarging myofibers (Moss and Leblond, 1971; White et al., 2010). In adult muscles, SCs are typically quiescent, but can be activated in response to muscle injury (Montarras et al., 2013; Schultz et al., 1978). Depending on the magnitude of tissue trauma, SCs may divide minimally to repair subtle damage within individual myofibers or produce a larger progeny pool that forms new myofibers in cases of overt muscle trauma (Grounds and Yablonka-Reuveni, 1993; Hawke and Garry, 2001). In addition to generating differentiated myogenic progeny, at least some SCs can self-renew, thereby meeting the defining criteria of bona fide resident stem cells (Collins et al., 2005; Kuang et al., 2007; Sacco et al., 2008).

Most SC studies, whether performed in vivo or with isolated cells, have focused on the easily accessible limb muscles. Such studies have provided significant insights into SC molecular/cellular signatures during growth, aging and repair (Chang and Rudnicki, 2014; Zammit et al., 2006). Limb muscles have also been the main focus in studies on the potential of SCs as donor cells to alleviate muscle wasting, especially in dystrophinopathy. Despite their myogenic aptitude, enthusiasm for using limb SC-derived myoblasts has been weakened by their limited expandability and engraftment outcomes (Konieczny et al., 2013; Miller et al., 1997; Tremblay et al., 1993; Wilschut et al., 2012). This drawback may be related to heterogeneity in limb SC "stemness" with only a minority of cells appearing capable of both progeny production and self-renewal (Day et al., 2010; Kuang et al., 2007). Indeed, in mouse transplantation studies only a very small fraction of donor SCs was able to contribute to myofiber formation and to enter the satellite cell niche within the host muscle (Beauchamp et al., 1999; Collins et al., 2005; Sacco et al., 2008). Subsequently, alternative (non-SC) sources of donor cells for muscle therapy have been explored (Negroni et al., 2011; Tedesco and Cossu, 2012), while bona fide SCs from non-limb muscle groups have received only limited attention. Significantly, insight into SCs in muscle groups that are spared from the impact of Duchenne muscular dystrophy could unveil new cellular and molecular players that may be beneficial for treating muscle wasting disorders.

Although the unique anatomical and physiological features of EOMs have drawn much interest (Andrade et al., 2000; da Silva Costa et al., 2012; Spencer and Porter, 2006; Zhou et al., 2010), knowledge about the EOM myogenic progenitors is currently limited. One study that has used transgenic Pax7-nGFP mice for isolating and characterizing myogenic progenitors, placed the Pax7^{+} SCs as the main myogenic population in adult EOM as in the other muscles (Sambasivan et al., 2009). Additionally, the isolation of a number of EOM populations (SP, MP, EECD34) containing myogenic cells has been described (Kallestad et al., 2011; Kallestad and McLoon, 2010; McDonald et al., 2014). Our interest in EOM SCs originated from an earlier study where we established EOM-derived myogenic cultures as a control for analysis of the myogenic potential of retina pericytes (Kirillova et al., 2007). Using our standard approach for isolating myogenic cells (Danoviz and Yablonka-Reuveni, 2012), we observed that EOM cultures produced numerous myogenic progeny despite the very small initial number of cells obtained from these tiny muscles (unpublished).

In the current study we aimed to specifically characterize EOM SCs and their regenerative capacity. We focus on their in-situ signature, in-vitro expansion, and in-vivo engraftment aptitude. To identify potential unique properties of EOM SCs, most aspects were analyzed in comparison to SCs from the limb and diaphragm somite-derived muscles.

Materials and methods

Mice

All mice were on C57BL/6 background and from colonies we have maintained long-term at the University of Washington. Mice were housed in a pathogen-free facility under 12:12-h light/dark cycle and were fed ad libitum Lab Diet 5053 (Purina Mills). Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington. Most studies were performed with adult mice (4–6 month old) but as indicated in the Results, some studies were done with younger (3-week old) or older (12–24 month old) mice, and unless otherwise noted, only males were used. The following strains (all described in our previous studies) were used for tissue and cell isolation: (i) Knockin heterozygous Cre males MyoD^{Cre} [Myod1$^{tm2.1(icre)Glh}$, (Kanisicak et al., 2009)], and Pax3^{Cre} [(Pax3$^{tm1(cre)Joe}$/J, (Engleka et al., 2005)] were crossed with knockin reporter females R26^{mTmG} [Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo}$/J, (Muzumdar et al., 2007)] to generate F1 animals (Stuelsatz et al., 2012; Stuelsatz et al., 2014). (ii) Transgenic, heterozygous Nestin-GFP (Mignone et al., 2004) and MLC3F-nLacZ [MLC3F=muscle-specific myosin light chain 3F; AKA 3F-nlacZ-2E and Tg(Myl1-lacZ)1Ibdml/J, (Beauchamp et al., 2000; Kelly et al., 1995)]. (iii) Myf5^{nLacZ}, knockin heterozygous (Tajbakhsh et al., 1996; Tajbakhsh et al., 1997). When indicated, reporter mice were crossed to generate double heterozygous Nestin-GFP/MLC3F-nLacZ and Nestin-GFP/Myf5^{nLacZ} (Day et al., 2010). (iv) Dystrophin-null mdx^{4cv} (Chapman et al., 1989; Im et al., 1996) were crossed with Nestin-GFP mice to produce mdx^{4cv}/Nestin-GFP experimental mice.

Additionally, Rag1$^{-/-}$/mdx^{5cv} double-null mice (8–9 week old) were used as hosts in cell transplantation studies. This dystrophin-null strain that also lacks mature T and B cells (Mombaerts et al., 1992) was initially developed at the Jackson Lab for studies of Dr. Emanuela Gussoni (Boston Children's Hospital, unpublished). Notably, the mdx^{4cv} and mdx^{5cv} alleles were generated by point mutations upon mutagen-treatment of male mice (Banks et al., 2010; Chapman et al., 1989; Im et al., 1996). These mdx "cv" strains have been preferred by some laboratories due to the reduced occurrence of revertants (i.e., spontaneously appearing dystrophin^{+} myofibers, Lu et al., 2000) compared to the "standard" mdx mice (spontaneous mutation) (Arpke et al., 2013; Danko et al., 1992; Decrouy et al., 1997; Im et al., 1996; Judge et al., 2006).

Tissue harvesting

Hindlimb muscles (LIMB; consisting of tibialis anterior [TA], extensor digitorum longus [EDL] and gastrocnemius) and whole diaphragm (DIA; includes the costal and crural muscles and the central tendon) were harvested according to our routine procedures for cell isolation or tissue sectioning (Danoviz and Yablonka-Reuveni, 2012; Day et al., 2007; Stuelsatz et al., 2012). For the EOM we processed two types of preparations, one for tissue sections and one for cell isolation as detailed in our recent publication (Stuelsatz et al., 2014). A typical EOM preparation for histology