Biochemical and Biophysical Research Communications 503 (2018) 109–115

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Fractal dimension analysis reveals skeletal muscle disorganization in *mdx* mice

Sarah Santiloni Cury [a], Paula Paccielli Freire [a], Bruno Martinucci [a], Veridiana Carvalho dos Santos [b], Grasieli de Oliveira [a], Renato Ferretti [b], Maeli Dal-Pai-Silva [a], Francis Lopes Pacagnelli [c], Flávia Karina Delella [a], Robson Francisco Carvalho [a, *]

[a] Department of Morphology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
[b] Department of Anatomy, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
[c] Department of Physiotherapy, University of Western São Paulo (UNOESTE), Presidente Prudente, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 22 May 2018
Accepted 28 May 2018
Available online 6 June 2018

Keywords:
Duchenne muscular dystrophy
Co-culture
Fractal dimension analysis
Histopathology
Osteoglycin

ABSTRACT

Duchenne Muscular Dystrophy (DMD) is characterized by muscle extracellular matrix disorganization due to the increased collagen deposition leading to fibrosis that significantly exacerbates disease progression. Fractal dimension analysis is a method that quantifies tissue/cellular disorganization and characterizes complex structures. The first objective of the present study was use fractal analysis to evaluate extracellular matrix disorganization in *mdx* mice soleus muscle. Next, we mimic a hyper-proliferation of fibrogenic cells by co-culturing NIH3T3 fibroblasts and C2C12 myoblasts to test whether fibroblasts induce disorganization in myoblast arrangement. Here, we show *mdx* presented high skeletal muscle disorganization as revealed by fractal analysis. Similarly, this method revealed that myoblasts co-cultured with fibroblast also presented cellular arrangement disorganization. We also reanalyzed skeletal muscle microarrays transcriptomic data from *mdx* and DMD patients that revealed transcripts related to extracellular matrix organization. This analysis also identified Osteoglycin, which was validated as a potential regulator of ECM organization in mdx dystrophic muscles. Our results demonstrate that fractal dimension is useful tool for the analysis of skeletal muscle disorganization in DMD and also reveal a fibroblast-myoblast cross-talk that contributes to "*in vitro*" myoblast disarrangement.

1. Introduction

Skeletal muscle primary diseases, such as muscular dystrophies, are characterized by muscle dysfunction due to muscle degeneration accompanied by chronic inflammation and fibrosis [1]. Fibrosis is a pathological feature of muscle biopsies from patients with Duchenne Muscular Dystrophy (DMD) that contributes to the lethal DMD phenotype [2]. Disrupted extracellular matrix (ECM) in dystrophic muscles is the result of increased collagen deposition and cross-linking [3] leading to fibrosis that significantly exacerbate disease progression [4]. This aberrant deposition of ECM in muscular dystrophies is also associated with hyper-proliferation and over-activation of fibrogenic cells that modifies tissue architecture [1]. However, to our knowledge, there is no study quantifying skeletal muscle disorganization in DMD, which can be used to better characterization of muscle structural changes.

Fractal dimension is a differentiated and innovative methodology that allows the quantification of tissue and cells disorganization with important clinical applications for histopathological studies in neurology, ophthalmology, and hepatology [5,6]. Fractal dimension has also been used to evaluate myocytes preservation in isolated rat hearts, to determine myocardial cellular rejection after transplantation, and most recently, to quantify right ventricular structural changes in a rat model of pulmonary arterial hypertension [7–9]. In skeletal muscles, fractal dimension analyses were performed in histological slides for fiber type characterization, and in electromyography studies to evaluate muscle fatigue [10–12].

* Corresponding author. Department of Morphology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, 18618-689, Brazil.
E-mail addresses: robson.carvalho@unesp.br, robsoncarvalho@yahoo.com (R.F. Carvalho).

https://doi.org/10.1016/j.bbrc.2018.05.189
0006-291X/

Considering the different cellular and molecular mechanisms underlying skeletal muscle tissue architecture modifications associated with dystrophin deficiency that have been described in patients and animals models [2], we hypothesized that ECM disorganization in *mdx* mice soleus muscle can be accessed by fractal dimension analysis. We also mimic a hyper-proliferation of fibrogenic cells by co-culturing NIH3T3 fibroblasts and C2C12 myoblasts, and applied fractal dimension analysis to test whether "*in vitro*" fibroblasts induce disorganization in myoblast arrangement. Our data demonstrate that fractal dimension is useful tool for the analysis of skeletal muscle disorganization in DMD and reveals a fibroblast-myoblast cross-talk that contributes to "*in vitro*" myoblast disarrangement. We also reanalyzed microarrays transcriptome data of skeletal muscle from *mdx* and DMD patients that revealed transcripts related to biological process of matrix organization. This analysis identified Osteoglycin (Ogn) as potential regulator of ECM organization in dystrophic muscles.

2. Materials and methods

2.1. Animals

Male C57BL/10-DMD^{mdx}/PasUnib (*mdx*) and C57BL/10-ScCr/PasUnib (Ctrl) mice obtained from the mouse breeding colony of the Sao Paulo State University (UNESP) were housed under controlled temperature conditions with a 12/12-h light/dark cycle and free access to food and water. Soleus muscles were dissected from three aged adults (13 months-old) *mdx* and three C57BL/10 (control) mice for histology analysis. All experiments were performed in accordance with the guidelines for the use of animals set forth by our institution. The study was approved by the local Ethics Committees (CEUA #461).

2.2. Histology

Cryostat transverse-sections of the soleus muscle (7 μm thick) were examined in *mdx* and Ctrl animals. Collected samples were fixed in Bouin or methanol for hematoxylin and eosin (HE) and Picrosirius red stained (PSR), respectively, according to standard laboratory histological protocol. Soleus sections stained with PSR were used to quantify ECM; analysis was performed using *ImageJ* following software instructions for collagen quantification. Tissue fractal dimension was assessed in both soleus sections stained with PSR or HE of three random sections from each animal, using a light microscope (Olympus, Japan) and 10X and 20X objective. Myoblasts images were analyzed in triplicate with four images per well by using phase-constrast inverted microscopy with coupled digital camera AxioCam ICc5 (Carl Zeiss, Germany).

2.3. Co-culture of myoblasts and fibroblasts

C2C12 mouse myoblasts (MB) and NIH3T3 mouse fibroblasts (FB) were first cultured separately in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, USA) with 1% Penicillin–Streptomycin (Thermo Fisher Scientific, USA) and 10% fetal bovine serum (FBS, Thermo Fisher Scientific, USA) at 37 °C and 5% CO2 for growth and expansion. After growth and harvest, the myoblasts were plated into six-well plates at a density of 1×10^5 cells/well in 2 ml of DMEM, and fibroblasts or myoblasts were seeded at same concentration into ThinCert™ Cell Culture Inserts (Greiner Bio-One) containing membrane with 0.4 μm pore size. After 50% of confluence of both cells, inserts were placed into six-well plates containing myoblasts. Experimental group were composed by fibroblasts on the inserts (FB/MB), and control group were composed by myoblasts on the inserts (MB/MB). Co-cultures were maintained for 24 h.

2.4. Fractal dimension analysis

To analyze the fractal dimension of soleus muscle and C2C12 myoblasts, the photographed slides and cell culture images were binarized by ImageJ, and the fractal dimension was estimated by using box-counting. This tool, in the image processing program, is required to quantify pixels distribution in the space, without considering image texture. All the steps in fractal dimension analysis in histological slides and cell culture images was based on Pacagnelli et al. (2016) [9]. The fractal dimension value is always expressed from 0 to 2, where values close to 2 represent higher tissue or cell disorganization.

2.5. Differential gene expression and ontology analysis

Microarray data from patients with DMD and *mdx* mice are available in Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo). The array data of GSE1007 included 20 samples of quadriceps from patients with DMD and normal skeletal muscle [13], and GSE466 are composed by 10 gastrocnemius samples of 16-weaks-old *mdx* and controls [14]. Those data were analyzed with GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/), which were used to determine the differentially expressed genes in patients or animals with muscular dystrophy [15]. Considering adj. $P < 0.05$ and |Fold Change (FC)|>2 as cut-off criterion for differential expressed genes. Gene enrichment analysis were performed by Gene Ontology Consortium database (http://geneontology.org/) [16] using the up-regulated transcripts in DMD and *mdx*, separately. Ontology terms with $P < 0.05$ were considered enriched.

2.6. Western blotting analysis

Soleus muscles from *mdx* and Ctrl were lysed in assay lysis buffer containing freshly added protease and phosphatase inhibitors (1% Triton X-100, 100 mM Tris-HCl, pH 7.4, 100 mM sodium pyrophosphate, 100 mM NaF, 10 mM sodium orthovanadium, 10 mM EDTA, 2 mM PMSF, and 10 μg/ml aprotinin). The samples were centrifuged at 12.581 × *g* for 20 min at 4 °C. Protein concentration in soleus muscle was determined by Bradford protein assay kit according to the standard protocol [17] (Bio-Rad Laboratories, USA). Subsequently, Laemmeli buffer (Sigma, USA) were added to each soleus muscle samples, and maintained at 100 °C for 10 min. After samples preparation, the proteins at 30 ng of concentration were placed on 10% polyacrylamide gels in SDS-PAGE electrophoresis. After electrophoresis, the separated proteins were subjected to nitrocellulose membranes (Bio-Rad, USA) for electrotransference. A 5% of nonfat dry milk solubilized in TBS buffer containing 0.5% Tween 20 (TBST) were used to block the electrotransferred membranes for 2 h at room temperature, and then incubated overnight at 4–8 °C with specific Ogn primary antibody (Santa Cruz Biotechnology, USA; 1:1000 dilution). Mouse secondary antibody was maintained for 2 h at room temperature to promote binding of the primary antibody with peroxidase conjugated secondary antibody. Proteins were detected by using enhanced chemiluminescence (Amersham Biosciences, USA) in ImageQuant™ LAS 4000 (GE Healthcare, USA) autoradiography equipment. Quantification analyses of blots were performed by ImageJ software. Ogn levels were normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.7. Statistical analysis

The data are expressed as the mean ± standard deviation (SD).

For the statistical analyzes that were not previously described, we analyzed data using Student's t-test to establish significance between data points. The values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Muscular dystrophy induces skeletal muscle fibrosis in aged-mdx mice

The cross-sectional analysis of soleus muscle stained by PSR from aged-*mdx* mice (13 months-old) demonstrated an increase of collagen content area in the ECM when compared to the control group (Fig. 1).

3.2. Fractal dimension reveals disorganization in mdx mice skeletal muscle and in myoblasts co-cultured with fibroblasts

To quantify dystrophic skeletal muscle tissue organization, we first assessed nuclear and extracellular matrix fractal dimension, by using soleus muscle histological sections stained with HE or PSR. This analysis revealed that *mdx* soleus muscle present disorganization in both nuclear localization and ECM arrangement, as demonstrated by a higher fractal dimension (increase in 18.7% for nuclear localization, and 7.3% for ECM arrangement) when compared to control group (Fig. 2a and b). Considering that *mdx* mice presented muscle tissue fibrosis, we mimic an *in vitro* hyperproliferation of fibrogenic cells condition to tested whether an increase in cultured fibroblast per si can induce C2C12 myoblasts disarrangement. This condition was simulated by co-culturing FB/MB or MB/MB for 24 h, followed by a fractal analysis of the cultured myoblasts. This experiment demonstrated that FB/MB present disorganization in cellular arrangement, as demonstrated by an increase of 6.9% in fractal dimension when compared to MB/MB group (Fig. 2c). Together, both *in vivo* and *in vitro* data indicate that fibrosis or hyper-proliferation of fibrogenic cells condition influence muscle cells organization.

3.3. Dystrophy induces up-regulation in skeletal muscle genes related to extracellular matrix organization in mdx mice and DMD patients

To further identify candidate genes as potential targets associated to muscle tissue disorganization in dystrophy, we reanalyzed two experiments that evaluated the transcriptome of rats and patients with this condition. This differential gene expression analysis identified 372 deregulated genes (adj. pvalue ≤ 0.05 and fold change ≥ 2.0), of which 266 and 106 were up- or down-regulated, respectively in DMD patients; additionally, *mdx* mice presented 808 deregulated genes (adj. pvalue ≤ 0.05 and fold change ≥ 2.0), of which 585 and 222 were up- or down-regulated, respectively (Supplemental Material). We next performed a gene ontology (GO) enrichment analysis that identified statistically distribution of up-regulated transcripts among GO categories in dystrophic muscles. The overrepresented categories are displayed in Fig. 3a and showed an up-regulation of genes related to extracellular matrix organization in both DMD patients and *mdx* mice. To verify transcripts that potentially regulate ECM, we compared both DMD patients and *mdx* data and asked whether a sub-set of up-regulated transcripts overlap in both studies. The intersection of both conditions tested showed 28 overlapping transcripts (Fig. 3b) and, among the transcripts with potential relevance in ECM regulation, we found Ogn (Table 1). This initial *in silico* transcriptomic screening pointed out Ogn as a potential regulator of skeletal muscle architecture changes observed in muscular dystrophy in humans and animal models of DMD. Finally, we evaluated the protein levels of Ogn in mdx mice, which confirmed the down-regulation of this ECM regulator in dystrophic muscles (Fig. 3c and d).

4. Discussion

The main finding of our study is that fractal dimension analysis can be used as an effective tool to quantify skeletal muscle disorganization in *mdx* dystrophic mice. We also used this methodology to demonstrate "*in vitro*" that fibroblasts induce disorganization in

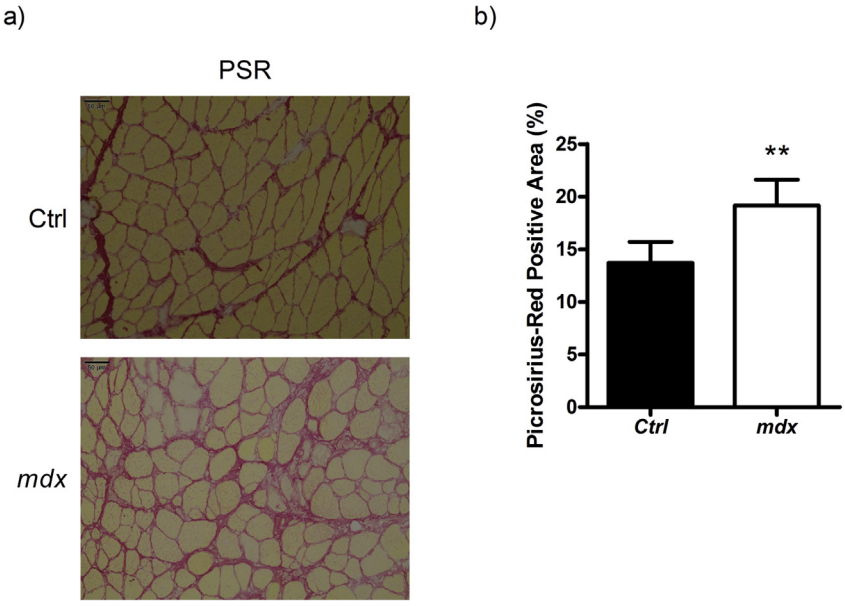

Fig. 1. Muscular dystrophy induces skeletal muscle fibrosis in aged-*mdx* mice. (a) Picrosirius-red staining of control soleus muscle and *mdx* soleus muscle assessing extracellular matrix fibrosis. (b) Quantitative analysis of PSR-stained sections. The data represent the mean ± standard deviation. Statistical analysis was performed using Student's t-test. ** represents a significance of $p < 0.001$. PSR: Picrosirius-red staining; Ctrl: control, *mdx*: dystrophic mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

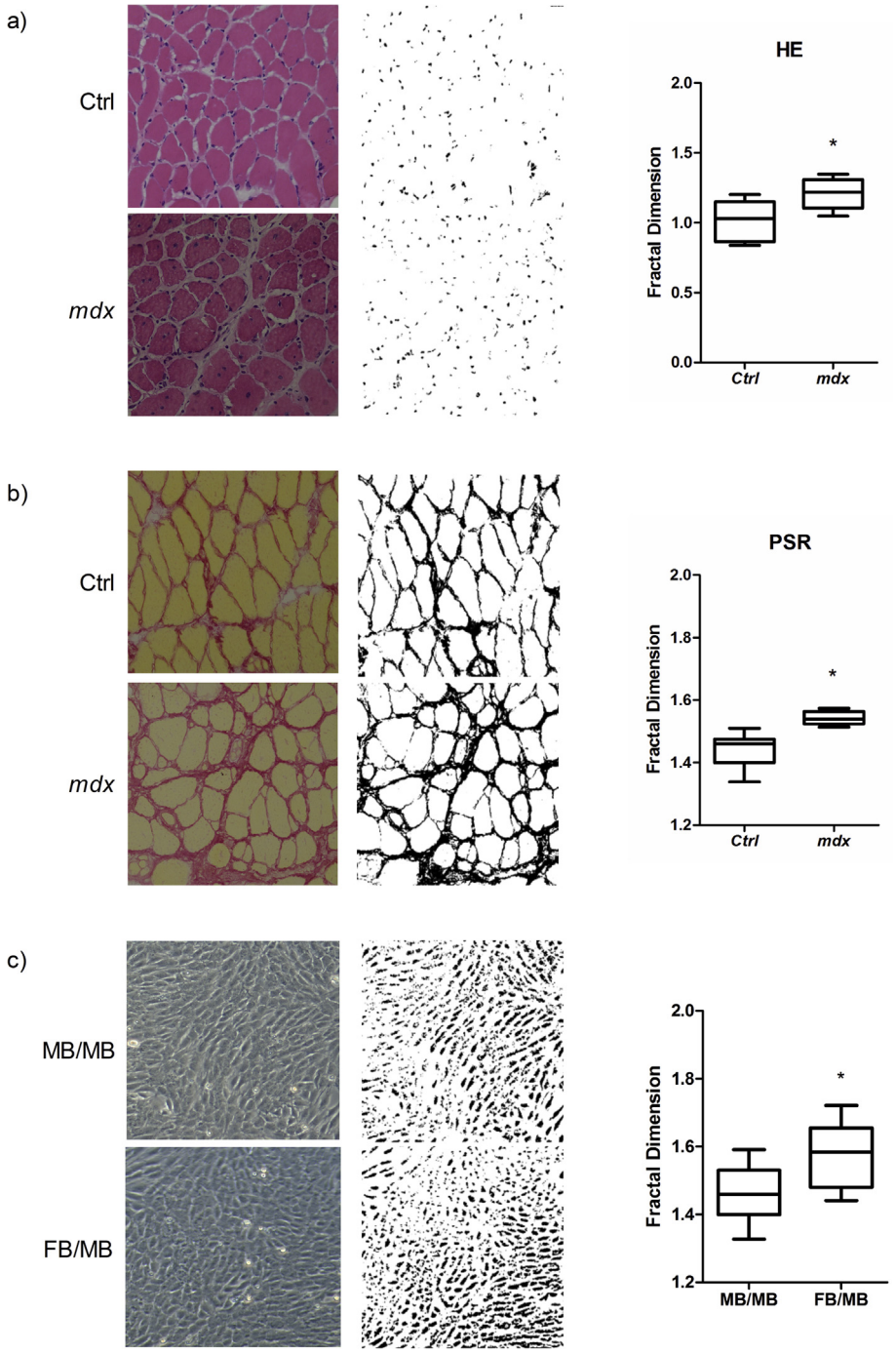

Fig. 2. Fractal dimension reveal disorganization in *mdx* mice skeletal muscle and in myoblasts co-cultured with fibroblast. (a) Histological sections of the soleus muscle stained with HE at 20x magnification, and the corresponding image after binarization. Fractal dimension analysis of *mdx*/control tissue stained with HE. (b) Histological sections of the soleus muscle stained with Picrosirius-red staining at 20x magnification, and corresponding image after binarization. Fractal dimension analysis of *mdx*/control tissue stained with PSR. (c) Co-cultured myoblasts MB/MB and FB/MB, at 20x original magnification, and corresponding image after binarization. Fractal dimension analysis of co-cultured myoblasts FB/MB and MB/MB. Data are expressed as box plot graphic. Statistical analysis was performed using Student's t-test $^{*}p < 0.05$. Ctrl: control; *mdx*: dystrophic mice; HE: Hematoxylin and Eosin; PSR: Picrosirius-red staining; MB: myoblasts; FB: fibroblasts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

myoblasts arrangement. Finally, we identify Ogn as potential regulator of ECM organization in dystrophy by re-analyzing publicly available muscle transcriptome data from dystrophic muscles from mice and humans.

We first characterized the *mdx* mice skeletal muscle phenotype and used fractal dimension analysis to quantify skeletal muscle tissue disorganization. This fractal dimension analysis demonstrated that *mdx* soleus muscle exhibit disorganization in nuclear localization and in ECM arrangement. These fractal dimension data are consistent with Huber et al. (2009), who showed that fractal dimension analysis was promising for the detection of muscle abnormalities in patients with neuromuscular disorders [18]. Additionally, fractal dimension analyses were previously used to characterize the distribution of muscle fiber type II

Table 1
Highly expressed transcripts shared by *mdx* mice and DMD patients skeletal muscles.

Gene Symbol	Description	FC (*mdx*)	FC (DMD)
Adam12	A disintegrin and metallopeptidase domain 12 (meltrin alpha)	2.55	3.16
Antxr2	Anthrax toxin receptor 2	2.62	2.16
Bok	BCL2-related ovarian killer	2.28	5.43
C1qa	Complement component 1, q subcomponent, alpha polypeptide	7.06	2.46
Ccdc80	Coiled-coil domain containing 80	3.36	5.74
Col1a2	Collagen, type I, alpha 2	3.92	7.36
Col8a1	Collagen, type VIII, alpha 1	4.29	3.81
Dab2	Disabled 2, mitogen-responsive phosphoprotein	2.13	3.16
Gpm6b	Glycoprotein m6b	2.58	2.97
Itgax	Integrin alpha X	2.85	2.58
Lat2	Linker for activation of T cells family, member 2	2.43	3.12
Lox	Lysyl oxidase	6.02	2.45
Maged1	Melanoma antigen, family D, 1	3.01	2.35
Marcks	Myristoylated alanine rich protein kinase C substrate	3.03	2.33
Mgp	Matrix Gla protein	3.27	2.30
Mpeg1	Macrophage expressed gene 1	9.99	2.64
Nes	Nestin	2.50	3.71
Ogn	Osteoglycin	2.39	3.46
Pdzd11	PDZ domain containing 11	2.43	3.14
Plekho1	Pleckstrin homology domain containing, family O member 1	2.38	2.06
Pqlc3	PQ loop repeat containing	4.26	2.06
Rab31	RAB31, member RAS oncogene family	3.41	2.04
Shkbp1	Sh3kbp1 binding protein 1	9.13	3.29
Sox11	SRY (sex determining region Y)-box 11	8.17	3.10
Tmem176a	Transmembrane protein 176A	6.11	2.30
Tmem176b	Transmembrane protein 176B	2.99	2.27
Tubb6	Tubulin, beta 6 class V	2.16	8.75
Ugcg	UDP-glucose ceramide glucosyltransferase	3.48	2.41

Fold change (FC) > 2 and significance adjusted p-values of <0.05.

in the extensor digitorum longus and soleus muscles of the adult rats [10]. Electromyography (EMC) data were also analyzed by fractal dimension analysis to determine the use of this methodology along with other parameters to better characterize the EMG signal, and to quantify motor unit recruitment patterns in biceps of normal patients [19,20]. Moreover, fractal analysis was used as a reliable measure of the skeletal muscle fatigue [11,12], and to assess changes in positron emission tomography (PET) data of peripheral vascular function of resting and exercising skeletal muscles [21]. Given the overall applicability of fractal analysis in myology, we emphasize the importance of this simple and low-cost methodology in translational medicine since it may be used as a differentiated tool for DMD diagnosis and disease staging.

Considering all these promising applications of fractal dimension analysis in evaluating different types of skeletal muscle data, including our DMD data, we also performed fractal dimension analysis to demonstrated *in vitro* that fibroblasts induce myoblasts arrangement disorganization. There are few studies describing the use of fractal analysis in the pattern of cellular organization *in vitro*; these studies applied this methodology to evaluate the effect of magnetic field application in promote saliences in astrocyte membranes [22], to characterize mammary gland epithelial cells migration patterns [23], and to determine indexes of dendrite branching complexity [24]. However, to our knowledge, this is the first study that used fractal dimension to demonstrate that C2C12 myoblasts arrangement *in vitro* is clearly influenced by the presence of fibroblasts. These fractal dimension results also indicate a paracrine signaling (cross-talk) between skeletal muscle cells and fibroblasts that promote myoblast arrangement disorganization.

To further explore and validate potential transcriptional changes related to tissue disorganization in dystrophic muscles, we reanalyzed microarrays transcriptome data of skeletal muscle from *mdx* mice and DMD patients and the respective controls [13,14]. Remarkably, the gene ontology analysis of these data confirmed a clear up-regulation of genes related to extracellular matrix organization. Previous transcriptomic study also revealed that mdx^{129} mice, which present a less severe DMD phenotype, has up-regulation in genes related to extracellular matrix functional category when compared to the more severe DMD phenotype of the mdx^{C57BL} mice. These authors pointed out that an increase in osteopontin protein levels, a extracellular matrix binding protein, can act as a protective factor to the less severe DMD muscle phenotype [25]. Another transcriptome analysis revealed that *mdx* dystrophin-down-regulation is responsible for modifications in the expression of genes that compromise proper development of striated muscles as an organized contractile unit [26]. Thus, exploring publicly available transcriptome data sets is an important strategy for the identification of ECM components associated with skeletal muscle organization/phenotype, and to reach new therapeutic targets of DMD.

In fact, by using our muscle transcriptome reanalysis from DMD patients and *mdx* mice, we identified shared up-regulated transcripts that are translated into muscle ECM and/or secretome components. Interestingly, this analysis revealed osteoglycin (Ogn), a ECM component with key role in collagen fibrillogenesis [27] and that belongs to the muscle cell secretome [28]. Ogn has also been implicated in corneal transparency [29], skin collagen fibril abnormalities [28]. Ogn is also a humoral factor secreted by skeletal muscle cells that induces bone anabolic effect [29]. Moreover, a transcriptome analysis of Ullrich congenital muscular dystrophy fibroblasts revealed increased expression of genes related to extracellular matrix, and Ogn was one of the Top 10 up-regulated transcript [30]. Although few studies have already identified differentially expressed secretome components in DMD [31,32], our muscle transcriptome reanalysis pointed out to Ogn as an important factor in regulating ECM organization in DMD, due to its role in coordinating appropriated collagen production, organization, and remodeling.

Considering that ECM modifications during the development of fibrosis in DMD results from ECM aberrant deposition [2], the

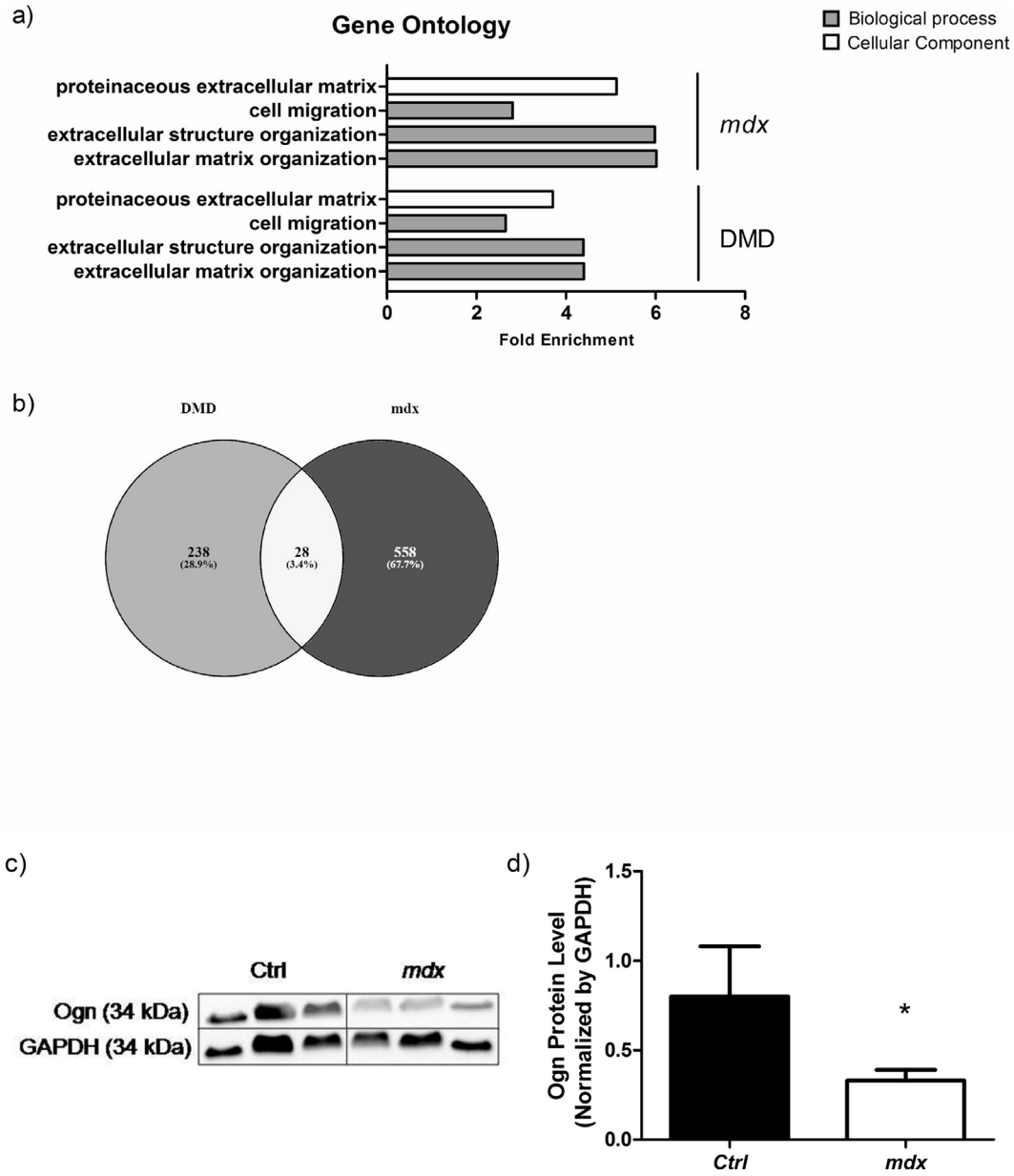

Fig. 3. Skeletal muscle genes associated to extracellular matrix organization in DMD. (a) Gene ontology of common biological process and cellular component enriched in *mdx* mice and patients with DMD. (b) Venn diagram showing the intersection containing 28 up-regulated transcripts shared by *mdx* and patients with DMD. (c) Representative blots normalized by GAPDH. (d) Extracts obtained from individual animals were used for densitometric analysis of the Osteoglycin levels following normalization to the GAPDH. Control (Ctrl, n = 3) and dystrophic mice (*mdx*, n = 3), and representative blots. Experiments were performed in triplicate, and the data represent the mean ± standard deviation. Statistical analysis was performed using Student's t-test. .* represents a significance of ($p < 0.05$). DMD: Duchenne Muscular Dystrophy; *mdx:* dystrophic mice; Ogn: osteoglycin; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; kDa: kilodalton.

identification of skeletal muscles secreted proteins that potentially control ECM organization may help to reveal mechanisms associated with the ECM remodeling in DMD. For a further understanding of Ogn in DMD, although we have not evaluated Ogn gene expression in the muscle samples used in this study, we found that Ogn protein levels is clearly reduced in our *mdx* model, suggesting that this protein contribute to ECM organization in dystrophic muscles.

The knowledge of the essential paracrine signaling components in muscle dystrophy is important for better understanding pathophysiological mechanisms of skeletal muscle tissue disorganization. Thus, future studies are needed to better understand the role of muscle cells secretome components in cellular communication and gene expression modulation in muscular dystrophies to bring new treatment strategies for muscle primary diseases, such DMD. Additionally, studies with longitudinal delineations are also necessary to better describe the use of fractal analysis in DMD once this study had a cross-sectional approach.

In conclusion, fractal dimension analysis can be used as an effective tool to quantify skeletal muscle disorganization in *mdx* dystrophic mice. We also showed that this methodology can be applied to demonstrate "*in vitro*" that fibroblast induce disorganization in myoblast localization. Our analysis also identified Ogn as potential regulator of ECM organization in dystrophic muscles.

Funding

This study was supported by the São Paulo Research Foundation (FAPESP, grant #2012/13961-6) and National Council for Scientific and Technological Development (CNPq, grants #476399/2013-0 and #800571/2016-9).

Acknowledgments

We would like to thank Dr. William Fernando Zambuzzi (IBB/UNESP) for giving us the fibroblasts NIH3T3, and Célio Júnior Fernandes for helping us with the management of this cell line.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.05.189.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.05.189.

References

[1] Y. Kharraz, J. Guerra, P. Pessina, A.L. Serrano, P. Muñoz-Cánoves, Understanding the process of fibrosis in Duchenne muscular dystrophy, BioMed Res. Int. 2014 (2014), https://doi.org/10.1155/2014/965631, 965631.
[2] L. Zhou, H. Lu, Targeting fibrosis in Duchenne muscular dystrophy, J. Neuropathol. Exp. Neurol. 69 (2010) 771–776, https://doi.org/10.1097/NEN.0b013e3181e9a34b.
[3] L.R. Smith, D.W. Hammers, H.L.E.E. Sweeney, E.R. Barton, Increased collagen cross-linking is a signature of dystrophin-deficient muscle, Muscle Nerve 54 (2016) 71–78, https://doi.org/10.1002/mus.24998.
[4] M. Quattrocelli, M.J. Spencer, E.M. Mcnally, Outside in: the matrix as a modifier of muscular dystrophy, Biochim. Biophys. Acta 1864 (2017) 572–579, https://doi.org/10.1016/j.bbamcr.2016.12.020 BBAMCR.
[5] M. Keipes, F. Ries, M. Dicato, Of the British coastline and the interest of fractals in medicine, Biomed. Pharmacother. 47 (1993) 409–415.
[6] N. Dioguardi, F. Grizzi, B. Franceschini, P. Bossi, C. Russo, N. Dioguardi, F. Grizzi, B. Franceschini, Liver fibrosis and tissue architectural change measurement using fractal-rectified metrics and Hurst ' s exponent, World J. Gastroenterol. 12 (2006) 2187–2194, https://doi.org/10.3748/wjg.v12.i14.2187.
[7] M. Oliveira, A. Brandi, C. Santos, P. Botelho, J. Cortez, M. Godoy, E. Al, Comparison of fractal dimension and Shannon entropy in myocytes from rats treated with histidine-tryptophan-glutamate and histidine- tryptophan cetoglutarate, Rev. Bras. Cir. Cardiovasc. 29 (2014) 156–162, https://doi.org/10.5935/1678-9741.20140052.
[8] R.D. G.M. Moreira, A.R. Moriel, L.O. Murta Junior, L.A. Neves, Fractal dimension in quantifying the degree of myocardial cellular rejection after cardiac transplantation, Rev. Bras. Cir. Cardiovasc. 26 (2011) 155–163.
[9] F.L. Pacagnelli, A. Karênina, D. De Almeida, T.B. Mariano, G. Akio, T. Ozaki, R.C. Castoldi, E. Maria, R.F. Carvalho, L. Casquel, K. Okoshi, L. Carlos, M. Vanderlei, Fractal dimension in quantifying experimental-pulmonary-hypertension-induced cardiac dysfunction in rats, Arq. Bras. Cardiol. 107 (2016) 33–39, https://doi.org/10.5935/abc.20160083.
[10] G.A. Arsos, P.P. Dimitriu, A fractal characterization of the type II fiber distribution in the extensor digitorum longus and soleus muscles of the adult rat, Muscle Nerve 18 (1995) 961–968, https://doi.org/10.1002/mus.880180907.
[11] M. Beretta-piccoli, G.D. Antona, M. Barbero, B. Fisher, R. Clijsen, C. Cescon, Evaluation of central and peripheral fatigue in the quadriceps using fractal dimension and conduction velocity in young females, PLoS One 10 (2015) 1–15, https://doi.org/10.1371/journal.pone.0123921.
[12] G. Boccia, M. Beretta-piccoli, G.D. Antona, C. Zampella, Muscle fiber conduction velocity and fractal dimension of EMG during fatiguing contraction of young and elderly active men, Physiol. Meas. 37 (2016) 162–174, https://doi.org/10.1088/0967-3334/37/1/162.
[13] J.N. Haslett, D. Sanoudou, A.T. Kho, M. Han, R.R. Bennett, I.S. Kohane, A.H. Beggs, L.M. Kunkel, Gene expression profiling of Duchenne muscular dystrophy skeletal muscle, Neurogenetics 4 (2003) 163–171, https://doi.org/10.1007/s10048-003-0148-x.
[14] B.S. Tseng, P. Zhao, J.S. Pattison, S.E. Gordon, J.A. Granchelli, R.W. Madsen, L.C. Folk, E.P. Hoffman, F.W. Booth, R.W. Madsen, L.C. Folk, E.P. Hoffman, F.W.B. Regenerated, Regenerated mdx mouse skeletal muscle shows differential mRNA expression, J. Appl. Physiol. 93 (2002) 537–545, https://doi.org/10.1152/japplphysiol.00202.2002.
[15] T. Barrett, S.E. Wilhite, P. Ledoux, C. Evangelista, I.F. Kim, M. Tomashevsky, K.A. Marshall, K.H. Phillippy, P.M. Sherman, M. Holko, A. Yefanov, H. Lee, N. Zhang, C.L. Robertson, N. Serova, S. Davis, A. Soboleva, NCBI GEO: archive for functional genomics data sets–update, Nucleic Acids Res. 41 (2013) D991–D995, https://doi.org/10.1093/nar/gks1193.
[16] J. Blake, K. Christie, M. Dolan, H. Drabkin, D. Hill, L. Ni, D. Sitnikov, M. Westerfield, Gene ontology Consortium: going forward, Nucleic Acids Res. 43 (2015) 1049–1056, https://doi.org/10.1093/nar/gku1179.
[17] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254. http://www.ncbi.nlm.nih.gov/pubmed/942051. (Accessed 9 July 2014).
[18] R. Huber, A.C. Ludolph, H. Ko, H. Gdynia, H. Mu, Quantitative muscle ultrasound in neuromuscular disorders using the parameters "intensity", "entropy", and "fractal dimension, Eur. J. Neurol. 16 (2009) 1151–1158, https://doi.org/10.1111/j.1468-1331.2009.02663.x.
[19] V. Gupta, S. Suryanarayanan, N. Reddy, Fractal analysis of surface EMG signals from the biceps, Int. J. Med. Inf. 45 (3) (1997) 185–192.
[20] J. Gitter, M. Czerniecki, Fractal analysis of the electromyographic interference pattern, J. Neurosci. Meth. 58 (1–2) (1995) 103–108.
[21] K. Kalliokoski, T. Kuusela, P. Nuutila, T. Tolvanen, V. Oikonen, M. Teräs, T. Takala, J. Knuuti, Perfusion heterogeneity in human skeletal muscle: fractal analysis of PET data, Eur. J. Nucl. Med. 28 (4) (2001) 450–456.
[22] I. Khodarahmi, H. Mobasheri, M. Firouzi, The effect of 2.1 T static magnetic field on astrocyte viability and morphology, Magn. Reson. Imag. 28 (6) (2010) 903–909 doi:0.1016/j.mri.2010.03.003.
[23] N. Wick, S. Thurner, K. Paiha, R. Sedivy, I. Vietor, L. Huber, Quantitative measurement of cell migration using time-lapse videomicroscopy and nonlinear system analysis, Histochem. Cell Biol. 119 (1) (2003) 15–20, https://doi.org/10.1007/s00418-002-0491-2.
[24] E. Neale, L. Bowers, T.J. Smith, Early dendrite development in spinal cord cell cultures: a quantitative study, J. Neurosci. Res. 34 (1) (1993) 54–66, https://doi.org/10.1002/jnr.490340107.
[25] P. Calyjur, C. Almeida, D. Ayub-Guerrieri, A.J. Ribeiro, S. Fernandes, R. Ishiba, A. Santos, P. Onofre-Oliveira, M. Vainzof, The mdx mutation in the 129/sv background results in a milder phenotype: transcriptome comparative analysis searching for the protective factors, PLoS One 8 (3) (2016), e0150748, https://doi.org/10.1371/journal.pone.0150748, 11.
[26] M. Seno, C. Trollet, T. Athanasopoulos, I. Graham, P. Hu, G. Dickson, Transcriptomic analysis of dystrophin RNAi knockdown reveals a central role for dystrophin in muscle differentiation and contractile apparatus organization, BMC Genom. 11 (2010) 345, https://doi.org/10.1186/1471-2164-11-345.
[27] J. Henningsen, K.T.G. Rigbolt, B. Blagoev, B.K. Pedersen, I. Kratchmarova, Dynamics of the skeletal muscle secretome during myoblast differentiation, Mol. Cell. Proteomics 9 (2010) 2482–2496, https://doi.org/10.1074/mcp.M110.002113.
[28] E.S. Tasheva, A. Koester, A.Q. Paulsen, A.S. Garrett, D.L. Boyle, H.J. Davidson, M. Song, N. Fox, G.W. Conrad, Mimecan/osteoglycin-deficient mice have collagen fibril abnormalities, Mol. Vis. 8 (2002) 407–415. http://www.ncbi.nlm.nih.gov/pubmed/12432342. (Accessed 1 November 2014).
[29] K. Tanaka, E. Matsumoto, Y. Higashimaki, T. Katagiri, T. Sugimoto, S. Seino, H. Kaji, Role of osteoglycin in the linkage between muscle and bone, J. Biol. Chem. 287 (2012) 11616–11628, https://doi.org/10.1074/jbc.M111.292193.
[30] S. Paco, T. Casserras, M.A. Rodríguez, C. Jou, Transcriptome analysis of Ullrich congenital muscular dystrophy fibroblasts reveals a disease extracellular matrix signature and key molecular regulators, PLoS One 10 (2015) 1–21, https://doi.org/10.1371/journal.pone.0145107.
[31] S. Lecompte, M. Abou-Samra, R. Boursereau, L. Noel, S. Brichard, Skeletal muscle secretome in Duchenne muscular dystrophy: a pivotal anti-inflammatory role of adiponectin, Cell. Mol. Life Sci. 74 (2017) 2487–2501, https://doi.org/10.1007/s00018-017-2465-5.
[32] S. Duguez, W. Duddy, H. Johnston, J. Lainé, M. Catherine, L. Bihan, K.J. Brown, A. Bigot, Y. Hathout, G.B. Terence, Dystrophin deficiency leads to disturbance of LAMP1-vesicle-associated protein secretion, Cell. Mol. Life Sci. 70 (2013) 2159–2174, https://doi.org/10.1007/s00018-012-1248-2.