Research Article
Volume 12:4, 2021

Journal of
Cytology & Histology

ISSN: 2157-7099 Open Access

Human Skeletal Muscle Satellite Cells Co-Express Aldehyde Dehydrogenase Isoforms Aldh1A1 & Aldh1A3

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Abstract

Satellite cells (SC) constitute the stem cell population of skeletal muscle and conduct myogenic growth and differentiation. Recently, aldehyde dehydrogenase 1 (ALDH1) has been identified as a novel myogenic factor in experimental models of SCs. ALDH1 constitutes a subfamily of the ALDH enzyme super family. The enzymatic functions of ALDH1 isoforms include both protection against oxidative stress products and regulation of differentiation as pacemaker enzyme in retinoic acid signaling. Although ALDH enzymatic activity has been demonstrated in SCs it is not clear which isoforms are important in human skeletal muscle. Here, we show that ALDH1A1 and ALDH1A3 are expressed in human SCs. Using antibodies directed against ALDH1 and its isoforms ALDH1A1 and ALDH1A3, respectively, we demonstrate immunohistochemical staining in peri-fascicular position matching the localization of SCs. Consistently, co-immunofluorescence reveals ALDH1 expression in CD56 positive stem cells and co-localization of the isoforms ALDH1A1 and ALDH1A3 in Pax7 positive SCs. Quantitative analysis of immunohistochemical staining showed no significant differences in the distribution of ALDH1 positive SCs in the skeletal muscle groups pectoralis, diaphragm and psoas that have been investigated in the present study. In conclusion, human SCs co-express the ALDH1 isoforms ALDH1A1 and ALDH1A3.

Keywords: Satellite Cells • Human Skeletal Muscle • ALDH1 • ALDH1A1 • ALDH1A3

Introduction

Satellite Cells (SC) constitute the stem cell population of skeletal muscle. The biological regulation of functional stem cell behavior, however, is not completely understood. Furthermore, exogenous and endogenous stimuli that control asymmetric division of SC have not been characterized in detail.

Aldehyde dehydrogenase (ALDH) has recently been identified as a novel myogenic factor in SC. It has been shown that ALDH1 expression correlates with growth and regenerative capacity in human and murine myogenic progenitor cells [1]. These results have been corroborated by experiments demonstrating high myogenic capacities and resistance to oxidative and inflammatory stress in ALDH1 expressing cells [2]. Most recently, Etienne et al. investigated various ALDH isoforms in different species and also included dystrophic muscle tissue from human and dog model system of DMD. They pointed out the important role of ALDH isoforms in muscle physiology and homeostasis, especially in the context of muscular dystrophies [3].

ALDH constitutes an enzyme super family with various isoforms and different functions in cell metabolism. ALDH2 is the most important enzyme in alcohol metabolism of the liver by detoxifying acetate aldehyde [4]. Other isoforms of ALDH are known as detoxifying agents in oxidative stress reaction after lipid peroxidation [5]. Thus, ALDH enzymes act as important factors in cellular protection. In addition, isoforms of the ALDH1 subfamily, especially ALDH1A1 and ALDH1A3, are most important regulators of retinoic acid (RA) metabolism, thereby activating differentiation [6,7].

These data indicate that ALDH1 seems to play an important role in skeletal muscle biology. The cellular function of ALDH1 might include both protection against oxidative stress and coordination of differentiation by regulation of RA signaling. Therefore, it is of particular interest to learn more about the functional expression of ALDH1 in SCs.

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Received 06 October 2020; **Accepted** 15 April 2021; **Published** 23 April 2021

Here, we show that ALDH1 is expressed in CD56 positive stem cells of human skeletal muscle and that its isoforms ALDH1A1 and ALDH1A3 co-localize with Pax7 positive SCs. Quantitative analysis of immunohistochemical stainings in different human muscles showed no significant differences in the distribution of ALDH1 positive Scs.

Material and Methods

21 skeletal muscle samples of 7 autopsy cases (3 male, 4 female, mean age 58,4 years, range 25-87) selected from the archives of TUM Institute of Pathology, were used. The study comply with the guidelines for human studies and the research was conducted in accordance with the World Medical Association Declaration of Helsinki. Patients' data have been completely anonymized before histological procedure.Tissue specimens from pectoralis, diaphragm and psoas muscles, respectively, were prepared during regular autopsy, formalin fixed and paraffin embedded.

Anti-CD56 (Cell Marque; monoclonal, MRQ-42, rabbit, dilution: 1:4, ready to use antibody), anti-Pax7 (Merck; polyclonal, rabbit, dilution: 1:500), anti-ALDH1 (BD Biosciences; monoclonal, clone 44, mouse, dilution: 1:250), anti-ALDH1A1 (Abcam; polyclonal, rabbit, dilution: 1:500), anti-ALDH1A3 (Thermo Fisher Scientific; polyclonal, rabbit, dilution: 1:250) and anti-CD68 (Dako; monoclonal, KP1, mouse, 1:15000) were used as primary antibodies for immunohistochemistry (IHC) and immunofluorescence (IF). Anti-mouse (Abcam; goat anti-mouse IgG; 1:400) and anti-rabbit (Abcam; goat anti-rabbit IgG, 1:400) served as secondary antibodies for IHC. Fluorescent anti-mouse (Invitrogen; Alexa Fluor 488, 1:2000; Life Technologies; Alexa Fluor 568, 1:2000, respectively) and fluorescent anti-rabbit (Life technologies; Alexa Fluor 488, 1:2000; Invitrogen; Alexa Fluor 568, 1:2000, respectively) served as secondary antibodies for IF.

Briefly, for IHC slides were boiled for heat-induced epitope uncovering in pH6.0 citrate buffer at 95°C for 30 minutes. After H_2O_2-incubation, primary antibodies were applied as indicated above. Slides were incubated overnight at 4°C with subsequent visualization using ABC-reagent (Vector laboratories) and 3,3'-diaminobenzidine-(DAB)-reagent (Agilent). Counterstaining with hematoxylin was conducted as last step. Positive controls served as quality assurance.

Immunohistochemical slides were examined with a light microscope Axioskop 2 (Zeiss), captured with an AxioCam and analyzed using AxioVision 4.8

software. For quantitative evaluation an area of 50 representative cross-orientated fibers per sample was randomly chosen and positive nucleic staining (Pax7) as well as cytoplasmatic staining (CD56, ALDH1) was counted. All 21 samples (7 autopsy cases with 3 different muscle types each) were evaluated in the expression-pattern of myogenic stem cell markers CD56, Pax7 and enzyme ALDH1 within isoforms ALDH1A1 and ALDH1A3.

For immunofluorescence (IF) staining secondary antibodies of CD56 and Pax7 were visualized with fluorescein isothiocynate (FITC) filter, secondary antibodies of ALDH1, ALDH1A1 and ALDH1A3 with rhodamine filter.

Briefly, slides were boiled in pH 6.0 citrate buffer at 95°C for 30 minutes for epitope uncovering. Primary antibodies were applied as indicated with overnight incubation at 4°C. Subsequently, fluorescent secondary antibodies were applied and incubated for 45 minutes. Slides were additionally treated with Quenching Kit (Vector) for 4 minutes and DAPI counterstaining. Positive controls served as quality assurance.

Immunofluorescent slides were examined with a Carl Zeiss Axioplan fluorescence microscope. Co-expression of CD56/ ALDH1, PAX7/ ALDH1A1 and Pax7/ ALDH1A3, respectively, was analyzed by co-immunofluorescence staining using different secondary antibodies for visualization.

Results

ALDH1 immunoreactive cells as well as cells positive for ALDH1A1 and ALDH1A3 isoforms were detected in all types of skeletal muscle tissue investigated in the present study, showing a peri-fascicular localization along the host muscle fiber, but above the sarcolemma. In general, staining pattern of ALDH1 did not show significant differences compared to its isoforms ALDH1A1 and ALDH1A3 (shown in Fig. 1. E-G) Especially in longitudinal orientation of single fibers cytoplasmic ALDH1 staining could be observed (shown in Fig. 1. E) although a faint staining of muscle fiber membranes was also visible on cross sections. SCs could be identified beneath the basal lamina in human skeletal muscle tissue using antibodies directed against CD56 and paired-box protein PAX7 (shown in Fig. 1. B-D). Additionally, SCs were often situated in a triangle position between fibers (shown in Fig. 1. A). In comparison, CD56-/ Pax7-positive SCs demonstrated the same distribution compared to ALDH1 and cells reactive to its isoforms ALDH1A1 and ALDH1A3, respectively (shown in Fig. 1.).

ALDH1 distribution pattern was nearly balanced in the muscle groups investigated in the present study (pectoralis, diaphragm, psoas; shown in Tab. 1. B). Quantitative analysis using the pan-ALDH1 antibody including all ALDH1 isoforms showed a mean value of 31.5 ALDH1 positive cells per 50 fibers, and there was a trend of more ALDH1A3 immunoreactive cells (mean value 10.2) than ALDH1A1 (mean value 7.6). The majority of ALDH1A1 (mean value 12) and ALDH1A3 positive cells (mean value 12.5) could be found in pectoralis muscle (shown in Tab. 1. B).

Analysis of SCs in skeletal muscle using CD56 and Pax7 antibodies showed a mean value of 19.4 CD56 and 10.8 Pax7 positive cells per 50 fibers. In detail, the majority of CD56 immunoreactive cells could be found in pectoralis muscle and diaphragm (mean 22.5 and 22, respectively) compared to psoas muscle (mean value 13.8; shown in Tab. 1. A). In contrast, a balanced distribution of Pax7 immunoreactive SC was observed in all muscle groups (shown in Tab. 1. A).

Co-immunofluorescent staining illustrated the majority of CD56 positive SCs with a co-expression of cytoplasmatic ALDH1 (shown in Fig. 2. A, B). The majority of Pax7 positive SCs co-expressed the isoforms ALDH1A1 and ALDH1A3, respectively (shown in Fig. 2 C, D). Non-SCs expressing ALDH1 were most likely to be immunohistochemically CD68 positive macrophages.

Discussion

Satellite cells (SCs) are the stem cells of skeletal muscle and therefore an important cell population for muscle growth and myotube formation. SCs are not only physiologically regulated through growth and differentiation signals, but also activated for regenerative responses by adverse stimuli, including inflammation or insults [2]. Interestingly, the enzyme aldehyde dehydrogenase 1 (ALDH1) plays a functional role both in differentiation by regulation of retinoic acid (RA) metabolism [7] and in detoxification of oxidative stress products [5]. ALDH enzymatic activity has been attributed to SCs in experimental studies [2,3]. Thus, the enzyme ALDH1 could be a potential mediator between physiological mechanisms and regenerative processes of SC regulation.

In the present study, we investigated human skeletal muscle tissues for the expression of ALDH1 and its isoforms ALDH1A1 and ALDH1A3. By immunohistochemistry we were able to demonstrate ALDH1 expression in all included muscle groups. More importantly, ALDH1 positive cells were found in the peri-fascicular stem cell niche and morphologically resembled SCs. They co-localized with CD56 and Pax7 expressing SCs, and CD56 and PAX7 immunoreactive cells showed ALDH1 expression by co-immunofluorescence. Evaluation of ALDH1A1 and ALDH1A3 isoforms did not show significant differences in the staining pattern compared to pan-ALDH1.

We used human skeletal muscle tissue from autopsies without clinical history of muscle disease or pathological finding in muscle tissue during autopsy. Therefore, tissue samples represented normal skeletal muscle and allowed an insight into ALDH1 histology in human SCs under physiological conditions. Our results clearly indicate that ALDH1 and its isoforms ALDH1A1 and ALDH1A3 are expressed by SCs in human skeletal muscle tissue.

Our data confirmed previous results from experimental findings [1,8]. In these experiments, myoblasts were isolated by their ALDH enzymatic activity using the Aldefluor assay and sorted by fluorescence-activated cell sorting (FACS). [1] (2009) showed that sorted human ALDH positive progenitor cells contained after cultivation a CD56- and ALDH-positive subpopulation with high myogenic differentiation potential both in vitro and in a xenograft transplantation model. In their study they used an anti-ALDH1 antibody. Based on the enzymatic activity and the immunofluorescence signal they claimed that this antibody was specific for the ALDH1A1 isoform. In our experiments, we used three different antibodies that were not available at the time of their study and could show the presence of ALDH1 and its isoforms by the use of a pan-ALDH1 antibody as well as antibodies against ALDH1A1 and ALDH1A3, respectively.

[8] (2011) used ALDH positive progenitors from a broad range of species including human muscle tissue. They confirmed the prior results and showed a high myogenic potential of ALDH expressing cells. Moreover, they were able to demonstrate that ALDH positive cells were more resistant against oxidative (H_2O_2) and inflammatory (TNF-α) stress, respectively, indicating a role in detoxification of SCs. By lentiviral knock-down they identified the ALDH1A1 as the isoform responsible for enzymatic activity.

More recently, [3] (2020) investigated human ALDH positive skeletal muscle cells after Aldefluor fluorescent activated cell sorting (FACS) for the expression of ALDH isoforms and identified different isoforms including ALDH1A1 and ALDH1A3 that have been also demonstrated in our results. Moreover, they showed changes in the expression pattern between control individuals and patients suffering from Duchenne's Muscle Dystrophy (DMD). However, these patterns were not identical in human DMD skeletal muscles compared with muscle tissue from a dog DMD model.

Quantitative analysis of the immunohistochemical staining results revealed higher numbers of CD56 positive SCs in pectoralis muscle and diaphragm compared to psoas muscle but a more homogenous distribution of Pax7 positive SCs in all muscle groups of our study. The number of CD56 positive cells was higher than Pax7 positivity. CD56 is an established marker for SCs in all developmental stages, whereas Pax7 is only expressed upon proliferative action [9]. ALDH1 was almost equally present in pectoralis and psoas muscle, and a trend of higher expression of isoform ALDH1A3 in comparison to ALDH1A1 could be detected. It is most likely that the low frequency of

ALDH1 positive non-SCs represent CD68 positive macrophages, which are known to be positive for ALDH1 [10].

Our data corroborate prior results from experimental studies and show that ALDH1A1 and ALDH1A3 are expressed in human skeletal muscle SCs where they could be involved in differentiation and regenerative functions. The identification of ALDH1 isoforms in SC biology is of particular interest since the functional processes regulating SC activation including asymmetric division and senescence are still poorly understood. Interference with ALDH enzymatic activity could then open novel approaches for the treatment of muscular diseases **(Figures 1 and 2) (Table 1).**

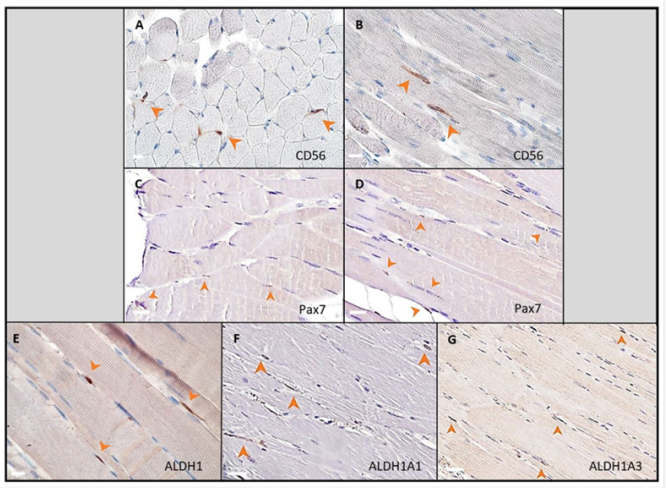

Figure 1: Immunostaining of CD56, Pax7, ALDH1 and isoforms ALDH1A1, ALDH1A3, respectively, in human skeletal muscle.

A, B: CD56 positive SC in cross (A) and longitudinal orientated (B) sections of psoas muscle | **C, D:** Pax7 positive SC in psoas (C) and pectoralis muscle (D)| **E, F:** ALDH1 positive cells in psoas (E) and pectoralis muscle (F) | **G:** ALDH1A1 positive cells in pectoralis muscle.

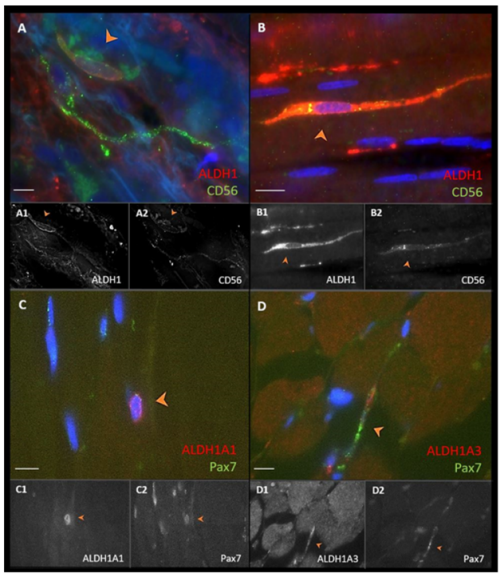

Figure 2: Co-immunofluorescent staining of CD56-ALDH1, Pax7-ALDH1A1 and Pax7-ALDH1A3 in longitudinal orientated human skeletal muscle.

A, B: Merge of CD56-ALDH1 coexpression in SC diaphragm (A) and psoas muscle (B) | **A1, A2:** Single captures of ALDH1 (A1) and CD56 (A2) positive cells | **B1, B2:** Single captures of ALDH1 (B1) and CD56 (B2) | **C:** Merge of Pax7-ALDH1A1 co-expression in SC in pectoralis muscle | **C1, C2:** Single captures of ALDH1A1 (C1) and Pax7 (C2) positive cells| **D:** Merge of Pax7-ALDH1A3 co-expression in SC pectoralis muscle | **D1, D2:** Single captures of ALDH1A3 (D1) and Pax7 (D2) positive cells.

Table 1 Quantitative analysis of immunohistochemistry: Mean value of immunoreactive cells of anti-CD56 and anti-Pax7 **(A)** as well as anti-ALDH1, anti-ALDH1A1, anti-ALDH1A3 **(B)** antibody staining counted in 50 fibres per sample.

A	**CD56 positive cells**		**Pax7 positive cells**	
	Tissue	**Mean value per 50 fibres**	**Mean value per 50 fibers**	
	M. pectoralis	22.5	12	
	Diaphragm	22	11.5	
	M. psoas	13.8	9	
	Total	19.4	10.8	
B	**ALDH1 positive cells**		**ALDH1A1 positive cells**	**ALDH1A3 positive cells**
	M. pectoralis	21.7	12	12.5
	Diaphragm	50	6.5	11.5
	M. psoas	22.7	4.5	6.5
	Total	31.5	7.6	10.2

References

1. Metze D, Oji V. “Palmoplantar keratodermas.” *Dermatology* 4th ed. *Philadelphia (PA): Elsevier Health Sciences* (2018) 924–43
2. Oztas Pinar, Nuran Alli, Muhterem Polat, Şerife Dagdelen and Hüseyin Üstün et al."Punctate Palmoplantar Keratoderma." *Am J Clin Dermatol* 8(2007):113-6.
3. Guerra L, M Castori, B Didona, D Castiglia and G. Zambruno. "Hereditary palmoplantar keratodermas. Part I. Non-syndromic palmoplantar keratodermas: classification, clinical and genetic features." *J Eur Acad Dermatol Venereol.*32(2018): 704-19.
4. Stanimirović Andrija, Aleksej Kansky, A Basta-Juzbašić, M Skerlev and Teodora Beck. "Hereditary palmoplantar keratoderma, type papulosa, in Croatia." *J Am Acad Dermatol.* 29 (1993): 435-7.
5. Gao M, S Yang, M Li, KL Yan and YX Jiang et al. "Refined localization of a punctate palmoplantar keratoderma gene to a 5· 06-cM region at 15q22. 2–15q22. 31." *Br J Dermatol.*152(2005): 874-8.
6. Griffiths WAD, Judge MR, Leigh IM. "Disorders of keratinization."*Textbook of Dermatology. 6th ed. Blackwell Science*(1998): 1483-588.
7. Horikoshi Mariko, Kei Kuroda, and Shingo Tajima. "Punctate palmoplantar keratoderma with pigmentary lesions on the dorsa of feet and ankles: successful treatment with a combination of low-dose oral etretinate and topical calcipotriol." *J Dermatol.* 31(2004): 469-72.
8. Giehl Kathrin A, Gertrud N Eckstein, Sandra M Pasternack, Silke Praetzel-Wunder and Thomas Ruzicka et al. "Nonsense mutations in AAGAB cause punctate palmoplantar keratoderma type Buschke-Fischer-Brauer." *Am J Hum Genet.*91(2012): 754-9.
9. Bennion Scott D and James W Patterson. "Keratosis punctata palmaris et plantaris and adenocarcinoma of the colon: a possible familial association of punctate keratoderma and gastrointestinal malignancy." *J Am Acad Dermatol* 10(1984): 587-91.
10. Stevens HP and IM Leigh. "The inherited keratodermas of palms and soles." *Dermatology in General Medicine,New York*, 1(1999):603-613.
11. Hesse S, PH Berbis and Y Privat. "Keratodermia palmo-plantaris papulosa (Buschke-Fischer's disease): efficacy of acitretin." *Br J Dermatol.* 128(1993): 104-5.

How to cite this article: Laura Sophie Rihani*, Friederike Liesche-StarneckerandJürgen Schlegel. “Human Skeletal Muscle Satellite Cells Co-Express Aldehyde Dehydrogenase Isoforms Aldh1A1 and Aldh1A3.” *J Cytol Histol* 12 (2021): 568.