Original Paper

Cellular activation patterns of CD10+ fibro-adipogenic progenitors across acquired disease states in human skeletal muscle biopsies

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Abstract

Background: Fibro-adipogenic progenitors (FAP) are muscle resident mesenchymal stem cells pivotal for regulation of myofiber repair. Experimental results show in addition involvement in a range of other pathological conditions and potential for pharmacological intervention. FAP histopathology in human muscle biopsies is largely unknown, but has potential to inform translational research.
Methods: CD10+ FAPs in 32 archival muscle biopsies from 8 groups (normal, dermatomyositis, inclusion body myositis (IBM), anti-synthetase syndrome, immune-mediated necrotizing myopathy (IMNM), denervation, type 2 atrophy, rhabdomyolysis) were visualized by CD10 immunohistochemistry and their histology compared. Groups are compared by semi-quantitative scoring.
Results: Histological activation of endomysial CD10+ FAPs includes prominent expansion of a network of cell processes surrounding muscle fibers, as well as endomysial cell clusters evidencing proliferation. Prominence of periarteriolar processes is a notable feature in some pathologies. FAP activation is often associated with fiber degeneration/regeneration, foci of inflammation, and denervation in keeping with experimental results. Type 2 atrophy shows no evidence of FAP activation. Dermatomyositis and anti-synthetase syndrome associated myositis demonstrate diffuse activation.
Conclusion: Assessment of CD10+ FAP activation is routinely possible using CD10 immunohistochemistry and demonstrates several patterns in keeping with preclinical results. Prominent expansion of FAP processes surrounding myofibers suggests enhanced interaction between myofiber/basement membranes and FAPs during activation. The presence of diffuse FAP activation in dermatomyositis biopsies unrelated to fiber repair raises the possibility of FAP activation as part of the autoimmune process. Future diagnostic applications, clinical significance and therapeutic potential remain to be elucidated.

Keywords: Fibro-adipogenic progenitor, Inflammatory myopathy, Denervation, CD10, Periarteriolar, Type 2 atrophy

Introduction

Fibro-adipogenic progenitor cells (FAPs) are muscle resident mesenchymal stem cells with potential to differentiate along a variety of lineages (fibrogenic, adipogenic, osteogenic) as well as several defined functions in muscle regeneration and tissue homeostasis. Elegant mouse studies have conclusively outlined their central role in determining muscle fiber repair or fibrosis after acute injury via cellular interactions with myogenic muscle stem cells (satellite cells) and macrophages [18]. Subsequent studies have demonstrated their involvement in mediating neurogenic atrophy, highlighting involvement in two very different types of tissue response [9, 20]. More specific pathomechanistic roles have been demonstrated experimentally in the context of muscular dystrophies (dystrophinopathies; dysferlinopathy, facioscapulohumeral muscular dystrophy, fibrodysplasia ossificans progressiva, posttraumatic fibroadipogenic muscle degeneration (rotator cuff tears), and fibro-fatty muscle degeneration in the context of systemic disease (chronic renal insufficiency, type 2 diabetes), and senescent cell states in inflammatory myopathies [5, 6, 14, 16-19, 21, 22, 25].

Quiescent FAPs are present in endomysial, perimysial, epimysial and perivascular locations [3]. Within the endomysium, FAPs are situated in the interstitial space outside basement membranes. Endomysial FAPs form an interconnected network of processes on the surface of muscle fibers and extending along capillaries. CD10 is a robust and widely available marker for FAPs in human muscle [12]. In response to activating signals, FAPs can initiate intercellular communications, clonal expansion, differentiation, or subsequent apoptosis [3, 30]. Morphological changes in response to activation in human skeletal muscle are not well delineated.

While the involvement of FAPs in various pathogenic contexts is coming to light, single-cell RNA sequencing has uncovered transcriptomic heterogeneity of molecular FAP subpopulations in animal models and in humans [7, 13, 15, 21]. Recent evidence indicates, for instance, that a CD90+ FAP subpopulation increases selectively in skeletal muscle of patients with type 2 diabetes [6]. In particular, CD10 has been recently reported to identify a subset of these cells with high adipogenic potential [7]. These data suggest that quiescent FAPs can evolve into a variety of transcriptomic subtypes in the context of a given pathological condition, whose exact biological significance and potential for therapeutic intervention often remain to be elucidated.

Experimental proof of concept studies have shown that targeting FAPs pharmacologically has therapeutic potential. Direct interruption of specific FAP signaling pathways with tyrosine kinase inhibitors can reduce fibrosis in a dystrophinopathy mouse model [18], indirect modulation of the muscular cytokine environment can result in FAP mediated augmentation of muscle regeneration [4], and the beneficial effects of exercise on senescent muscle are partly mediated by FAPs [25]. In view of these results, understanding pathogenetic contributions of FAPs in a wider range of human myopathies is of translational interest.

Little is known about the histological appearance of activated FAPs in human skeletal muscle biopsies across various myopathies. The majority of FAP studies have been performed in well controlled mouse models, mostly with changes in FAP cell numbers as a measure of activation [17, 18, 20, 21, 25]. Better understanding of histopathological reaction patterns of FAPs on muscle biopsy may help to screen a wide range of conditions for potentially pathological FAP activation. The aim of this study was to delineate histological activation patterns of FAPs in human skeletal muscle biopsies to allow for immunohistochemical screening and evaluation of FAP activation in various conditions. We compared histopathological changes of CD10+ FAPs in six frequent disease conditions seen in clinical muscle biopsies.

Methods

The study was approved by the Clinical Research Ethics Board of the University of British Columbia.

Chart review and biopsy selection

Muscle biopsies taken between 2010 and 2018 were selected from the archive of the Muscle Pathology Laboratory at Vancouver General Hospital

for nine groups: Normal (N), anti-synthetase syndrome associated myositis (ASA-M), dermatomyositis (DM), inclusion body myositis (IBM), immune mediated necrotizing myopathy (IMNM), type 2 fiber atrophy (T2A), neurogenic atrophy (NA), and rhabdomyolysis (RM). Pathological categories were selected to represent histopathologically well defined acquired myopathic conditions. Electronically available medical records were reviewed. Normal biopsies were chosen based on the absence of abnormalities on routine clinical biopsy evaluation and chart review negative for muscular conditions.

Histology and chromogen immunohistochemistry

Paraffin sections (4 µm) were used for evaluation in order to take advantage of established CD10 immunohistochemical protocols. HE stained sections were prepared in the usual manner. CD10 was used as marker for FAPs based on poor performance of PDGFR-α immunohistochemistry in human muscle samples and previous work by Hejbol et al lending support to the use of CD10 as FAP marker in human muscle tissue [12]. All immunohistochemical stains were performed on a Dako Omnis instrument with heat epitope retrieval for 30 min at 97C and pH 9.0. The CD10 antibody used was Dako ready to use monoclonal, clone 56C6 (Agilent, Santa Clara, USA), with DAB chromogen.

Fluorescence immunohistochemistry

Normal human muscle frozen section (8 µm) was fixed in cold Acetone/ Ethanol (1:1) for 10 min, followed by washing in PBS for 30 min. After blocking in 20% normal goat serum for 30 min, sections were concomitantly incubated overnight at 4°C with CD10 antibody, clone 56C6 (Bio-Rad, MCA1806T, isotype mouse IgG1, dilution 1:40), and Laminin-2-alpha antibody, clone 4H8-2 (Abcam, ab11576, isotype rat IgG1, dilution: 1:200). After washing, section was stained for 2 hrs at room temperature with secondary antibodies goat –anti mouse IgG1 Alexa Fluor 647 (Thermo Fisher, 21240, dilution 1:1000) and Goat-anti-Rat IgG Alexa Fluor 488 (Thermo Fisher, A-11006, dilution 1:1000). Hoechst 33342 was used for nuclear staining.

Histopathological review

Stained sections were reviewed by an experienced myopathologist (PWS). Qualitative changes were captured descriptively on initial review. The type of qualitative changes found was suggestive of a semi-quantitative scale to compare changes between biopsies (Table 2. Figure 4.). This semi-quantitative scale could be consistently applied to all biopsies. The scoring system is entirely descriptive at this stage and any potential pathological significance could be addressed in subsequent studies.

Results

Patient characteristics

We reviewed muscle biopsies from 32 patients summarized in table 1. The age range was 39 – 82 years, biopsied muscles included quadriceps, biceps, gastrocnemius, and, in a single case, deltoid. Gastrocnemius biopsies are over-represented in the neurogenic atrophy group since these biopsies were often taken concomitantly with sural nerve biopsies. There is a slight male predominance in the IBM group in keeping with disease epidemiology. The selection of normal controls was based on normal myopathological examination and absence of neuromuscular disease upon retrospective chart review. Clinical indications for biopsies with normal muscle histology are listed. Serological information for patients with inflammatory myopathies was available in 6/9 cases and is presented in table 1. Diagnosis of inflammatory myopathies in cases without serological information is based on clinico-pathological correlation.

CD10+ FAPs in normal skeletal muscle biopsies

The histological appearance of FAPs revealed by CD10 immunohistochemistry was consistent with expectations based on previous studies [12]. Cross sections of skeletal muscle showed scattered concave triangular or quadrangular cell bodies in endomysial corners between muscle fibers (Fig. 1A).

Case	Diagnosis	Age /years	Sex	Muscle	Clinical	MSA
1	N	58	F	Q	Chronic fatigue	
2	N	56	M	Q	Sensory-motor polyneuropathy	
3	N	71	F	B	SLE, sclerosing cholangitis, and liver Tx	
4	N	51	F	Q	Chronic fatigue	
5	N	67	F	B	Suspect mitochondrial disease	
6	ASA-M	42	F	Q	Anti-synthetase syndrome	Jo1
7	ASA-M	52	M	B	Polymyositis	Jo1
8	ASA-M	52	M	Q	Polymyositis with ILD	Jo1
9	DM	81	F	B	Dermatomyositis	N/A
10	DM	61	F	B	Dermatomyositis	Mi2
11	DM	50	M	B	Dermatomyositis	N/A
12	IBM	67	M	B	IBM	
13	IBM	82	M	B	IBM	
14	IBM	73	M	B	IBM	
15	IBM	74	M	B	IBM	
16	IBM	61	F	B	IBM	
17	IBM	82	F	Q	IBM	
18	IMNM	53	M	Q	Statin associated myositis	HMGCR
19	IMNM	57	M	B	Statin associated myositis	N/A
20	IMNM	66	F	B	Statin associated myositis	HMGCR
21	T2A	58	F	n/a	Multiple morbidities	
22	T2A	74	F	B	Suspect Parkinson plus syndrome	
23	T2A	82	M	Q	Gait disturbance	
24	T2A	44	F	Q	Leg pain	
25	T2A	75	M	D	Recurrent lung carcinoma	
26	NA	39	F	G	Peripheral neuropathy	
27	NA	57	M	G	ALS	
28	NA	67	M	Q	Motorneuron-syndrome	
29	NA	58	F	G	Polyneuropathy	
30	RM	72	M	Q	Rhabdomyolysis uncertain cause	
31	RM	68	F	n/a	Rhabdomyolysis and renal failure	
32	RM	72	F	n/a	Rhabdomyolysis and sepsis	

Table 1. Clinical and serological characteristics of study cases. N: Normal; ASA-M: Anti-synthetase autoantibody associated myositis; DM: Dermatomyositis; IBM: Inclusion body myositis; IMNM: Immune mediated necrotizing myopathy; T2A: Type 2 fiber atrophy; NA: Neurogenic atrophy; RM: Rhabdomyolysis; MSA: Myositis-specific-antibody; Q: Quadriceps femoris; B: Biceps brachii; D: Deltoid; G: Gastrocnemius.

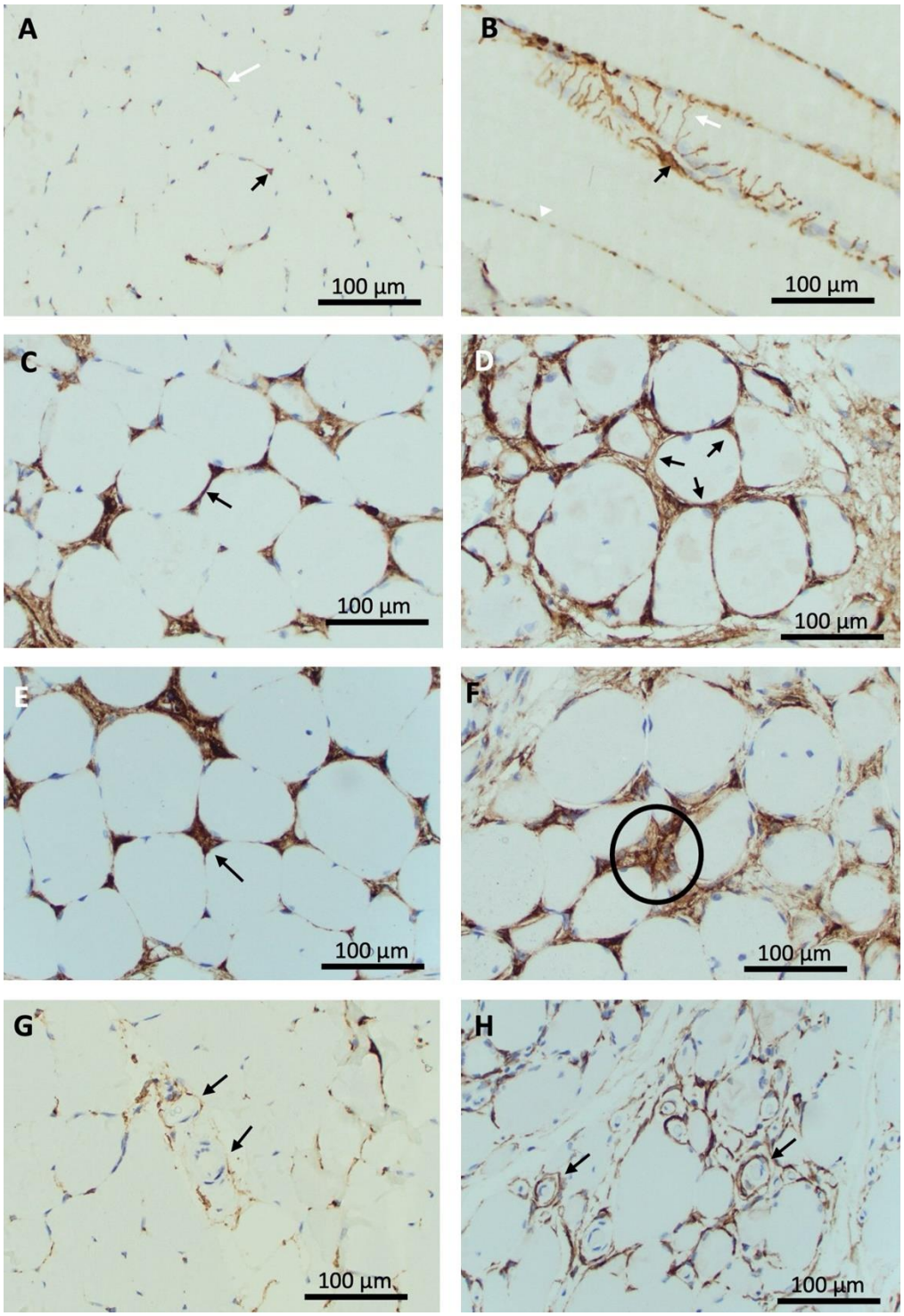

Figure 1. Resting and activated histology of CD10+ fibro-adipogenic progenitors in human skeletal muscle.

A-B: Normal muscle demonstrates occasional triangular staining cell bodies in endomysial corners (black arrow) and delicate linear staining processes along endomysial lines (white arrow) on cross section (**A**). Longitudinal sections (**B**) show plump cell bodies (black arrow) with processes extending around muscle fibers (white arrow). These appear as dot-like staining on deeper planes (white arrowhead).
C-D: Activated FAPs can show segmental ropey expansion of endomysial processes (black arrow) along one side of a muscle fiber only (**C**), or circumferentially (**D**, black arrows).
E-F: Endomysial corners are significantly expanded in **E** (black arrow), indicating enlarged or aggregated cell bodies. Multiple nuclei in endomysial corners in **F** (black circle) are consistent with cell proliferation.
G-H: Periarteriolar staining is sparse in normal biopsies (**G**, black arrow), but can increase significantly in activated states (**H**, black arrow).

Linear endomysial spaces between muscle fibers show occasional wispy lines of CD10 positivity, corresponding FAP cell processes running along the surface of muscle fibers in a transverse plane. These processes are clearly visible on longitudinal sections tangential to the surface of muscle fibers, which show delicate branching processes embracing muscle fibers (Fig. 1B). Longitudinal sections through the body of muscle fibers demonstrate dot-like staining in the endomysial space, representing delicate processes in cross section. There is band-like or coarsely granular staining along capillaries, which likely reflects the known proclivity of FAPs to arrange along capillaries. Small vessels with thicker walls, likely arteriolar, show delicate sparse perivascular staining (Fig. 1G).

Localization of CD10+ FAPs outside basement membrane was confirmed on immunofluorescent triple labelling (Fig. 2).

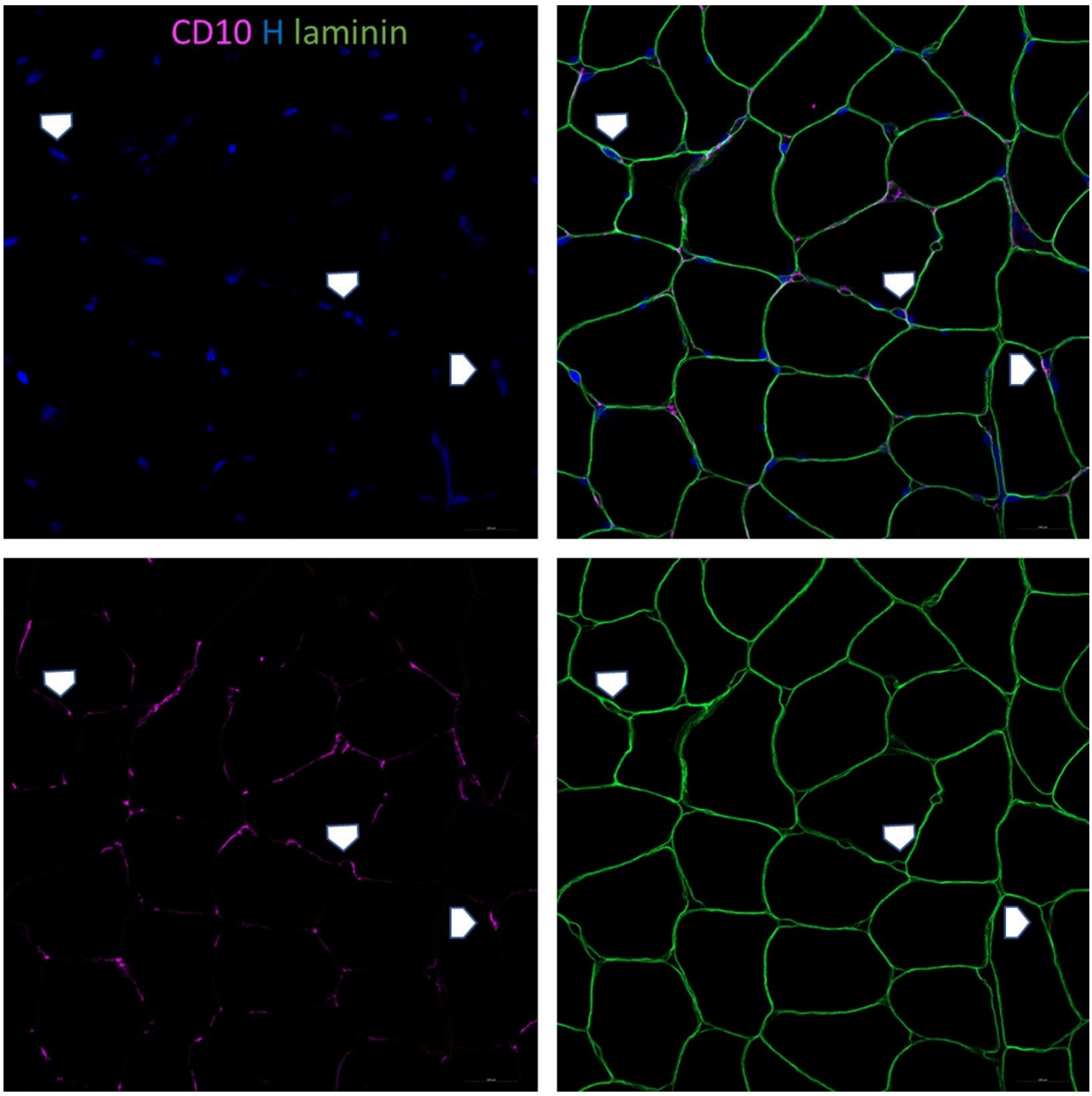

Figure 2. Normal muscle biopsy with immunofluorescent labelling for laminin, CD10, and Hoechst demonstrates location of plump shaped CD10+ cells bodies and wispy processes outside the basal lamina in the endomysial space, in keeping with the expected location of CD10+ FAPs.

Cellular changes of CD10+ FAPs in pathological muscle biopsies

Qualitative review showed changes to the appearance of cell processes as well as to cell bodies in pathological biopsies. Processes frequently appear as ropey and coarse linear staining along endomysial lines, either segmental or circumferential around muscle fibers, indicative of expanded, hypertrophic FAP process networks (Fig. 1C, D). Longitudinal sections demonstrate broad sheets of CD10 positivity instead of delicate thread-like processes. Endomysial corners can show enlarged, prominent, bulky staining, indicative of expanded or conglomerated cell bodies. Several biopsies show clusters of nuclei in a CD10 positive background, indicating cell clusters as opposed to single, slender angulated cell bodies in normal biopsies (Fig. 1E, F).

A subset of biopsies demonstrated strong circumferential staining around small vessels with a thin muscularis, consistent with enhanced periarteriolar cell processes. This contrasts with sparse delicate periarteriolar staining in normal biopsies (Fig. 1G, H).

Overall, the observed changes can be placed into three categories: (i) Expansion of FAP processes represented by ropey thickening and circumferential staining along endomysial lines; (ii) Proliferation and hypertrophy of cell bodies in endomysial corners, represented by the presence of cell clusters; (iii) Expansion of periarteriolar processes, indicated by circumferential coarse staining around thicker-walled small vessels. These changes did not occur in unison and each component could be seen in isolation or in conjunction with others.

For semi-quantitative evaluation of the cellular activation of FAPs we developed a scoring system based on these observations, capturing process expansion, cell clusters, and periarteriolar staining. The scoring system is summarized in table 2. Exemplary images for semi-quantitative evaluation are presented in Fig. 3.

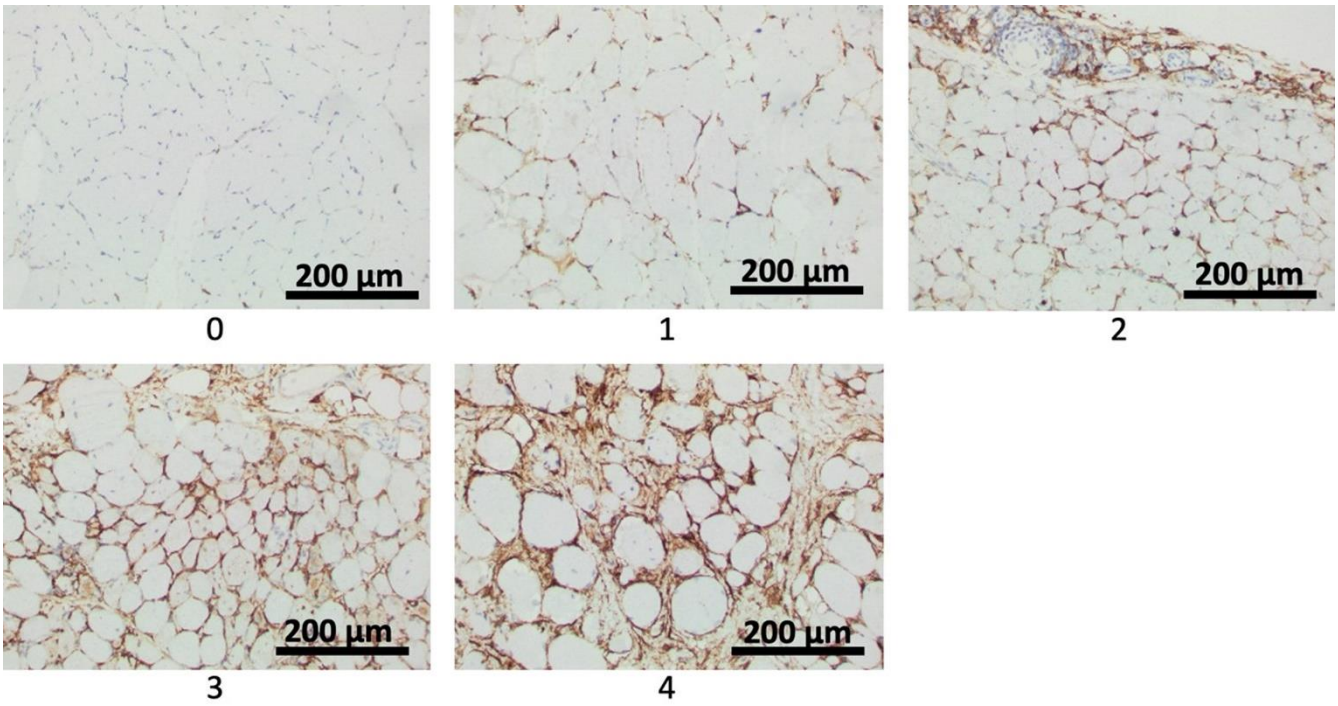

Figure 3. Exemplary photographs representing semiquantitative scores 1 to 4 of endomysial FAP process activation as defined in Table 2. With increasing severity, ropey processes are seen along increasing numbers of myofibers, eventually showing circumferential staining around a few and many fibers for scores 3 and 4, respectively.

Endomysial FAP Score	
FAP Processes	
0	Wispy processes along rare fibers
1	Ropey processes along occasional fibers, not circumferential
2	Ropey processes along numerous fibers, not circumferential
3	Ropey processes along numerous and circumferential processes around occasional fibers
4	Circumferential ropey processes around numerous fibers
FAP Cell Clusters	
0	None
1	Single isolated
2	Occasional (1-2/mm^2)
3	Few (3-6/mm^2)
4	Frequent (>6/mm^2)
Periarteriolar FAP Score	
0	None
1	Sparse
2	Occasional
3	Moderate
4	Frequent
Definitions and Comments	
Scoring	Highest score typically associated with areas of pathology on a given section in an area of 1 mm^2.
Ropey	Thickening of cell processes along periphery of muscle fiber, often with expanded staining of endomysial corners; exclude staining related to vessels or to Schwann cells of endomysial nerve twigs.
Cell Cluster	Two or more adjacent nuclei with CD10+ cell bodies, often in endomysial corners.
Periarteriolar	Circumferential arteriolar CD10+ processes.

Table 2. Semi-quantitative scoring system for endomysial and periarteriolar FAP activation on CD10 immunohistochemically stained cross sections of skeletal muscle. Endomysial activation involves cell process expansion and/or evidence of proliferation in the form of cell clusters. Periarteriolar activation is represented by arterioles entirely surrounded by CD10+ processes. For illustration of histological changes see figure 1. For exemplary photos of endomysial FAP process activation scores see figure 4.

Immunoreactivity for CD10 of non-FAP structures in muscle biopsies

CD10 labels FAPs in human muscle biopsies, but it is not a specific marker. Its utility derives from a combination of the characteristic morphology of FAPs with CD10 positivity. Other histologically easily identifiable structures can label for CD10. Sarcoplasm of regenerating fibers can show positivity for CD10 [2], as was apparent in biopsies for IMNM and rhabdomyolysis (Fig. 4E, H). Myelin sheaths and perineurium label with CD10 [12], seen in a small nerve branch in the normal biopsy represented in Fig. 4A. Although CD10 is expressed in early lymphoid progenitors and germinal center cells, these are not normally part of inflammatory infiltrates in muscle, unless histologically distinct lymph-follicle-like-structures are present as part of an inflammatory process, or there is involvement by CD10+ lymphoma.

Endomysial and periarteriolar CD10+ FAP activation across pathological groups

Patterns of CD10+ FAP activation differed across disease groups. Typical photographs are presented in Fig. 4, scoring results are summarized in Fig. 5. Detailed correlation of scoring results, HE pathology, and distribution of CD10+ FAP activation is presented in table 3.

Biopsies from the anti-synthetase syndrome group showed strong diffuse activation of FAPs with often circumferential processes and prominent FAP cell clusters. On HE stained sections, scattered or perifascicular de- and regenerating fibers were apparent, which demonstrated sarcoplasmic labelling on CD 10 immunohistochemistry. FAP activation was diffuse and as such included activation around normal appearing fibers as well as activation adjacent to degenerating and regenerating fibers (Fig. 4B).

Dermatomyositis biopsies showed impressive diffuse circumferential ropey staining in regions of fiber atrophy but notably also in areas of fibers without evidence of de- or regeneration. In addition, there were clusters of cell proliferation, though less pronounced than in ASA-M biopsies. The cause of diffuse and pronounced FAP activation was not obvious on this study in the absence of evidence of fiber de- or regeneration, inflammatory infiltrates, or neurogenic change (Fig. 4C).

IBM biopsies showed staining associated with inflammatory foci or regions of chronic myopathic change and fibrosis only, often with cell clusters. This could be interpreted as in keeping with a role of FAPs in fiber repair and fibrosis (Fig. 4D). Interestingly, IBM biopsies tended to show prominent periarteriolar staining, the significance of which is uncertain (Fig. 5B).

Immune mediated necrotizing myopathies showed multifocal increased staining restricted to degenerating/regenerating fibers, with a mix of linear coarseness and expanded endomysial corners, consistent with fiber repair (Fig. 4E).

Type 2 fiber atrophy did not show significant abnormal staining and has the same profile on scores as normal biopsies (Figs. 4F; 5A). In contrast, biopsies with neurogenic atrophy clearly exhibited increased ropey staining surrounding muscle fibers without evidence of FAP proliferation, as is also apparent on the scoring profile (Figs. 4G; 5A). Neurogenic biopsies showed in addition significant periarteriolar activation (Fig. 5B). Experimental evidence suggests a causal role of FAPs in neurogenic fiber atrophy. The absence of evidence of FAP proliferation raises the possibility that this effect may primarily go along with cell process expansion in human skeletal muscle.

Biopsies from patients with rhabdomyolysis represent a histologically more diverse group with biopsies taken at various stages of degeneration or regeneration and with overall variable severity. These biopsies showed variable interstitial staining centered on abnormal fibers and frequent fibers with sarcoplasmic positivity in keeping with fiber regeneration (Fig. 4H).

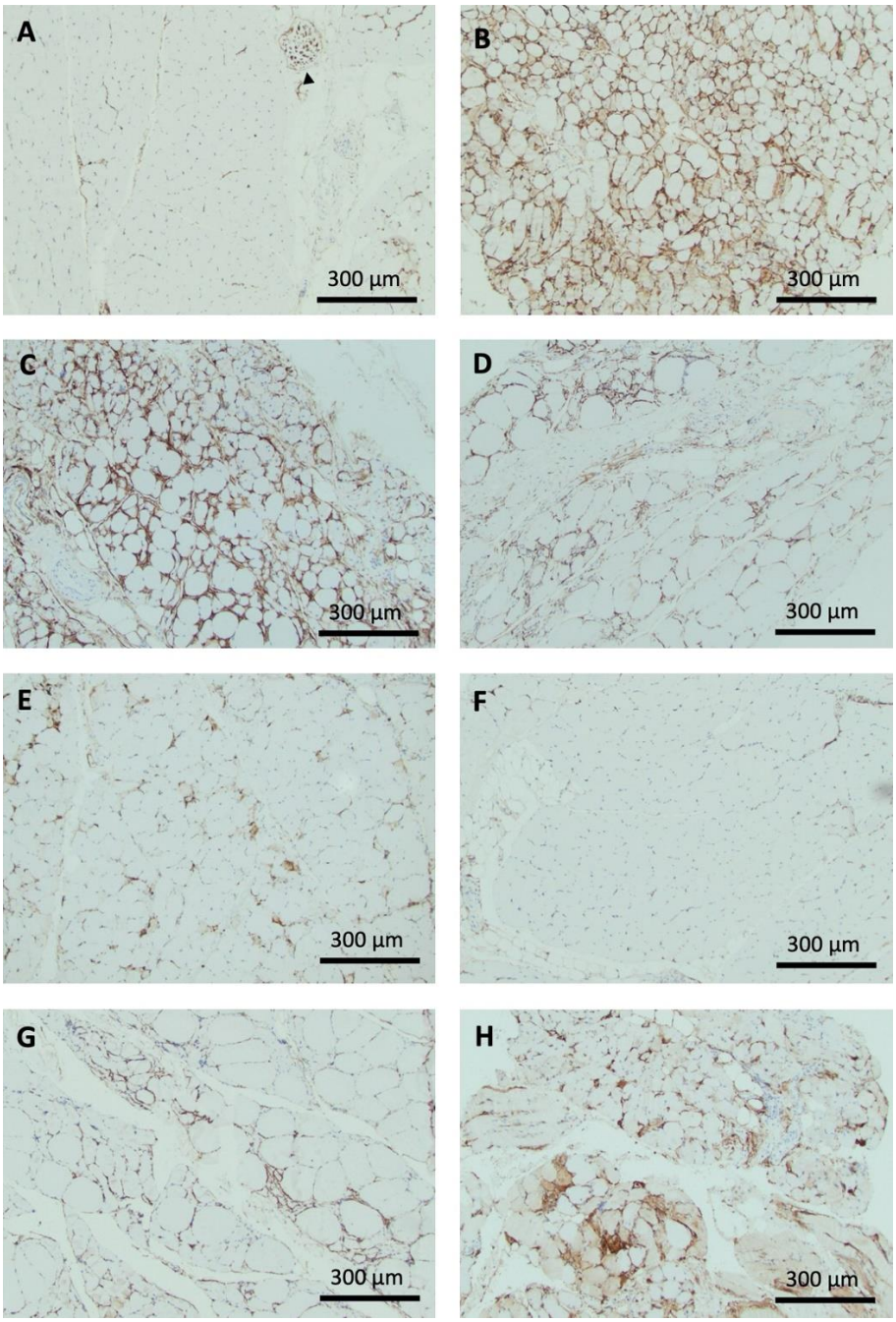

Figure 4. Exemplary patterns of CD10+ FAP activation across pathological groups.
A: Normal muscle biopsy. Occasional triangular cell bodies in endomysial corners and granular staining along capillaries running transversely. There is in addition a cross section of peripheral nerve with CD10 immunoreactivity of perineurium and myelin sheaths (black arrowhead).
B: Anti-synthetase syndrome. Strong immunoreactivity of endomysial processes and expansion of endomysial corners with circumferential staining around several fibers.
C: Dermatomyositis. Diffuse presence of ropey endomysial staining, some circumferential. Several expanded endomysial corners.
D: Inclusion Body Myositis. Multifocal ropey positivity associated with endomysial cellular infiltrates and fiber size variability.
E: Immune mediated necrotizing myopathy. Multifocal ropey positivity associated with scattered degenerating and regenerating fibers with sarcoplasmic staining in regenerating fibers.
F: Type 2 atrophy. No significantly abnormal CD10 staining.
G: Neurogenic atrophy. Increased ropey staining in particular in regions of atrophic fibers.
H: Rhabdomyolysis. Multifocal ropey positivity or expansion of endomysial corners. Note strong sarcoplasmic labelling in several regenerating fibers.

Case	Diagnosis	FAP Processes	FAP Cell Clusters	FAP Periarteriolar	HE Pathology	Distribution of CD10 staining
1	N	1	0	0	normal	normally scattered
2	N	1	0	0	normal	normally scattered
3	N	0	0	0	normal	normally scattered
4	N	1	0	0	normal	normally scattered
5	N	0	0	1	normal	normally scattered
6	ASA-M	4	3	3	pf and scattered d&r, numerous, lymphocytic clusters	around d&r; focally without obvious correlation with fiber pathology
7	ASA-M	2	3	2	scattered d&r, occasional	around d&r; focally without obvious correlation with fiber pathology
8	ASA-M	4	3	0	pf and scattered d&r, numerous, lymphocytic clusters	around d&r; focally without obvious correlation with fiber pathology
9	DM	4	2	2	pf atrophy, fibrosis, pm lymphocytes	diffusely without obvious correlation with fiber pathology or atrophic regions
10	DM	2	0	0	pf atrophy, focal mild	diffusely without obvious correlation with fiber pathology or atrophic regions
11	DM	2	1	0	pf atrophy, focal mild	diffusely without obvious correlation with fiber pathology or atrophic regions
12	IBM	2	2	2	cm, endomysial lymphocytes, mild	regions of lymphocytic inflammation
13	IBM	2	1	2	cm, endomysial lymphocytes, mild	regions of lymphocytic inflammation
14	IBM	3	3	3	cm, endomysial lymphocytes, moderate	regions of lymphocytic inflammation
15	IBM	2	1	3	cm, endomysial lymphocytes, moderate	regions of lymphocytic inflammation
16	IBM	1	1	3	multifocal endomysial lymphocytes	regions of lymphocytic inflammation
17	IBM	2	2	4	cm, endomysial lymphocytes, moderate to severe	regions of lymphocytic inflammation

18	IMNM	2	1	0	scattered d&r, mild	around d&r
19	IMNM	1	1	0	scattered r, mild	around d&r
20	IMNM	2	2	0	scattered d&r, prominent capillaries, moderate	around d&r
21	T2A	0	0	0	biphasic fiber size distribution, moderate	normally scattered
22	T2A	0	0	0	biphasic fiber size distribution, severe	normally scattered
23	T2A	1	0	0	biphasic fiber size distribution, moderate	normally scattered
24	T2A	0	0	0	biphasic fiber size distribution, moderate	normally scattered
25	T2A	1	0	0	biphasic fiber size distribution, mild	normally scattered
26	NA	2	0	3	regional angulated atrophic fibers, severe	around atrophic fibers
27	NA	3	0	4	severe neurogenic atrophy, mild compensatory hypertrophy	around atrophic fibers; distinct staining around hypertrophic fibers
28	NA	2	0	1	moderate neurogenic atrophy	around atrophic fibers; some clusters without CD10 reaction
29	NA	2	0	2	rare small clusters of angulated atrophic fibers	normally scattered
30	RM	2	2	0	Early necrosis, numerous, calcification, scattered regen, severe	around injured fibers
31	RM	2	0	0	Scattered fibers in late myophagocytosis/early regeneration	around injured fibers
32	RM	2	1	1	d&r, focal inflammation around vessels	around injured fibers

Table 3. Endomysial and periarteriolar activation scores for each case in correlation with HE pathology and observed distribution of CD10 staining. N: Normal; ASA-M: Antisynthetase autoantibody associated myositis; DM: Dermatomyositis; IBM: Inclusion body myositis; IMNM: Immune mediated necrotizing myopathy; T2A: Type 2 fiber atrophy; NA: Neurogenic atrophy; RM: Rhabdomyolysis; cm: Chronic myopathic change, in particular fiber size variability and endomysial fibrosis; d: Degenerating/necrotic fibers; r: Regenerating fibers; pf: Perifascicular.

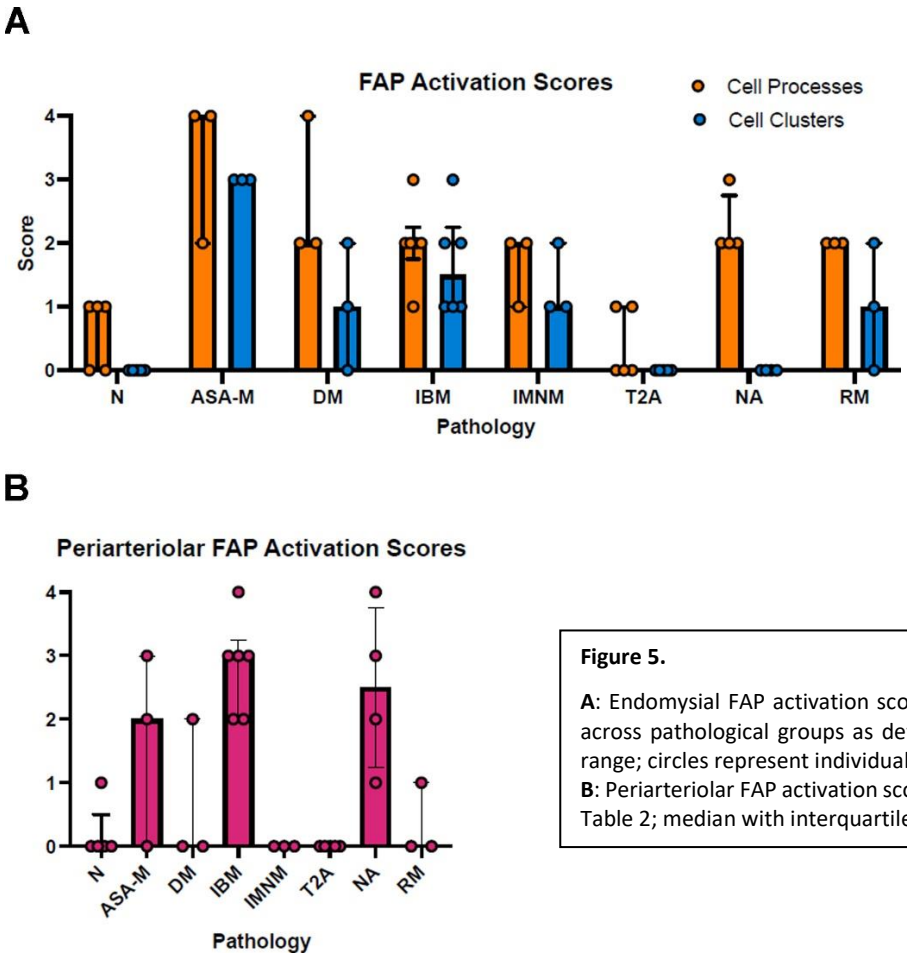

Figure 5.

A: Endomysial FAP activation scores for cell processes and cell proliferation across pathological groups as defined in Table 2; median with interquartile range; circles represent individual cases.
B: Periarteriolar FAP activation scores across pathological groups as defined in Table 2; median with interquartile range; circles represent individual cases.

Discussion

Morphological changes of CD10+ FAPs upon activation across a range of pathological conditions in human skeletal muscle biopsies in our study combine expansion of endomysial cell processes, clustering of cell bodies between muscle fibers, and prominent circumferential periarteriolar cell processes. FAP proliferation in response to myofiber injury is well described in experimental studies [18, 25]. Expansion of FAP processes has received less attention, but is a prominent histological feature in our study of endomysial CD10+ FAP activation in human muscle biopsies. Morphologically, the expanded network of processes surrounding myofibers suggests enhanced FAP-myofiber/basement membrane interactions, including surveillance and signaling. This is in keeping with a general role for multipotent stromal cells as damage sensors and responders, modulating the local cellular environment [27].

Several observations of cellular FAP reactions are in keeping with previously reported results. Normal muscle biopsies showed delicate CD10+ FAPs without features of histological activation or at most segmental ropey processes along occasional fibers [3, 12]. Disease groups with muscle fiber degeneration and regeneration as part of their histopathology (IMNM, ASA-M, RM) revealed FAP activation of processes and some evidence of cell proliferation mostly in proximity of degenerating or regenerating fibers, in keeping with their role in muscle fiber repair [18]. Neurogenic atrophy biopsies demonstrate activation of endomysial FAP processes without evidence of FAP proliferation in regions of atrophy and, interestingly, around hypertrophic fibers in one biopsy. Mouse work has previously suggested a distinct and pivotal role of FAP activation for denervation atrophy involving IL6-STAT3 signaling, possibly reflected in an expanded network of processes [20]. It is thought that FAP activation may be triggered by disintegration of neuromuscular junctions as a result

of nerve degeneration. Taken together, our findings confirm the applicability of major preclinical insights to clinical muscle biopsies, and support the validity of our approach. They also suggest that the type of cellular reaction (proliferation and expansion of processes) could be modulated by the pathogenetic context. It is tempting to speculate that some of this variability could be related to transcriptomic plasticity emerging in several studies [7, 13, 15, 21].

Dermatomyositis biopsies reveal extensive, diffusely distributed CD10 staining of endomysial circumferential processes and cell clusters. The overall degree of CD10 staining was to some extent related to the general severity of underlying pathological change in terms of fiber atrophy, size variability and fibrosis on HE stained sections. However, normal appearing fibers and fibers without evidence of necrosis, regeneration, or inflammatory infiltrates showed pronounced adjacent and circumferential FAP activation. This pattern cannot be easily explained by experimentally and clinically delineated roles for known FAP activation in fiber degeneration, neurogenic atrophy, or post-traumatic or systemic fibrofatty degeneration. Rather it suggests extensive pathological FAP activation in dermatomyositis related to an unknown cause. Abnormal IFN1-signaling activation is an important component of the partially understood pathogenesis of dermatomyositis [8, 10, 23], and diffuse distribution of FAP activation could indicate its involvement in the underlying autoimmune process. This would be in keeping with a posited role of FAPs as damage sensors interacting with the immune system [27] [18].

Biopsies of type 2 fiber atrophy showed the same scoring profile as normal biopsies, indicating the absence of FAP activation in this pathology group. This result forms a contrast to obvious FAP activation as part of neurogenic atrophy. Type 2 fiber atrophy is a common pathological reaction pattern in muscle disuse or in various, often metabolic or toxic, myopathic conditions. The absence of FAP activation in type 2 atrophy as opposed to neurogenic atrophy indicates a different level of involvement of interstitial cells in both types of atrophy.

Antisynthetase-antibody associated (ASA) myositis shows the highest scores for endomysial FAP activation. This can partly be explained by the presence of degenerating and regenerating fibers. In addition, several regions without obvious fiber necrosis or regeneration demonstrated signs of FAP activation, which is more difficult to understand. ASA-myositis is known to show interferon activation on the analysis of cell homogenates in a different pattern from dermatomyositis, with more prominent interferon 2 and less prominent but active interferon 1 activation [1, 23]. This may be part of an explanation for similarly diffuse FAP activation in antisynthetase syndrome and dermatomyositis. Overall, results point to FAP activation as part of an autoimmune process, perhaps in addition to activation caused by fiber necrosis.

Periarteriolar FAP scores revealed variable activation across groups with prominent activation in neurogenic atrophy and IBM biopsies, some activation in dermatomyositis and anti-synthetase syndrome, and no activation in type 2 atrophy and immune mediated necrotizing myopathy. Periarteriolar prominence of FAP processes suggests cellular interaction with perivascular basement membrane, vascular mural cells, or endothelial cells. Experimental evidence supports a specific compartmentalized interaction between FAPs and capillaries mediated by VEGF in a model of ischemic skeletal muscle injury. This interaction mediates capillary remodeling and vascularization [11, 26]. Periarteriolar prominence of FAP processes would be in keeping with the spatial organization of endothelial cell interaction. In addition, the role of vascular mural cells for skeletal muscle tissue homeostasis has been suggested [24]. In this context, periarteriolar FAP processes raise the possibility of mural cell – FAP interaction. While experimental data support the existence of spatially compartmentalized interaction between FAPs and vascular structures, it remains unclear why there is differential periarteriolar FAP activation across groups of more chronic myopathies, for instance prominent periarteriolar activation in IBM and none in IMNM. It is tempting to speculate that a specific patho-mechanistic context plays a role.

The use of CD10 as immunohistochemical marker to visualize FAPs in muscle biopsies has the advantage of easy applicability to a wide variety of archival biopsies. The fact that the method works well on formalin-fixed, paraffin embedded tissue,

expands the spectrum of immunohistochemical stains for paraffin embedded muscle biopsies [28, 29], with the advantage that these can be used in laboratories outside dedicated muscle pathology centers. Although CD10 is not specific for FAPs, their characteristic histological appearance allows reliable identification of reaction patterns. Other CD10-positive, non-FAP structures in muscle biopsies were easily identified in our study.

Recently, single cell sequencing technologies have yielded insights into transcriptomic heterogeneity of FAP subpopulations in response to various pathological stimuli [7, 13, 15, 21]. These studies suggest that FAP activation results in a dynamic profile of FAP subtypes, which varies with pathological context. More specifically, a recent publication demonstrated in this regard that CD10+ FAPs in humans represent a large FAP subpopulation with adipogenic potential [7]. It is therefore possible that our approach does not capture the entire population of cells, which could be identified as FAPs on a transcriptomic level. Bearing these limitations in mind, cellular reaction patterns delineated by CD10 immunohistochemistry remain a valid insight into CD10+ FAP activation in various disease groups, even if it may not capture the entire FAP spectrum.

Conclusion

In conclusion, CD10 immunohistochemistry is a robust technique to analyze histological reaction patterns of CD10+ FAPs in routine clinical muscle biopsies. Histopathological features of activation of endomysial CD10+ FAPs in human muscle biopsies include prominent expansion of the network of cell processes surrounding muscle fibers as well as endomysial cell clusters as evidence of proliferation. In addition, prominence of periarteriolar processes is a notable feature in some pathologies. A scoring system for endomysial and periarteriolar activation is proposed.

Analysis of biopsies from a range of pathological groups confirms cellular reaction patterns in keeping with preclinical insights in many instances. Other observations raise the possibilities of pathological FAP activation in dermatomyositis, interactions between FAPs and vascular mural cells or endothelial cells in particular in the context of denervation and IBM, and absence of FAP activation in type 2 fiber atrophy.

Reliable diagnostic applications, pathomechanistical significance and therapeutic potential remain to be elucidated.

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