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Satellite cells isolated from aged or dystrophic muscle exhibit a reduced capacity to promote angiogenesis *in vitro*



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ABSTRACT

Deficits in skeletal muscle function exist during aging and muscular dystrophy, and suboptimal function has been related to factors such as atrophy, excessive inflammation and fibrosis. Ineffective muscle regeneration underlies each condition and has been attributed to a deficit in myogenic potential of resident stem cells or satellite cells. In addition to reduced myogenic activity, satellite cells may also lose the ability to communicate with vascular cells for coordination of myogenesis and angiogenesis and restoration of proper muscle function. Objectives of the current study were to determine the angiogenic-promoting capacity of satellite cells from two states characterized by dysfunctional skeletal muscle repair, aging and Duchenne muscular dystrophy. An *in vitro* culture model composed of satellite cells or their conditioned media and rat adipose tissue microvascular fragments (MVF) was used to examine this relationship. Microvascular fragments cultured in the presence of rat satellite cells from adult muscle donors (9–12 month of age) exhibited greater indices of angiogenesis (endothelial cell sprouting, tubule formation and extensive branching) than MVF co-cultured with satellite cells from aged muscle donors (24 month of age). We sought to determine if the differential degree of angiogenesis we observed in the co-culture setting was due to soluble factors produced by each satellite cell age group. Similar to the co-culture experiment, conditioned media produced by adult satellite cells promoted greater angiogenesis than that of aged satellite cells. Next, we examined differences in angiogenesis-stimulating ability of satellite cells from 12 mo old MDX mice or age-matched wild-type mice. A reduction in angiogenesis activity of media conditioned by satellite cells from dystrophic muscle was observed as compared to healthy muscle. Finally, we found reduced gene expression of hypoxia-inducible factor 1 α (HIF-1 α) and vascular endothelial growth factor (VEGF) in both aged and dystrophic satellite cells compared to their adult and normal counterparts, respectively. These results indicate that functional deficits in satellite cell activities during aging and diseased muscle may extend to their ability to communicate with other cells in their environment, in this case cells involved in angiogenesis.

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1. Introduction

Healthy adult skeletal muscle demonstrates the remarkable capacity to adapt to trauma and injury via muscle regeneration and repair. This ability is largely attributed to the stem cell within adult skeletal muscle called satellite cells [1]. Such myogenic cells lie in quiescence until activated by a stress-related event such as injury. Once activated, satellite cells undergo multiple rounds of proliferation where some cells ultimately differentiate into muscle

tissue while others return to quiescence to replenish and thus maintain the satellite cell pool [2]. Studies involving satellite cell biology have traditionally investigated myogenic properties, however recent studies demonstrate significant cross-talk between muscle precursor cells and vascular cells including the capacity for satellite cells to stimulate angiogenesis from pre-existing vascular segments [3,4]. Classical studies have indicated that spatial and temporal coordination of myogenesis as well as angiogenesis must occur in order to avoid the development of fibrosis and achieve functional muscle repair [5–7].

Aging or disease states, such as Duchenne muscular dystrophy, significantly alter the regenerative capacity of skeletal muscle [8,9]. The causes of this diminished regenerative capacity are likely to be numerous, but a common factor to both conditions is an alteration in the absolute number of satellite cells [10,11] as well

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as a diminished myogenic capacity [12,13]. A clear consensus on the role of intrinsic versus extrinsic factors during the weakened regenerative response is not evident in the literature [14,15]. However, a contributing factor to the reduced ability for skeletal muscle regeneration may involve satellite cell senescence that follows numerous bouts of activation and proliferation [14,16].

While numerous studies have been conducted looking at the diminished myogenic capacity of satellite cells in aging and disease states, other satellite cell attributes may underlie the delayed or defective skeletal muscle regeneration. Satellite cells appear capable of contributing to the revascularization of damaged muscle tissue by secreting soluble proteins known to be angiogenic in nature, such as VEGF [4]. Indeed, both dystrophic and aged muscle exhibit reduced capillary density [17,18]. Taken together, it is conceivable that there is not only a diminished myogenic capacity in satellite cells, but also a diminished angiogenic capacity contributing to an overall decrease in capillary density within the muscle and a delay in return to a fully functional state.

We hypothesized that satellite cells from aged or dystrophic skeletal muscle would exhibit a reduced capacity to stimulate angiogenesis. In order to examine the angiogenic potential of aged or dystrophic satellite cells, we utilized a three-dimensional microvascular fragment (MVF) construct combined with satellite cell conditioned media. Data herein indicate a diminished angiogenic response from satellite cells that were isolated from muscles with reduced regenerative competence. This intrinsic defect may be an important factor in the diminished revascularization that accompanies aged or dystrophic muscle and may represent a promising target for therapeutic intervention in the future.

2. Materials and methods

2.1. Materials

All animal care and use was conducted according to National Research Council guidelines and under the supervision of the University of Arizona Institutional Animal Care and Use Committee. In the following experiments, 9 or 24 month-old male Sprague Dawley rats and 14–15 month old dystrophic (C57BL/10ScSn-Dmd^{mdx}/J) and age-matched control (C57BL/10ScSn) mice (The Jackson Laboratory, Bar Harbor, ME) were used. Cell culture medium and fetal bovine serum (FBS) were purchased from Hyclone® (Logan, Utah).

2.2. Satellite cell isolation and culture

Satellite cells were isolated according to Allen et al. [19] and Rathbone et al. [20]. Briefly, muscle groups from the hind limb and back were excised, trimmed of fat and connective tissue, hand minced with sterile scissors and digested for 1 h at 37 °C with 1.25 mg/ml pronase. Cells were separated from muscle fiber fragments and tissue debris by differential centrifugation and plated in tissue culture dishes coated with 20 µl/cm² poly-L-lysine (0.1 mg/ml in distilled water; Sigma, St. Louis, MO) and a 10 µg/ml solution of fibronectin (Sigma) in sterile phosphate buffered saline (PBS). Following isolation, satellite cells were cultured for 48 h prior to re-seeding at equal cell densities in tissue culture dishes for co-culture or conditioned medium collection experiments (described below).

2.3. In vitro microvascular fragment culture model

Microvascular fragments (MVFs) were isolated from male rats as previously described [4,21] with slight modifications. Briefly, epididymal fat pads collected from euthanized rats were minced and digested in a collagenase solution [2 mg/ml in PBS containing

0.1% bovine serum albumin (BSA)] for 8–10 min. MVFs were pelleted by centrifugation, washed and resuspended in PBS containing 0.1% BSA. Isolation of MVF from tissue debris and single cells was achieved by differential selection through a 500 and 30 µm screen, respectively. MVFs were suspended in ice-cold, pH-neutralized rat tail type I collagen [3 mg/ml final concentration using 2.5× Dulbecco's Modified Eagle Medium (DMEM)] at approximately 15,000 MVF/ml for plating (0.25 ml/well) in 48-well culture plates. Following collagen polymerization, an equal volume of media (DMEM containing 2% FBS or satellite cell conditioned media) was added.

Angiogenic growth of MVFs in collagen gels is characterized by the formation of smooth vascular sprouts that are morphologically distinct from the rough, smooth-muscle associated appearance of the parent vessel [4,22]. The sprouting assay has been described previously [22]. Briefly, images of phase contrast microscopic fields (10× or 20×) of selected fragments from each gel were captured using a digital camera. Vessel sprout length was determined by measurement of a line traced along each sprout using the computer program ImageJ 1.32s (National Institutes of Health, USA). During measurement of sprout length, the number of sprouts per parent fragment was also recorded. Selection of fragments was based on two criteria: (1) fragment and sprouts must be physically independent of neighboring fragments; (2) the parent fragment must be clearly distinct. Sprout length and number were averaged from replicate experiments within each treatment group and included a minimum of 30 sprouts per treatment.

2.4. Co-culture model

An *in vitro* co-culture model composed of satellite cells and MVF was used as described previously [4]. Isolated MVF were suspended in pH-neutralized rat tail type I collagen (3 mg/ml final concentration using 2.5× DMEM) at approximately 15,000 MVF/ml for plating (0.25 ml/well) in 48-well culture plates over a satellite cell monolayer (at 50% confluency) and cultured in DMEM with 2% FBS.

2.5. Collection of satellite cell conditioned medium

Conditioned media (CM) was collected from satellite cells as described previously [4]. Following isolation, satellite cells were cultured for 48 h prior to re-seeding at equal cell densities in tissue culture plates. Briefly, plates were rinsed twice with pre-CM (DMEM + 0.1% penicillin/streptomycin + 1× insulin/transferrin/selenium (ITS); Cambrex Bio Science, Walkersville, Maryland) to remove residual serum. Following rinses, cells were cultured in pre-CM for 24 h, the medium was removed, and centrifuged for 5 min at 1500×g to remove any cellular debris. Conditioned medium was concentrated 10-fold (10×) using conical filter units (10,000 molecular weight cut-off, Amicon® Ultra Centrifugal Filter Devices, Millipore, Bedford, Massachusetts). Pre-conditioned medium that was not used on cells was also placed in conical filter units for control conditions. The medium was then filtered using Steriflip® Filter Units (0.22 µm, Millipore, Bedford, Massachusetts) and FBS was added to each type of medium to a final concentration of 2%. The medium was then added to MVFs or stored at 4 °C for less than 2 weeks.

2.6. Analysis of gene expression

Analysis of gene expression was performed as described previously [4]. Briefly, total RNA was isolated from cells using the QIAGEN RNeasy Mini Kit (QIAGEN, Germantown, Maryland) with on-column DNase digestion to remove genomic DNA contamination. Quantity and quality of RNA was assessed by absorbance at 260 nm and

verified using the Experion System (Bio-Rad Laboratories, Inc., Hercules, CA). Total RNA (500 ng) was reverse-transcribed using SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, California). Real-time SYBR green PCR assays were performed with rat-specific primers for VEGF (forward primer: 5-actccaggctcatcattg-3, reverse primer: 5-aattgagacctggtggaca-3), HIF-1 α (forward primer: 5-TGCTCATCAGTTGCCACTTC-3, reverse primer: 5-CCATCCAGGGCTTTCAGATA-3), and HPRT (forward primer: 5-GTCTGTTGCTGCGTCCCTTT-3, reverse primer: 5-CTGTCTGTCTCACAAGGGAAGTG-3) or mouse-specific primers for VEGF (forward primer: 5-CACGACAGAAGGAGAGCAGAA-3, reverse primer: 5-CGCTGGTAGACGTCCATGA-3), HIF-1 α (forward primer: 5-CAACGTGGAAGGTGCTTCA-3, reverse primer: 5-TGAGTTGGT-TACTGTTGGTATCA-3), and HPRT (forward primer: 5-AAACCTTGCTTCCCTGGTGA-3, reverse primer: 5-AGGCTTGTATTTGGCTTTTC-3). PCR reactions were performed in a 25 μ l volume using iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, California). Reactions contained 1 μ l primer set and diluted cDNA (5 ng). PCR quantification of each sample was performed in triplicate and SYBER Green fluorescence was quantified with the iQ5 Real Time PCR Detection System (Bio-Rad Laboratories, Inc.). For each assay 40 PCR cycles were run and a dissociation curve was included to verify the amplification of a single PCR product. Analyses of amplification plots were performed with the iQ5 Optical System Software version 2.0 (Bio-Rad Laboratories, Inc.). Data were analyzed using a five point relative standard curve generated using serial 10-fold dilutions of cDNA prepared and pooled from experimental samples. Each assay plate contained negative controls and a standard curve (five serial dilutions of a pool cDNA sample) to determine amplification efficiency of the respective primer pair. Unknown sample expression was then determined from the standard curve, normalized for HPRT expression and expressed relative to adult or control (wild-type) expression.

3. Results

Satellite cells have been previously demonstrated to cause angiogenesis using a three dimensional microvascular fragment model [4]. In order to investigate age-related alterations to satellite cell-mediated angiogenesis, we employed this co-culture model using satellite cells and MVFs isolated from adult and aged hosts. Adult satellite cells elicited a greater angiogenic response (endothelial cell sprouting, tubule formation, and extensive branching) than aged satellite cells, irrespective of the age of MVF examined ($P < 0.05$; Fig. 1A). Next, we sought to determine if the differential angiogenesis observed was due to soluble factors produced by satellite cells or direct participation of cells from the satellite cell preparation, we cultured MVF in the presence of adult (9 month) or aged (24 month) satellite cell conditioned medium (CM) harvested from proliferating cells of equivalent cell number. Adult satellite cell CM was more effective in promoting MVF growth than aged satellite cell CM ($P < 0.05$; Fig. 1B), indicating that satellite cell secretion of soluble-acting angiogenic factor(s) is altered by age.

The basis for the reduced angiogenic capacity of aged satellite cells is unclear; however, one possibility may involve activation of the transcription factor HIF-1 that drives a cascade of pro-angiogenic processes such as production of vascular endothelial growth factor (VEGF). To determine if this cascade is affected by aging in satellite cells, HIF-1 α and VEGF gene expression was determined in adult and aged proliferating satellite cells. Aged satellite cells exhibit a lower abundance of HIF-1 α and VEGF mRNA ($P < 0.05$; Fig. 2).

Dystrophic skeletal muscle is marked by fibrotic lesions and reduced vascularity despite extensive satellite cell activity [9]. To determine if satellite cells from dystrophic muscle have a reduced

propensity to stimulate angiogenesis, we collected conditioned media from MDX or wild-type satellite cells for use in the MVF angiogenic assay using the same protocol as with aging satellite cells, above. Both sprout length and number were reduced in MVF exposed to CM from MDX satellite cells compared to wild-type ($P < 0.05$; Fig. 3).

Finally, we chose to investigate HIF-1 α and VEGF gene expression in dystrophic and wild-type satellite cells to determine if alteration of this pathway may underlie the angiogenic deficiencies similar to that for aging above. Abundance of HIF-1 α and VEGF mRNA were decreased in proliferating satellite cells from the dystrophic muscle compared to wild-type ($P < 0.05$; Fig. 4) indicating that a dystrophic muscle environment diminishes satellite cell angiogenic capacity, partly through a mechanism involving decreased VEGF expression.

4. Discussion

In the current study we derived satellite cells from skeletal muscle of aged rats and mdx mice, two distinct models for conditions characterized by pathophysiological derangement in skeletal muscle architecture. In each condition, a component of the altered architecture involves remodeling of the skeletal muscle vasculature resulting in a reduced capillary network [17,18]. The altered vasculature within the muscle niche may involve satellite cells given that these stem cells normally lie in close proximity to capillaries and cross-talk between myogenic and angiogenic cell types is evident [3,4,23]. In support of this notion, we observed that both aged and dystrophic-derived satellite cells exhibited a decreased ability to induce angiogenesis. These data are consistent with intrinsic changes within the satellite cell that alter its functionality. In the case of aged individuals this is an important distinction since potential changes in satellite cell number cannot be causally linked to poor regeneration due to variable reports of enumeration in aged muscle (reviewed in [14]). It is not clear, however, if the lost angiogenic function is an inherent defect within the satellite cell or a response to extrinsic factors within the stem cell niche.

The mdx mouse model used in these studies, like human dystrophy, is an X-linked myopathic mutant, which lacks the dystrophin protein [24,25]. Interestingly, in young and adult mdx mice, there is continuous muscle fiber necrosis but this is compensated by vigorous regeneration leading to hypertrophic muscles. However, in older mdx mice regeneration declines leading to abnormal muscle fiber morphology, muscle degeneration, skeletal muscle necrosis, and abnormal muscle physiology [26–28]. Consistent with these observations, there is a reduced number of satellite cells in dystrophic muscle and the remaining satellite cells appear to have diminished myogenic capacity [10,13,29,30]. Our data indicate that satellite cells derived from dystrophic muscle alter the secretion of soluble factors that promote angiogenesis and this is associated with a reduced HIF-1 and VEGF gene expression profiles. Other studies have shown that administration of recombinant VEGF adeno associated virus into dystrophic skeletal muscle improved pathophysiology of the mdx mouse including increased capillary density, a decrease in necrotic fiber area and an increase in regenerating fiber area [31]. A more recent study specifically overexpressed VEGF in muscle-derived stem cells using retroviral expressing vectors and found an increase in skeletal muscle repair through increased angiogenesis in mdx skeletal muscle [32]. Taken together, it appears that the reduced VEGF gene expression in satellite cells from mdx mice may be a contributing factor to the reduction in capillary density previously observed in dystrophic muscle and thus the ability to restore VEGF expression may provide a promising area for future therapeutic intervention.

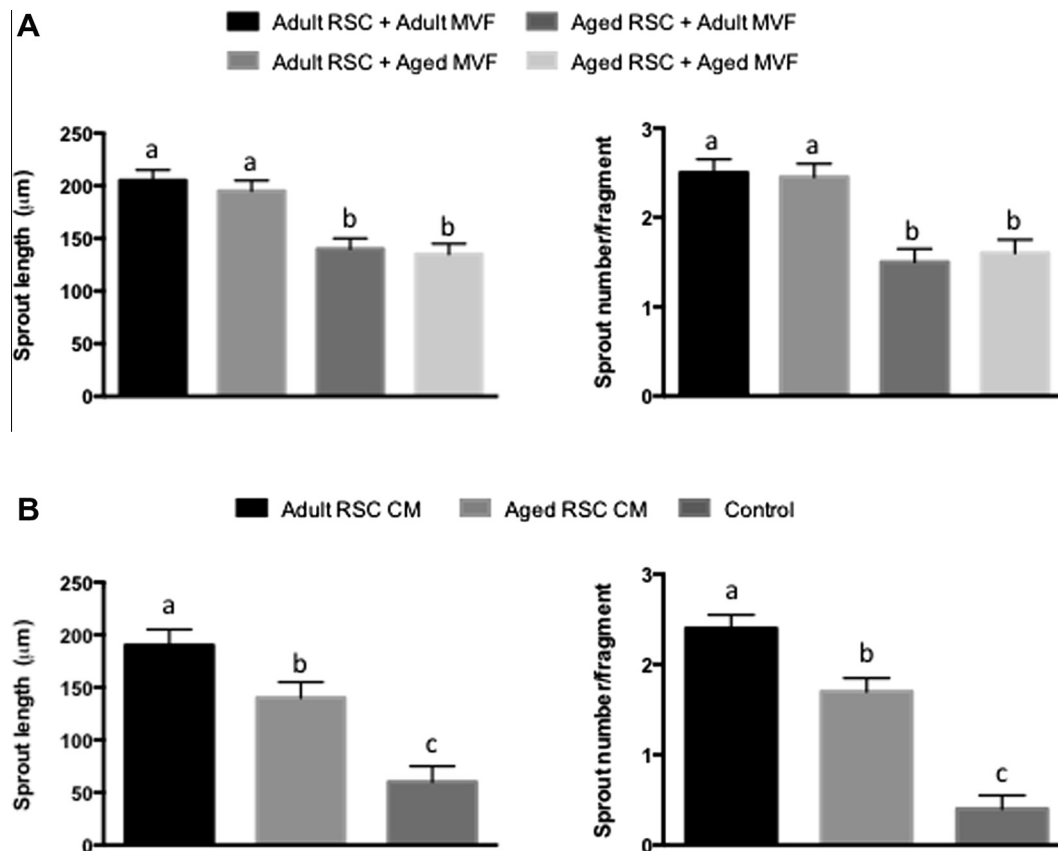


Fig. 1. Effect of rat age on satellite cell stimulation of *in vitro* angiogenesis. Panel A: satellite cell cultures were prepared from adult (9 month) and aged (24 month) rats and seeded at the same density. At 48 h post-plating, microvascular fragments (MVFs) from adult and aged rats were prepared, embedded in collagen and layered over satellite cell monolayers for 5 days. After 5 days of co-culture, MVF images were captured and angiogenesis quantified. Left: bars represent mean sprout length \pm SE. Right: bars represent mean sprout number \pm SE. Panel B: promotion of *in vitro* angiogenesis by conditioned medium from secondary cultures of proliferating satellite cells isolated from aged or adult rat skeletal muscle. Microvascular fragments (MVFs) were cultured with satellite cell-derived conditioned medium (CM) of satellite cell cultures prepared from adult (9 month) and aged (24 month) rats and seeded at the same density. Left: bars represent mean sprout length \pm SE. Right: bars represent mean sprout number \pm SE. Bars with different letters differ at $P < 0.05$.

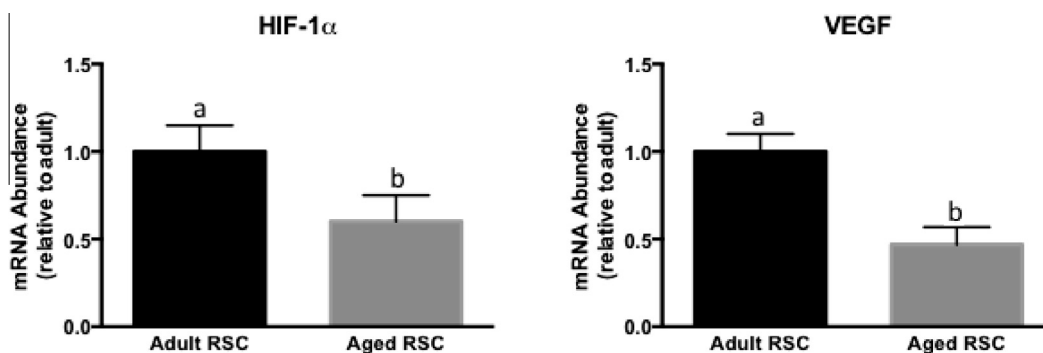


Fig. 2. Modulation of hypoxia-inducible factor-1 α (HIF-1 α , left panel) and vascular endothelial growth factor (VEGF, right panel) gene expression in secondary cultures of proliferating rat satellite cell (RSC) isolated from adult (9 month) and aged (24 month) rat skeletal muscle. Cells were cultured for 48 h prior to RNA isolation and real-time PCR analysis. For each gene, mRNA abundance is normalized to the housekeeping gene HPRT and expressed relative to adult expression. Bars with different letters differ at $P < 0.05$.

A novel observation from the current study is the reduced ability of satellite cells from aged muscle to properly stimulate angiogenesis whether in close proximity to the microvascular fragments or via the production of soluble factors. In addition, our observation that VEGF mRNA abundance is reduced in aged satellite cells is consistent with prior work indicating a deficit in VEGF and other angiogenic factors from aged skeletal muscle [18,33]. These observations take on additional importance when viewed in the context

of a recent report by Lee et al. [15] examining the ability of aged skeletal muscle to fully regenerate a functional architecture. The investigators demonstrated that the regenerative response from a muscle injury involving damage to the neurovascular supply was delayed and less effective. Although their focus was on net outcome and not the regenerative process per se, our data provide a potential mechanism underlying potential age-related differences in the regenerative capacity of the skeletal muscle

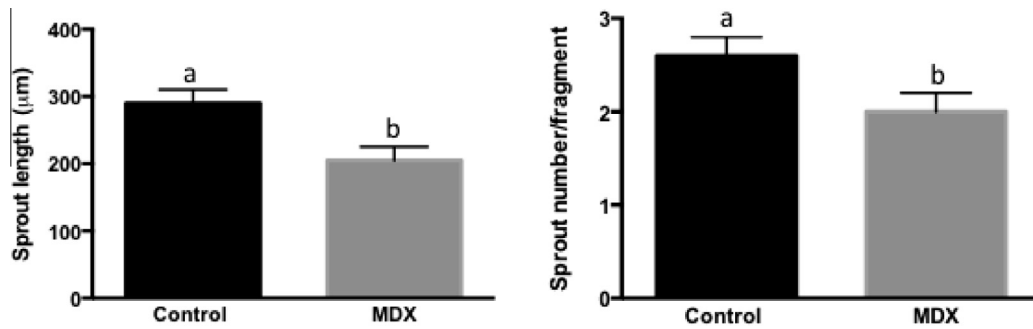


Fig. 3. Promotion of *in vitro* angiogenesis by conditioned medium from secondary cultures of proliferating satellite cells isolated from dystrophic or wild-type mouse skeletal muscle. Microvascular fragments (MVFs) were cultured with satellite cell-derived conditioned medium (CM) of satellite cell cultures prepared from dystrophic (MDX) and control (wild-type) mouse skeletal muscle and seeded at the same density. Left: bars represent mean sprout length \pm SE. Right: bars represent mean sprout number \pm SE. Bars with different letters differ at $P < 0.05$.

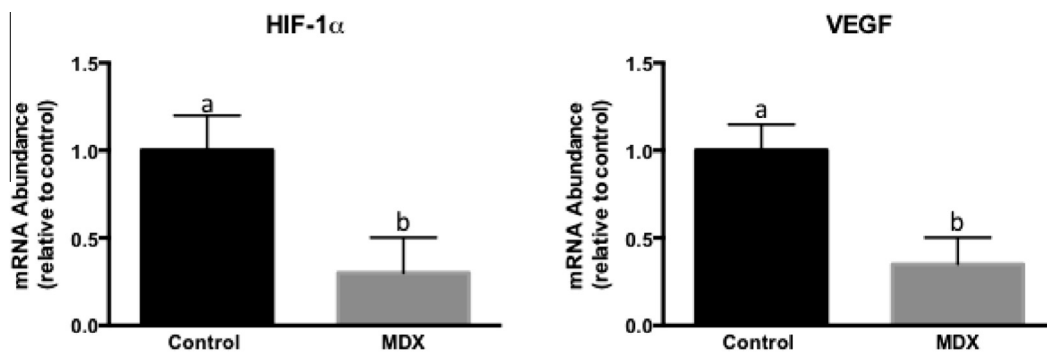


Fig. 4. Modulation of hypoxia-inducible factor-1 α (HIF-1 α , left panel) and vascular endothelial growth factor (VEGF, right panel) gene expression in primary mouse satellite cell cultures isolated from dystrophic (MDX) and control (wild-type) mouse skeletal muscle. Cells were cultured for 48 h prior to RNA isolation and real-time PCR analysis. For each gene, mRNA abundance is normalized to the housekeeping gene HPRT and expressed relative to control expression. Bars with different letters differ at $P < 0.05$.

blood supply. Additional studies are needed to determine the role of soluble factors, both pro- and anti-angiogenic, involved in satellite cell-mediated angiogenesis with aging.

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References

- [1] S.B. Chargé, M.A. Rudnicki, Cellular and molecular regulation of muscle regeneration, *Physiol. Rev.* 84 (2004) 209–238.
- [2] F. Relaix, P.S. Zammit, Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage, *Development* 139 (2012) 2845–2856.
- [3] C. Christov, F. Chretien, R. Abou-Khalil, G. Bassez, G. Vallet, F.J. Authier, Y. Bassaglia, V. Shinin, S. Tajbakhsh, B. Chazaud, R.K. Gherardi, Muscle satellite cells and endothelial cells: close neighbors and privileged partners, *Mol. Biol. Cell* 18 (2007) 1397–1409.
- [4] R.P. Rhoads, R.M. Johnson, C.R. Rathbone, X. Liu, C. Temm-Grove, S.M. Sheehan, J.B. Hoying, R.E. Allen, Satellite cell mediated angiogenesis coincides with a functional hypoxia-inducible factor (HIF) pathway, *Am. J. Physiol. Cell Physiol.* 296 (2009) C1321–8.
- [5] J.M. Markley, J.A. Faulkner, B.M. Carlson, Regeneration of skeletal muscle after grafting in monkeys, *Plast. Reconstr. Surg.* 62 (1978) 415–422.
- [6] F.M. Hansen-Smith, B.M. Carlson, Cellular responses to free grafting of the extensor digitorum longus muscle of the rat, *J. Neurol. Sci.* 41 (1979) 149–173.
- [7] F.M. Hansen-Smith, B.M. Carlson, K.L. Irwin, Revascularization of the freely grafted extensor digitorum longus muscle in the rat, *Am. J. Anat.* 158 (1980) 65–82.
- [8] Y.C. Jang, M. Sinha, M. Cerletti, C. Dall'Osso, A.J. Wagers, Skeletal muscle stem cells: effects of aging and metabolism on muscle regenerative function, *Cold Spring Harb. Symp. Quant. Biol.* 76 (2011) 101–111.
- [9] M.A. Rüegg, D.J. Glass, Molecular mechanisms and treatment options for muscle wasting diseases, *Annu. Rev. Pharmacol. Toxicol.* 51 (2011) 373–395.
- [10] C. Webster, H.M. Blau, Accelerated age-related decline in replicative life-span of Duchenne muscular dystrophy myoblasts: implications for cell and gene therapy, *Somat. Cell Mol. Genet.* 16 (1990) 557–565.
- [11] M.H. Snow, The effects of aging on satellite cells in skeletal muscles of mice and rats, *Cell Tissue Res.* 185 (1977) 399–408.
- [12] E. Schultz, B.H. Lipton, Skeletal muscle satellite cells: changes in proliferation potential as a function of age, *Mech. Ageing Dev.* 20 (1982) 377–383.
- [13] E. Schultz, D.L. Jaryszak, Effects of skeletal muscle regeneration on the proliferation potential of satellite cells, *Mech. Ageing Dev.* 30 (1985) 63–72.
- [14] A.S. Brack, T.A. Rando, Intrinsic changes and extrinsic influences of myogenic stem cell function during aging, *Stem Cell Rev.* 3 (2007) 226–237.
- [15] A.S. Lee, J.E. Anderson, J.E. Joya, S.I. Head, N. Pather, A.J. Kee, P.W. Gunning, E.C. Hardeman, Aged skeletal muscle retains the ability to fully regenerate functional architecture, *Bioarchitecture* 3 (2013) 25–37.
- [16] S. Decary, V. Mouly, C.B. Hamida, A. Sautet, J.P. Barbet, G.S. Butler-Browne, Replicative potential and telomere length in human muscle: implications for satellite cell mediated gene therapy, *Hum. Gene Ther.* 8 (1997) 1429–1438.
- [17] F. Nguyen, L. Guigand, I. Goubault-Leroux, M. Wyers, Y. Cherel, Microvessel density in muscles of dogs with golden retriever muscular dystrophy, *Neuromuscul. Disord.* 15 (2005) 154–163.
- [18] N.A. Ryan, K.A. Zwetsloot, L.M. Westerkamp, R.C. Hickner, W.E. Pofahl, T.P. Gavin, Lower skeletal muscle capillarization and VEGF expression in aged vs. young men, *J. Appl. Physiol.* 100 (2006) 178–185.
- [19] R.E. Allen, C.J. Temm-Grove, S.M. Sheehan, G. Rice, Skeletal muscle satellite cell cultures, *Methods Cell Biol.* 52 (1998) 155–176.
- [20] C.R. Rathbone, K. Yamanouchi, X.K. Chen, C.J. Nevoret-Bell, R.P. Rhoads, R.E. Allen, Effects of transforming growth factor-beta (TGF- β 1) on satellite cell activation and survival during oxidative stress, *J. Muscle Res. Cell Motil.* 32 (2011) 99–109.
- [21] J.B. Hoying, C.A. Boswell, S.K. Williams, Angiogenic potential of microvessel fragments established in three-dimensional collagen gels, *In Vitro Cell. Dev. Biol. Anim.* 32 (1996) 409–419.
- [22] K.B. Vartanian, H.Y.S. Chen, J. Kennedy, S.K. Beck, J.T. Ryaby, H. Wang, J.B. Hoying, The non-proteolytically active thrombin peptide TP508 stimulates angiogenic sprouting, *J. Cell. Physiol.* 206 (2006) 175–180.
- [23] H. Schmalbruch, U. Hellhammer, The number of satellite cells in normal human muscle, *Anat. Rec.* 185 (1976) 279–287.
- [24] E.P. Hoffman, R.H. Brown, L.M. Kunkel, Dystrophin: the protein product of the Duchenne muscular dystrophy locus, *Cell* 51 (1987) 919–928.

- [25] P. Sicinski, Y. Geng, A.S. Ryder-Cook, E.A. Barnard, M.G. Darlison, P.J. Barnard, The molecular basis of muscular dystrophy in the mdx mouse: a point mutation, *Science* 244 (1989) 1578–1580.
- [26] C. Pastoret, A. Sebille, Age-related differences in regeneration of dystrophic (mdx) and normal muscle in the mouse, *Muscle Nerve* 18 (1995) 1147–1154.
- [27] C. Pastoret, A. Sebille, Mdx mice show progressive weakness and muscle deterioration with age, *J. Neurosci.* 129 (1995) 97–105.
- [28] J.P. Lefaucheur, C. Pastoret, A. Sebille, Phenotype of dystrophinopathy in old mdx mice, *Anat. Rec.* 242 (1995) 70–76.
- [29] H.M. Blau, C. Webster, G.K. Pavlath, Defective myoblasts identified in Duchenne muscular dystrophy, *Proc. Natl. Acad. Sci. USA* 80 (1983) 4856–4860.
- [30] L. Heslop, J.E. Morgan, T.A. Partridge, Evidence for a myogenic stem cell that is exhausted in dystrophic muscle, *J. Cell Sci.* 113 (2000) 2299–2308.
- [31] S. Messina, A. Mazzeo, A. Bitto, M. Aguenouz, A. Migliorato, M.G. De Pasquale, L. Minutoli, D. Altavilla, L. Zentilin, M. Giacca, F. Squadrito, G. Vita, VEGF overexpression via adeno-associated virus gene transfer promotes skeletal muscle regeneration and enhances muscle function in mdx mice, *FASEB J.* 21 (2007) 3737–3746.
- [32] B.M. Deasy, J.M. Feduska, T.R. Payne, Y. Li, F. Ambrosio, J. Huard, Effect of VEGF on the regenerative capacity of muscle stem cells in dystrophic skeletal muscle, *Mol. Ther.* 17 (2009) 1788–1798.
- [33] A. Wagatsuma, Effect of aging on expression of angiogenesis-related factors in mouse skeletal muscle, *Exp. Gerontol.* 41 (2006) 49–54.