Adipose tissue is a source of fibro-adipogenic progenitors for regenerating skeletal muscles

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Article

Keywords: Adipose stromal cells, Fibro/adipogenic progenitors, platelets, cell migration, acute muscle stress

Posted Date: August 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-754516/v1>

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Version of Record: A version of this preprint was published at Nature Communications on January 5th, 2023. See the published version at <https://doi.org/10.1038/s41467-022-35524-7>.

Abstract

Fibro adipogenic progenitors (FAPs) play a crucial role in skeletal muscle regeneration, as they generate a favorable niche that allows satellite cells to perform efficient muscle regeneration. After muscle injury, FAP content increases rapidly within the injured muscle, the origin of which has been attributed to their proliferation. Recently, single-cell RNAseq approaches have revealed phenotype and functional heterogeneity in FAPs. Here we report that FAP-like cells residing in subcutaneous adipose tissue (ScAT), the adipose stromal cells (ASCs), are rapidly released from ScAT in response to muscle injury. In parallel, we show in healthy humans that exercise-induced muscle stress response triggers the migration of human native ASCs. Additionally, we find that released ASCs infiltrate the damaged muscle, via a platelet dependent mechanism and that blocking ASC infiltration impairs muscle regeneration. Collectively, our data reveal that ScAT is an unsuspected physiological reservoir of regenerative cells that support skeletal muscle regeneration.

Introduction

Skeletal muscle exhibits a remarkable regenerative capacity in adult mammals and a large number of studies are running to better characterize and understand the underlying mechanisms controlling this process. The regenerative potential of skeletal muscle relies on a pool of resident adult stem cells, the satellite cells which proliferate and differentiate to allow muscle growth and remodeling in response to exercise or following trauma¹⁻³. Recent studies identified mesenchymal progenitors, termed “fibro-adipogenic progenitors” (FAPs), providing a key functional support to satellite cells⁴⁻⁷. Beside their supportive role, the regulation of FAP content is also crucial since their absence leads to regeneration impairment, whereas FAP maintenance in the late phase of regeneration leads to fibrosis and/or fatty degeneration of the injured muscle⁸⁻¹⁰. The evolution of FAP number upon damage exhibits distinct wave⁹. A first wave occurring 1 day post-injury (dpi) followed by a second wave at 3 dpi⁹. Interestingly, single cell analysis technology largely revealed the heterogeneity of FAPs¹¹⁻¹⁴ and also reported the first wave of FAP. Indeed, Oprescu et al. elegantly identified a subpopulation of “activated” FAPs in the very early phases following muscle injury (0.5 dpi)¹⁴. These “activated” FAPs were considered by the authors to be muscle residents, although direct demonstration was not done. Moreover, it is very well described that FAPs proliferation starts 2dpi for a period of 72–96 h^{4,13,15-17}. Consequently, the cellular origin of the first wave of FAP increase observed at 1dpi is not characterized and has not been studied so far^{8,9}.

White adipose tissue (AT) houses a mesenchymal cell population, which resembles FAPs, the adipose stromal cells (ASCs). Various studies, including ours show that ASCs exhibit similar cell surface antigen combination, same clonogenic activity and differentiation potentials¹⁸⁻²⁰. ASCs attract a lot of interest due to their regenerative potential and represent very promising tools for cell-based therapies²¹. Whether FAPs and ASCs are distinct cell types remains unclear²²⁻²⁴. The circulation of stromal cells has been reported^{25,26} and we and others have shown that ectopic fat cells partly arise from these circulating stromal cells particularly upon weight gain²⁷⁻²⁹. We demonstrated that subcutaneous AT (ScAT) releases

ASCs in response to inflammatory stimuli^{18,30} or to high fat diet²⁸. Consequently, we questioned here whether the first wave of FAP number increase in response to acute muscle injury is the result of ASC release from ScAT followed by their further infiltration into the damaged muscle.

In the present study, we report that ScAT releases ASCs in response to acute muscle damage and that released ASCs infiltrate the injured muscle via a platelet dependent mechanism. We also show that blunting the infiltration of ASCs impairs muscle regeneration suggesting that ScAT is an unsuspected partner of muscle regeneration.

Results

Acute muscle injury induces a rise in FAP muscle content mirrored by a specific and rapid decrease in ASC content in the ScAT

The number of FAPs was evaluated in the quadriceps muscle 24h following damage using glycerol (Gly) or myotoxin (cardiotoxin, CTX) both well characterized murine models of muscle injury (Figure S1A,B). The cell composition of whole muscle-derived stroma-vascular fraction (SVF) was analyzed by flow cytometry and FAPs content (identified as Sca1⁺/CD34⁺/CD140a⁺/CD31⁻/CD45⁻ cells (Fig. 1A)) was measured from 1 to 9 days post-injury (dpi) (Fig. 1B). As previously reported^{4,5,31} and whatever the muscle injury model, muscle FAP number peaked at 3 dpi and reached back basal value at 9 dpi (Fig. 1B). Also in accordance with previous studies, FAP number drastically increased by 1 dpi in the injured muscle (> 5 fold, Fig. 1A-B, without being affected in the controlateral non-injured side, Fig. 1C) and we noticed that most of them were podoplanin-positive (Fig. 1A). The rise in FAPs at 1 dpi was not due to their proliferation, since no differences in EdU incorporation -a nucleotide analogue- between FAPs from injured and control mice were detected (Fig. 1C, S1C), whereas other cell population, such as leucocytes start proliferating by 1 dpi (Figure S1D). The FAP increase evidenced by flow cytometry 1 dpi was then confirmed *in situ*, by immunohistochemistry of the damaged muscle (Fig. 1D, S1E). Moreover, given that FAPs are the only cell type expressing adipogenic potential in the muscle^{5,24,31,32}, *in vitro* adipogenesis of whole muscle-derived SVF from control or 1 dpi injured animals would directly and functionally reflects the quantity of FAPs inside (Fig. 1E). Upon adipogenic induction muscle-derived SVF originating from injured animals clearly accumulated more lipids and expressed higher levels of adipogenic markers (Fig. 1F) confirming the rise in FAP content in the injured muscle at 1 dpi.

In parallel, we studied the evolution of ASCs content in ScAT following muscle injury. ASCs were identified as Sca1⁺/CD34⁺/CD140a⁺/CD31⁻/CD45⁻ cells as previously reported (Fig. 2A)^{18,19}. Strikingly, concomitant with the increase in FAP number in the injured muscle, the number of ASCs significantly decreased by 20–25% in the ScAT at 1dpi (Fig. 2B), both following Gly or CTX damage. Importantly, the gain in muscle FAP number was found equivalent to the loss in ScAT ASCs, whatever the injury model used (Fig. 2C). To verify that muscle injury specifically triggered the decrease in ASCs and to rule out any impact of the injury procedure on the cell content of ScAT, NaCL was injected into the quadriceps muscle. NaCL neither induced muscle damage (Figure S2A, B) nor modified ASC and/or leucocyte content in ScAT

(Figure S2C, D), demonstrating that muscle injury *per se* triggers the fall in ASCs content observed in the ScAT at 1 dpi. Moreover, the ScAT was specifically impacted by muscle damage since neither perigonadic AT (PGAT) nor the bone marrow (BM), another tissue source of MSCs, were affected (Fig. 2D). To examine whether programmed cell death could be responsible of the drop of ASCs content in the ScAT, we measured DNA fragmentation (TUNEL) after Gly or CTX muscle damage at 1 dpi. Frequencies of TUNEL positive ASCs were not modified in the ScAT (Fig. 2E-F). As for the muscle, ASCs are the only cell population in AT-derived SVF capable of adipogenic potential^{19,33}. Therefore, *in vitro* adipogenesis of whole ScAT-derived SVF from control or injured animals were tested and compared (Fig. 2G) to assess ASC content. In agreement with flow cytometry results the drop in ASCs content was associated with a decrease in lipid accumulation and expression of adipogenic markers in ScAT-derived SVF originating from injured animals (Fig. 2G-H). To rule out putative effects of CTX or Gly on the metabolic status of the animals, and as thus on the biology of AT, the body weights as well as plasmatic glucose levels of the animals were monitored during 28 days following muscle injury. Neither the body weights of the animals nor their glycaemia were modified by muscle lesion (Figure S2E, F). Collectively our data strongly suggest that in response to quadriceps injury the ScAT releases ASCs that might infiltrate the injured muscle within 24h after injury, corresponding to the first wave of FAP rise.

The muscle stress response after injury or exercise triggers ASC chemotaxis in humans and mice

We hypothesized that the drop in ASCs in response to muscle injury resulted from their egress from the ScAT. Consequently, we investigated *in vitro* whether serum from injured animals could trigger ASC chemotaxis. We thus collected the serum from control or injured (1dpi) animals and ASCs chemotaxis was studied and compared. We found that whatever the injury model, serum from injured animals strongly induced the chemotaxis of ASCs (Fig. 3A-B), supporting the notion that muscle injury may trigger the egress of ASCs from ScAT via the production of blood circulating factors. Whether such mechanism may occur in human was also questioned. To do so, we took advantage of blood samples originating from a clinical study investigating the impact of an acute bout of continuous exercise³⁴ in young active men (Table 1) as a putative source of muscle damage and/or fatigue. As for mice, the chemotactic response of human native ASCs towards the serum of each individuals before and after the exercise was studied. As shown in Fig. 3C, the chemotactic response of ASCs toward serum at post-exercise was increased by 2 to 5 fold compared to the pre-exercise condition, though heterogeneity was observed from one individual to another. We then wondered whether such a disparity could be explained by inter-individual variability to exercise-mediated stress response. Growth differentiation factor 15 (GDF-15) is induced under stress conditions, to maintain cell and tissue homeostasis³⁵. Recent studies also suggested that GDF15 is an exerkine³⁴ exhibiting possible protective role in exercise-induced muscle injury or inflammation^{36,37}. Therefore, circulating blood levels of GDF15 were measured before and after exercise to assess the exercise-induced stress response intensity of each individuals (Table 2). In agreement with our hypothesis and the results obtained in mice, the chemotactic activity of ASCs is strongly correlated with GDF15 levels akin to an index of the exercise-induced stress response (Fig. 3D). These findings indicate that blood factors released after muscle injury/stress may trigger the

mobilization of ASCs from the ScAT, in both mice and humans, further reaching the injured or stressed muscle.

Table 1
Anthropometry and body composition of the individuals enrolled in the clinical study

Subject ID	Age	Weight (kg)	Height (cm)	BMI (kg/m ²)	%Fat Mass	Fat Mass (kg)	Free Fat Mass (kg)
A	22	79	184	23.33	12.16	8.5	62.1
B	19	87	193	23.36	17	13.6	63.9
D	19	70	171	23.94	17.8	11.1	49.4
I	27	87.2	185	24,54	15.9	12.8	64.8
J	24	79.3	191	21.7	15.6	10.6	60.7
M	29	71.3	176	23.01	14.3	10.4	59.2

Table 2
GDF 15 blood levels in individuals before (pre) and after (post) an exercise on a bicycle ergometer at 60% VO₂ peak for 1-hr. Δ corresponds to the difference of GDF15 blood level, before and after the exercise.

GDF15 (pg/ml)			
Subject ID	Pre	Post	Δ (Post - Pre)
A	153,4	168,1	14,7
B	417,0	457,5	40,5
D	209,4	313,2	103,8
I	341,2	446,5	105,3
J	451,6	437,7	-14,0
M	263,8	329,5	65,6

Upon muscle injury ASCs egress the ScAT and infiltrate the damaged muscle

To determine whether ASCs can leave the ScAT in response to muscle injury to further infiltrate it, we performed *in vivo* experiment in mice. However, due to the absence of unique specific ASC marker, no mouse model is so far available to visualize, *in vivo*, the trafficking of native ASCs from AT to any other tissue compartments. To overcome this obstacle we took advantage of the Tg(Cd34-EGFP)MF6Gsat/Mmucd (referred to as CD34-GFP, an ASC surface marker) from which a piece of ScAT was grafted into the ScAT of a non-GFP recipient mouse (Fig. 3E). Fat graft revascularization, an index of

viability, was verified by using retro-orbital injection of Rhodamin-lectin into grafted animals (Fig. 3E, S3A). At day 7 post-graft, muscle was damaged and the presence of GFP⁺ cells into the injured muscles was assessed at 1 dpi. CD31⁻/CD45⁻/Sca-1⁺/GFP⁺ cells were found in injured muscles by flow cytometry (roughly 8% of CD31⁻/CD45⁻/Sca-1⁺ cells, Fig. 3F, S3B) while by contrast no GFP cells were detected neither in the muscle of a CD34-GFP ScAT grafted non-injured control animal (Fig. 3F) nor in a non-grafted mouse (Figure S3B). Further analysis demonstrated that GFP⁺-ASCs were mainly in the Sca-1 low subpopulation (Fig. 3D, right panel). This result clearly demonstrates that following muscle injury ScAT releases CD34⁺ cells, which infiltrate specifically the damaged muscle. Further analysis of their cell surface immunophenotype showed that the infiltrated GFP cells were Sca1⁺/CD34⁺/Podoplanin⁺/CD140a⁺/CD31⁻/CD45⁻, corresponding to ASCs (Fig. 3E-G). To verify that GFP expression was specific, FAPs from grafted injured (1 dpi) or non-injured animals were sorted and genomic GFP expression was analysed. In agreement with flow cytometry and immunohistochemistry data, genomic GFP expression was found in FAPs of injured muscles (Figure S3C), in contrast to control muscles from grafted animals (Fig. 3G) or other distant organs (Figure S3D). Finally, we performed immunohistochemistry analysis of muscle originating from grafted animals at 1dpi and showed the presence of GFP⁺-ASC- (Fig. 3E) that also expressed podoplanin (Fig. 3F) and CD140a (Fig. 3G) such as observed in previous cytometry experiments. Of note, the observation of infiltrated ASCs post muscle injury (1 dpi) was also made with the ScAT graft from another fluorescent mouse model, the Rosa26^{mT/mG} animals (Figure S3E). Here again, we observed ~ 10% of Tomato⁺-cells among the CD45⁻/CD31⁻/Sca-1⁺ cells, corresponding to infiltrated ASCs originating from the red fluorescent mT/mG graft pad (Figure S3F). Interestingly, the Tomato⁺-cells laid preferentially among the CD45⁻/CD31⁻/Sca-1^{low} cell subpopulation (Figure S3F, lower panel) such as observed previously in Fig. 3D. Altogether, these findings demonstrate that the first wave of FAP increase following muscle injury at 1dpi largely results from ASC mobilization and infiltration into the damaged muscle.

ASC early infiltration into the injured muscle involves platelets and is crucial for effective muscle regeneration

Our current understanding of *in vivo* trafficking of native ASCs/MSCs is still very incomplete³⁸ and mostly derives from *in vitro* experiments. However, some studies indicate that platelets can control MSC trafficking and/or recruitment to sites of injury³⁹⁻⁴¹. Interestingly, we observed an up-regulation of podoplanin expression at the cell surface of ASCs from injured animals measured by flow cytometry (Fig. 4A). Podoplanin is an endogenous ligand for C-type lectin-like receptor 2 (CLEC-2) which is an essential platelet-activating receptor⁴². Consequently, we next assessed whether ASCs and platelets interaction is necessary for the recruitment of ASCs to the injured muscle. We used an *in vitro* assay whereby platelets, isolated from control or injured animals (1 dpi) were fluorescently labelled and co-incubated with ASCs (isolated from ScAT). The adhesion of platelets to ASCs was evaluated and compared. The results show that platelets originating from injured animals adhered more to ASCs *in vitro* (Fig. 4B-C), a physical interaction that was completely abolished when an antibody directed against

podoplanin was added (Fig. 4C). Given our results showing that both ASC mobilization and infiltration strongly account for the first wave of FAP rise in injured muscle and that platelets originating from injured animals interact more with ASCs, we next investigated the consequences of platelet depletion *in vivo* on FAP augmentation following muscle injury. Muscle damage was induced as described earlier and platelet depletion was performed 1h later (Fig. 4D-E, S4A). FAP content was then quantified by flow cytometry at 1dpi, according to the immunophenotype described earlier. Our data show that platelet depletion diminished by more than twofold the rise of FAPs in the muscle at 1 dpi (Fig. 4F) suggesting that the infiltration of ASCs into the injured muscle involves platelets.

Previous studies have demonstrated that FAP facilitate myogenesis by promoting differentiation of muscle progenitors and myotube formation^{4,10,17}. Hence, we next studied the impact of FAP rise disruption on the muscle regeneration process. To do so, the expression of genes involved in the molecular program of muscle regeneration was studied following muscle injury and concomitant platelet depletion until 14 dpi. Though the expression patterns of early genetic markers of the muscle regeneration program (Pax7, Myf5) were not modified (Fig. 4G), the expression of late myogenic markers (MyoD, Myogenin, eMyHC) was clearly modified in platelet depleted animals compared with controls (Fig. 4H). These results strongly suggest that when the early infiltration of ASCs is disrupted, the regeneration program is impaired. To reinforce these results, we quantified the number of newly synthesized centrally nucleated fibers, a marker of the regenerative process and found that the proportion of centrally nucleated fibers was dramatically diminished in platelet depleted animals (Fig. 5A-B). Next, we quantified the cross-sectional area distribution of regenerating fibers with centralized nuclei, as well as the total area of these regenerating fibers and we showed that they were dramatically diminished in animals where the rise in FAP was impaired (Fig. 5C, D). Upon activation, platelets secrete more than 300 active substances, among which is PDGF, an important factor for FAPs expansion and survival^{15,43-45}. To rule out that a reduction in PDGF was the main effect of platelet depletion rather than the absence of platelet *per se*, ScAT from both sides were removed in order to take away the reservoir of ASCs. Muscle injury was performed and the expression of genes involved in the molecular program of muscle regeneration was studied. As for platelet depleted animals, the expression pattern of Pax7 was not modified (Fig. 5E) while the one of late myogenic markers (myogenin, eMYHC) was clearly affected (Fig. 5E), strongly suggesting that an interaction between platelets and ASCs is necessary. To finally demonstrate that ASCs early infiltration was responsible for both early FAP rise and effective muscle regeneration, ASCs were injected into the injured muscle (1h after muscle injury) of platelet depleted animals in order to mimic the first wave of FAP increase. The number of newly synthesized centrally nucleated fibers, the cross-sectional area distribution of regenerating fibers and the total area of regenerating fibers were evaluated and the injection of ASCs completely restored those parameters (Fig. 5B-D) strongly supporting a proregenerative role of ASC early infiltration in the damaged skeletal muscle.⁷

Discussion

The regenerative capacity of skeletal muscle mostly relies on satellite cells (SCs), which proliferate in response to exercise or following myotrauma to repair the injured muscle ^{3,46}. However, in the past decade many other cell types have been shown to contribute to this process in order to maintain skeletal muscle integrity and functions ⁴⁷.

Among those diverse and heterogeneous cell types, resident mesenchymal progenitors named fibro-adipogenic progenitors (FAPs) have emerged as key players in skeletal muscle regeneration and disease by providing functional support to SCs to perform efficient muscle regeneration ^{7,48}. Upon muscle injury, FAPs become activated and expand rapidly ^{4,5}. Indeed, the number of FAPs peaks 96h post-muscle damage ⁹ together with their proliferative activity ⁴. Surprisingly, while FAPs proliferation is not yet activated ^{4,5,8,9,49}, FAPs number dramatically increases within the 24h following muscle injury ^{8,9,11,47}. The results presented here provide a mechanism that explains the rapid FAP number increase observed 24h after muscle injury without proliferation and could correspond to the subpopulation of “activated” FAPs reported by Oprescu et al ¹⁴. Indeed, we show that in response to acute muscle injury ASCs egress ScAT and infiltrate the damaged muscle, a mechanism necessary for efficient muscle regeneration. Our data also suggest that such a mechanism may also occur in humans in response to exercise-induced muscle stress response.

FAPs and ASCs share many molecular and functional properties with MSCs ^{47,50}. Importantly, the lack of unique specific marker so far prevents the specific genetic targeting of these cell types and as thus the simultaneous investigation of their fates *in vivo*. To overcome this obstacle, we have developed and used a ScAT grafting approach, to track specifically endogenous ASCs *in vivo*. To the best of our knowledge, using this model allowed us to visualize for the first time the specific mobilization and homing of ASCs from ScAT within injured muscle. Interestingly, it was recently identified that *Drosophila* fat body cells, the equivalent to the vertebrate adipocyte precursors, are also motile and migrate to distant wounded sites to drive repair ⁵¹. Until now, FAPs are considered as resident-muscle progenitors ²³, however, we propose here that a subpopulation of mesenchymal progenitors originating from ScAT participates to the pool of FAPs. Various recent studies addressed the question of muscle resident cell heterogeneity in homeostasis and regenerative conditions using single cell analysis ^{11,14,52-54}. In that context, Malecova et al. identified in the mouse the appearance of a subpopulation of Tie2^{low}FAPs within the 24h following muscle injury, which they considered as reflecting cell state of FAPs during muscle regeneration ¹¹. The analogous in human samples was recently identified and it expresses FBN1 gene (fibrillin 1) ⁵⁴. Based on our results it is tempting to speculate that this FAP subpopulation originates from ScAT rather than being a transitory cell state of resident FAPs. Also, it has been reported that FAPs can differentiate into brown-like adipocytes ^{55,56}, whether the muscle infiltrating ASCs exhibit a brown/beige adipogenic potential remains to be determined particularly in light of the closer association between brown adipocytes and the skeletal muscle lineage ^{31,57}.

The mechanisms controlling the trafficking of endogenous MSCs and/or ASCs are poorly understood and our current understanding mostly derives from *in vitro* studies ³⁸. We show here that in response to

muscle injury, ASCs within ScAT overexpress podoplanin, which is a ligand for the platelet receptor CLEC-2 (for C-type lectin-like receptor 2)⁵⁸. In agreement with this result, several studies reported that the majority of infused MSCs can be found within the circulation in close contact with platelets^{40,41} to facilitate their homing to inflamed tissues⁵⁹. Our data show that platelets originating from muscle-injured animals adhere more *in vitro* to ASCs and that neutralizing the CLEC-2/podoplanin interaction completely abolished this effect, suggesting a role for platelets in ASC trafficking *in vivo*. This was confirmed in platelet-depleted animals where FAP increase was strongly inhibited in response to muscle injury. Platelets maintain endothelial permeability and the trafficking of infused MSCs to inflamed sites is facilitated by increased endothelial permeability⁶⁰. However, it was recently demonstrated that the extravasation of infused MSCs to inflamed sites is not dependent on endothelial gaps or permeability⁴¹. Hence, the precise mechanisms by which platelets control ASC egress and/or infiltration are still unclear.

In conclusion, our work identifies a novel and unsuspected role of ScAT. These findings introduce the concept of adipose tissue as an endogenous supplier of regenerative cells allowing skeletal muscle to regenerate efficiently. The well-timed clearance of FAPs during the regeneration process is essential⁹. Indeed, abnormal persistence of FAPs leads to excessive matrix deposition and thus to muscle fibrosis contributing to subsequent pathogenesis^{7,61}. Our work strongly supports a model in which the finely tuned progression of FAP increase is necessary for healthy muscle regeneration where ScAT plays a crucial role in this precise temporal succession.

Declarations

Acknowledgments.

We thank the Cell sorting platform of RESTORE and Marie-Laure Renoud for technical help. We also thank the Cellular Imaging Facility-I2MC/TRI platform and Alexia Zakaroff-Girard and Elodie Riant as well as Imag'IN platform and François-Xavier Frenois for helpful assistance.

References

1. Chargé, S. B. P. & Rudnicki, M. A. Cellular and molecular regulation of muscle regeneration. *Physiol. Rev.* **84**, 209–238 (2004).
2. Hawke, T. J. & Garry, D. J. Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol* (1985) **91**, 534–51 (2001).
3. Mauro, A. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* **9**, 493–5 (1961).
4. Joe, A. W. B. *et al.* Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat. Cell Biol.* **12**, 153–163 (2010).
5. Uezumi, A., Fukada, S., Yamamoto, N., Takeda, S. & Tsuchida, K. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nat Cell Biol* **12**, 143–

52 (2010).

6. Wosczyzna, M. N., Biswas, A. A., Cogswell, C. A. & Goldhamer, D. J. Multipotent progenitors resident in the skeletal muscle interstitium exhibit robust BMP-dependent osteogenic activity and mediate heterotopic ossification. *J Bone Miner Res* **27**, 1004–17 (2012).
7. Theret, M., Rossi, F. M. V. & Contreras, O. Evolving Roles of Muscle-Resident Fibro-Adipogenic Progenitors in Health, Regeneration, Neuromuscular Disorders, and Aging. *Front. Physiol.* **12**, (2021).
8. Fiore, D. *et al.* Pharmacological blockage of fibro/adipogenic progenitor expansion and suppression of regenerative fibrogenesis is associated with impaired skeletal muscle regeneration. *Stem Cell Res* **17**, 161–169 (2016).
9. Lemos, D. R. *et al.* Nilotinib reduces muscle fibrosis in chronic muscle injury by promoting TNF-mediated apoptosis of fibro/adipogenic progenitors. *Nat. Med.* **21**, 786–794 (2015).
10. Murphy, M. M., Lawson, J. A., Mathew, S. J., Hutcheson, D. A. & Kardon, G. C. Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. *Development (Cambridge, England)* **138**, 3625–3637 (2011).
11. Malecova, B. *et al.* Dynamics of cellular states of fibro-adipogenic progenitors during myogenesis and muscular dystrophy. *Nature communications* **9**, 3670 (2018).
12. Stumm, J. *et al.* Odd skipped-related 1 (Osr1) identifies muscle-interstitial fibro-adipogenic progenitors (FAPs) activated by acute injury. *Stem Cell Research* **32**, 8–16 (2018).
13. Scott, R. W., Arostegui, M., Schweitzer, R., Rossi, F. M. V. & Underhill, T. M. Hic1 Defines Quiescent Mesenchymal Progenitor Subpopulations with Distinct Functions and Fates in Skeletal Muscle Regeneration. *Cell Stem Cell* **25**, 797–813.e9 (2019).
14. Oprescu, S. N., Yue, F., Qiu, J., Brito, L. F. & Kuang, S. Temporal Dynamics and Heterogeneity of Cell Populations during Skeletal Muscle Regeneration. *iScience* **23**, 100993 (2020).
15. Contreras, O. *et al.* Cross-talk between TGF- β and PDGFR α signaling pathways regulates the fate of stromal fibro–adipogenic progenitors. *Journal of Cell Science* **132**, (2019).
16. Dulauroy, S., Di Carlo, S. E., Langa, F., Eberl, G. & Peduto, L. Lineage tracing and genetic ablation of ADAM12(+) perivascular cells identify a major source of profibrotic cells during acute tissue injury. *Nat Med* **18**, 1262–70 (2012).
17. Mathew, S. J. *et al.* Connective tissue fibroblasts and Tcf4 regulate myogenesis. *Development* **138**, 371–84 (2011).
18. Gil-Ortega, M. *et al.* Native adipose stromal cells egress from adipose tissue in vivo: evidence during lymph node activation. *Stem Cells* **31**, 1309–1320 (2013).
19. Rodeheffer, M. S., Birsoy, K. & Friedman, J. M. Identification of white adipocyte progenitor cells in vivo. *Cell* **135**, 240–9 (2008).
20. Tang, W. *et al.* White fat progenitor cells reside in the adipose vasculature. *Science* **322**, 583–6 (2008).

21. Gimble, J. M., Bunnell, B. A., Chiu, E. S. & Guilak, F. Concise review: Adipose-derived stromal vascular fraction cells and stem cells: let's not get lost in translation. *Stem Cells* **29**, 749–54 (2011).
22. Arrighi, N. *et al.* Characterization of adipocytes derived from fibro/adipogenic progenitors resident in human skeletal muscle. *Cell Death Dis* **6**, e1733 (2015).
23. Judson, R. N., Zhang, R.-H. H. & Rossi, F. M. Tissue-resident mesenchymal stem/progenitor cells in skeletal muscle: collaborators or saboteurs? *The FEBS journal* **280**, 4100–4108 (2013).
24. Laurens, C. *et al.* Adipogenic progenitors from obese human skeletal muscle give rise to functional white adipocytes that contribute to insulin resistance. *Int J Obes (Lond)* **40**, 497–506 (2016).
25. Bellows, C. F., Zhang, Y., Simmons, P. J., Khalsa, A. S. & Kolonin, M. G. Influence of BMI on level of circulating progenitor cells. *Obesity* **19**, 1722–6 (2011).
26. Kolonin, M. G., Evans, K. W., Mani, S. A. & Gomer, R. H. Alternative origins of stroma in normal organs and disease. *Stem Cell Res* **8**, 312–23 (2012).
27. Crossno, J. T., Jr., Majka, S. M., Grazia, T., Gill, R. G. & Klemm, D. J. Rosiglitazone promotes development of a novel adipocyte population from bone marrow-derived circulating progenitor cells. *J Clin Invest* **116**, 3220–8 (2006).
28. Grousse, A. *et al.* The Release of Adipose Stromal Cells from Subcutaneous Adipose Tissue Regulates Ectopic Intramuscular Adipocyte Deposition. *Cell Rep* **27**, 323–333.e5 (2019).
29. Rydén, M. On the origin of human adipocytes and the contribution of bone marrow-derived cells. *Adipocyte* **5**, 312–317 (2016).
30. Gil-Ortega, M., Fernández-Alfonso, M. S., Somoza, B., Casteilla, L. & Sengenès, C. Ex vivo microperfusion system of the adipose organ: a new approach to studying the mobilization of adipose cell populations. *Int J Obes (Lond)* **38**, 1255–1262 (2014).
31. Pannérec, A., Formicola, L., Besson, V., Marazzi, G. & Sassoon, D. A. Defining skeletal muscle resident progenitors and their cell fate potentials. *Development* **140**, 2879–2891 (2013).
32. Schulz, T. J. *et al.* Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 143–148 (2011).
33. Sengenès, C., Lomède, K., Zakaroff-Girard, A., Busse, R. & Bouloumié, A. Preadipocytes in the human subcutaneous adipose tissue display distinct features from the adult mesenchymal and hematopoietic stem cells. *J. Cell. Physiol.* **205**, 114–122 (2005).
34. Laurens, C. *et al.* Growth and differentiation factor 15 is secreted by skeletal muscle during exercise and promotes lipolysis in humans. *JCI Insight* **5**, (2020).
35. Wischhusen, J., Melero, I. & Fridman, W. H. Growth/Differentiation Factor-15 (GDF-15): From Biomarker to Novel Targetable Immune Checkpoint. *Front Immunol* **11**, 951 (2020).
36. Gil, C. I. *et al.* Role of GDF15 in active lifestyle induced metabolic adaptations and acute exercise response in mice. *Sci Rep* **9**, 20120 (2019).
37. Klein, A. B. *et al.* Pharmacological but not physiological GDF15 suppresses feeding and the motivation to exercise. *Nat Commun* **12**, 1041 (2021).

38. Girusse, A. *et al.* Endogenous Mobilization of Mesenchymal Stromal Cells: A Pathway for Interorgan Communication? *Front. Cell Dev. Biol.* **8**, (2021).
39. Massberg, S. *et al.* Platelets secrete stromal cell-derived factor 1alpha and recruit bone marrow-derived progenitor cells to arterial thrombi in vivo. *J Exp Med* **203**, 1221–33 (2006).
40. Sheriff, L. *et al.* Origin-Specific Adhesive Interactions of Mesenchymal Stem Cells with Platelets Influence Their Behavior After Infusion. *Stem Cells* **36**, 1062–1074 (2018).
41. Teo, G. S. L., Yang, Z., Carman, C. V., Karp, J. M. & Lin, C. P. Intravital imaging of mesenchymal stem cell trafficking and association with platelets and neutrophils. *Stem Cells* **33**, 265–277 (2015).
42. Suzuki-Inoue, K. *et al.* Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. *J Biol Chem* **282**, 25993–6001 (2007).
43. Piñol-Jurado, P. *et al.* Platelet-Derived Growth Factor BB Influences Muscle Regeneration in Duchenne Muscle Dystrophy. *The American Journal of Pathology* **187**, 1814–1827 (2017).
44. Uezumi, A. *et al.* Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle. *J. Cell. Sci.* **124**, 3654–3664 (2011).
45. Contreras, O., Córdova-Casanova, A. & Brandan, E. PDGF-PDGFR network differentially regulates the fate, migration, proliferation, and cell cycle progression of myogenic cells. *Cellular Signalling* **84**, 110036 (2021).
46. Sambasivan, R. *et al.* Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development* **138**, 3647–56 (2011).
47. Wosczyzna, M. N. & Rando, T. A. A Muscle Stem Cell Support Group: Coordinated Cellular Responses in Muscle Regeneration. *Developmental cell* **46**, 135–143 (2018).
48. Biferali, B., Proietti, D., Mozzetta, C. & Madaro, L. Fibro–Adipogenic Progenitors Cross-Talk in Skeletal Muscle: The Social Network. *Front. Physiol.* **10**, (2019).
49. Dong, Y., Silva, K. A., Dong, Y. & Zhang, L. Glucocorticoids increase adipocytes in muscle by affecting IL-4 regulated FAP activity. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology* **28**, 4123–4132 (2014).
50. Gimble, J. M., Grayson, W., Guilak, F., Lopez, M. J. & Vunjak-Novakovic, G. Adipose tissue as a stem cell source for musculoskeletal regeneration. *Front Biosci (Schol Ed)* **3**, 69–81 (2011).
51. Franz, A., Wood, W. & Martin, P. Fat Body Cells Are Motile and Actively Migrate to Wounds to Drive Repair and Prevent Infection. *Dev. Cell* **44**, 460–470.e3 (2018).
52. De Micheli, A. J. *et al.* Single-Cell Analysis of the Muscle Stem Cell Hierarchy Identifies Heterotypic Communication Signals Involved in Skeletal Muscle Regeneration. *Cell Rep* **30**, 3583–3595.e5 (2020).
53. De Micheli, A. J., Spector, J. A., Elemento, O. & Cosgrove, B. D. A reference single-cell transcriptomic atlas of human skeletal muscle tissue reveals bifurcated muscle stem cell populations. *Skeletal Muscle* **10**, 19 (2020).

54. Rubenstein, A. B. *et al.* Single-cell transcriptional profiles in human skeletal muscle. *Sci Rep* **10**, 229 (2020).
55. Gorski, T., Mathes, S. & Krützfeldt, J. Uncoupling protein 1 expression in adipocytes derived from skeletal muscle fibro/adipogenic progenitors is under genetic and hormonal control. *J Cachexia Sarcopenia Muscle* **9**, 384–399 (2018).
56. Lau, A.-M., Tseng, Y.-H. & Schulz, T. J. Adipogenic Fate Commitment of Muscle-Derived Progenitor Cells: Isolation, Culture, and Differentiation. *Methods Mol Biol* **1213**, 229–243 (2014).
57. Hepler, C., Vishvanath, L. & Gupta, R. K. Sorting out adipocyte precursors and their role in physiology and disease. *Genes Dev.* **31**, 127–140 (2017).
58. Astarita, J. L., Acton, S. E. & Turley, S. J. C. Podoplanin: emerging functions in development, the immune system, and cancer. *Frontiers in immunology* **3**, 283 (2012).
59. Jiang, L. *et al.* Platelet-mediated mesenchymal stem cells homing to the lung reduces monocrotaline-induced rat pulmonary hypertension. *Cell Transplant* **21**, 1463–75 (2012).
60. Karp, J. M. & Leng Teo, G. S. Mesenchymal stem cell homing: the devil is in the details. *Cell Stem Cell* **4**, 206–16 (2009).
61. Kramann, R., Dirocco, D. P. & Humphreys, B. D. Understanding the origin, activation and regulation of matrix-producing myofibroblasts for treatment of fibrotic disease. *J Pathol* **231**, 273–289 (2013).

Material And Methods

Animal experiments

This work was submitted to and approved by the Regional Ethic Committee and registered to the French Ministère de la Recherche. For muscle injuries, 8-12 weeks old male C57BL/6 mice (Janvier) were anesthetized with isoflurane and 80µL of 10 µM cardiotoxin (Sigma, C9759) or 50% glycerol in saline solution (NaCl 0.9%) were injected into the right quadriceps. For ScAT grafting, small pieces of ScAT (10mg) from Tg(Cd34-EGFP)MF6Gsat/Mmcd referred to as CD34-GFP mice (MMRRC) were grafted into ScAT of WT C57BL/6 mice by surgically skin incision, and after 7 days grafted mice were injured. For platelet depletion, mice were injected intraperitoneally with platelet depleting antibody (anti-GPIb, EMFRET) in saline solution (2mg/kg). Blood was collected under anesthesia in inferior cava vein with G25 needle and 1mL syringe coated with PBS/Heparin (20U/mL), and then samples were stored at 4°C to perform plasma or platelets isolation. Quadriceps muscle, sub cutaneous (Sc) and perigonadic (PG) adipose tissues (AT) were harvested for cell isolation. Liver, heart, kidney and front limb were dissected and fresh frozen at -80°C for genomic DNA or RNAs extraction. For lipectomy model, animals were anesthetized with isoflurane and a skin incision was performed above ScAT lymph node. ScAT was then removed using forceps to disrupt conjunctive tissue adherences, and blood vessels located at the extremities were cauterized. Wound was closed using surgical clips, and animals were monitored daily for 5 to 7 days before removal of the clips. Muscle injured results were compared to non-injured animals, and lipectomy results were compared to sham (skin incision only) animals.

Human Clinical Study.

Young active men (age 23.3 ± 1.7 years; BMI $23.3 \pm 0.4 \text{ kg.m}^{-2}$; $\text{VO}_{2\text{max}}$ $47.5 \pm 1.6 \text{ ml.kg}^{-1}.\text{min}^{-1}$) were recruited to partake in this study. The protocol was approved by the Dublin City University Ethics Committee and all subjects gave written informed consent. Participants were instructed to refrain from exercise and to replicate food intake the day before each trial. In the morning, following an overnight fast, participants lay on a bed for 1-hr after arriving at the lab. A blood sample was taken and they then exercised on a bicycle ergometer at 60% VO_2 peak for 1-hr. VO_2 peak was determined on a cycle ergometer starting at 70 watts and increasing in 30-watt increments every 3 minutes until exhaustion. A blood sample was taken at the end of the exercise. The intensity was determined using the results of an incremental exercise test to exhaustion. GDF15 protein levels in blood samples were determined by ELISA (R&D Systems).

Human adipose tissue samples.

AT was obtained from patients who provided prior written informed consent according to the ethics committees of Toulouse Hospitals. AT was harvested during plastic surgery (abdominoplasty) from 3 adult patients (female, age 47.6 ± 3.7 years, BMI $26.7 \pm 0.9 \text{ kg.m}^{-2}$) at Rangueil Hospital (part of CHU of Toulouse).

Human AT-SVF isolation.

Human adipose tissue was digested for 45 minutes in α -MEM (GIBCO) containing collagenase (NB 4, Coger, 0.4 IU/mL), Dispase II (Roche, 1.6 UI/mL, Basel), in a water-shaking bath at 37°C. After digestion, an equal volume of α -MEM was added to stop enzymatic digestion. The cells were passed through a 100 μm filter (Steriflip, Millipore, Billerica, MA) and then centrifuged. The pellet was resuspended in α -MEM containing 2% PLP and the total number of cells in the SVF was counted.

Murine AT- or muscle- SVF isolation.

Freshly harvested tissues were minced and stroma vascular fractions (SVF) were obtained by enzymatic digestion. PGAT and ScAT were digested with collagenase (NB4, Coger; 0.4 U/mL) and DNase (1%, Roche) in α MEM (GIBCO) at 37°C for 45 and 60 min respectively under constant agitation. After centrifugation (300g, 10min, RT) and elimination of the floating adipocytes and the supernatant, the pellet containing the SVF was resuspended in erythrocyte lysis buffer (155 mmol/L NH_4Cl ; 5.7 mmol/L K_2HPO_4 ; 0.1 mmol/L EDTA, pH 7.3). After filtration through 34 μm sieve and centrifugation (300g, 10min, RT), cells were resuspended in autoMACS® Running Buffer (Miltenyi Biotec). Quadriceps muscles were digested with collagenase B (0.5 U/mL, Roche) and dispase II (2.4 U/mL; Roche) in Hank's Balanced Saline Solution (HBSS)+2.5 mM Ca^{2+} for 2 rounds of 30 min at 37°C under agitation separated by mechanical dissociation through G18 syringe. Reaction was stopped by adding a 2 volumes of α MEM + 10%NCS, then samples were filtered through 34 μm sieve and centrifuged (300g, 10 min, RT) to eliminate supernatant and to resuspend the pellets in autoMACS® Running Buffer.

ASCs, FAPs, Platelets isolation.

For ASCs sorting, ScAT-derived SVF was depleted in CD45⁺ and CD31⁺ cells using anti-CD45-FITC and anti-CD31-FITC microbeads and autoMACS® Pro Separator (MACS Cell Separation, Miltenyi Biotec SAS) according to the manufacturer's instructions. ASCs were plated at a density of 80,000 cells/cm² in αMEM + 10% NCS for 3 days with 5% CO₂ for further co-incubation with platelets.

For FAPs sorting, muscle derived SVF cells were stained with anti-CD45-PE, anti-CD31-PE and anti-Sca1-BV421 for 20 min and sorted with FACS ARIA (BD Biosciences). Sorted cells were stored at -20°C for further DNA extraction.

For platelets isolation, blood was centrifuged (5 min, 500g, RT), and the plasma rich platelets (PRP) was resuspended in Tyrode's Buffer. Samples were centrifuged for 2 rounds (1900g, 8 min) and platelets were stained with PKH26 for 5min at RT°. After another centrifugation step (1900, 8 min, twice), platelets were resuspended in αMEM + 10%NCS at 10⁶ platelets/mL.

Cell culture.

Plated ASCs were incubated with blocking antibody anti-Podoplanin (10µg/mL) before being co-cultured with 100.000 platelets for 1 hour (37°C, 5%CO₂). Then, medium was removed, cells were rinsed with phosphate-buffered saline (PBS) and fixed with 3,7% PFA. Nuclei were stained with DAPI. Acquisition and analysis of data were performed with Operetta® system (Perkin Elmer).

For adipogenic differentiation, SVF cells were plated at a density of 60 000 cells/cm² in αMEM + 10%NCS at 37°C with 5% CO₂. After 24 hours, medium was removed to eliminate the non-adherent cells, and replaced by adipogenic medium (αMEM, 2% NCS, Dexamethazone 33nM, Insulin 5mg/mL, Rosiglitazone 1µM, T3 10µM, Transferrin 10µg/mL). After 3 days of incubation, medium was removed and cells were frozen at -20°C for RNAs extraction.

Cell Migration Assay.

ASC migration assay was performed using the IncuCyte® S3 Live-Cell Analysis System (Essenbioscience). ASCs were isolated from mice ScAT or human samples as described above and were plated at a density of 2000 cell per well in a 96 well plate including a reservoir and an optically clear membrane insert assembly with laser-etched 8µm pore. For mice migration assay, the reservoirs were loaded with 200 µl of plasma (50%) obtained from animals injured or not. For human migration assay, the reservoirs were loaded with 200 µl of human serum at 25 % from healthy volunteers before or 1 hour after exercise. Cell migration across the pores was automatically quantified for over 30h or 40h for human and mouse ASCs respectively. Migration was analyzed via the Incucyte S3 software (Essenbioscience) and area under curves (AUC) were calculated and compared using GraphPad Prism software (GraphPad Software).

Flow Cytometry Analysis.

Isolated cells from ATs or BM were incubated (25 minutes, 4°C, dark) with Phycoerythrin (PE) CD31, CD45 or $\alpha 7$ int, Allophycocyanin (APC)-Podoplanin or CD140a, Fluorescein isothiocyanate (FITC)-CD31, CD45 or Sca1, Sca1-V500 (BD Bio-sciences), Brilliant violet (BV) 421-CXCR4 or Sca1 antibodies or the appropriate isotype controls. After washing, the labeled cells were quantified on LSR Fortessa flow cytometer and analyzed using FACSDiva software (BD Biosciences).

Proliferation in vivo was assessed with the Click Plus EdU 488 Flow Kit (Life Technologies) following the manufacturer's instructions. Briefly, animals were injected IP with Edu (40 mg/g) just after the muscle injury, (i.e. 24 hr before sacrifice, such as described by Lemos et al.⁹). Isolated muscle SVF-derived cells were fixed and permeabilized and further incubated for 30min with a Click It reaction cocktail to reveal Edu staining with Alexa Fluor 488 in proliferative cells.

For apoptose/necrose evaluation, *In Situ* Cell Death Detection Kit (ROCHE) was used following manufacturer's instructions. Shortly, isolated SVF-derived cells were fixed and permeabilized before the TUNEL staining (labelling solution + enzyme) for 1 hour at 37°C. Stained cells were quantified by flow cytometry on LSR Fortessa (BD Biosciences).

RNAs and genomic DNA extraction, and RT-qPCR.

Extraction was realized on frozen cells or whole tissues using RNA Extractions Mini kit (QiaGEN) following manufacturer's instructions. Briefly, samples were unfrozen in RLT and lysed with tissue lyser® (QIAGEN). Samples were passed through columns with washing steps and DNA incubation to purify RNAs. Elution was performed with RNase free water, and RNAs concentration was evaluated with Nanodrop® 2000c (Thermo Scientific).

Genomic DNA was obtained with QiaAmp DNA Mini Kit (QiaGEN) following manufacturer's instructions. Cells were lysed and passed through column to bind DNA, and after two washing steps genomic material was eluted in Elution Buffer. Genomic DNA concentration was measured using n Nanodrop® 2000c; and then stored at -20°C.

For qPCR, RNAs were reverse transcribed using High capacity reverse transcriptase (Invitrogen) and diluted at 50ng/ μ L in RNase free water. Then, qPCR was performed using Fast SYBR® Green Mix (Applied Biosystems) on 384 wells plate, results were acquired with Viia7 device (Life Technologies) and data were analyzed with Real-Time qPCR Studio (Life Technologies) using the $2^{-\Delta\Delta C_t}$ method compared to CTL condition.

Immunohistochemistry.

FAP/ASC immunohistology

Paraformaldehyde (PFA) (4%) fixed muscles and ScAT were embedded in agarose 3% for 1 hour and sliced with Vibroslicer® 5100mz (Campden instruments) (300µm thick). Samples were permeabilized with Triton X100 (0,2% in PBS) before non-specific antigen saturation with BSA 3% and Goat serum in PBS. Tissue sections were incubated with Hamster anti mouse podoplanin, anti Sca1, anti CD45, anti-GFP primary antibodies for 24 hours at 4°C under constant agitation. Several washing with PBS were performed, and then sections were incubated with secondary antibodies (see Supplementary Informations) for 8 hours at RT. After several washes, nucleus were stained with DAPI and images were obtained using ZEISS LSM780 Confocal microscope (Plateforme I2MC) and analyzed with IMARIS® 8 (Bitplane).

Regeneration evaluation

Mouse muscle samples were cryopreserved in OCT frozen in liquid nitrogen-cooled isopentane. Samples were then sectioned at 10 µ on a cryostat and post fixed with 4% PFA for 15 min at RT. Slides were incubated with PBS containing Triton 0.5% (10 min, RT). They were incubated with 2% BSA (1h, RT) and overnight with a rabbit anti laminin antibody (1:200, Sigma Aldrich) (4°C, moist chamber) Slides were washed three times with PBS and incubated with FITC conjugated anti-rabbit secondary antibody (1:200, Invitrogen) (37°C, 45 min). Section were soaked for 5 min in DAPI solution and washed once with PBS before mounting with antifading mounting medium. Images were captured using Operetta High Content Imaging System (Perkin Elmer). The size and distribution of myofibers with central nuclei was calculated from laminin-DAPI staining on all fibers of the section and area determination were performed across the entire sections using an automated image processing algorithm (Harmony 4.5 software, Perkin Elmer).

Statistical Analysis.

Data are expressed as the mean ± s.e.m. Statistical analyses were performed using Student's t-test (two-tailed paired or unpaired) or one-way ANOVA followed by the post hoc Dunnett's and Tukey's test with GraphPad Prism software (GraphPad Software). P values less than * p<0,05 ; ** p<0,01 and *** p<0,001 were statistically significant.

Tables

Table 1 : Anthropometry and body composition of the individuals enrolled in the clinical study

Subject ID	Age	Weight (kg)	Height (cm)	BMI (kg/m ²)	%Fat Mass	Fat Mass (kg)	Free Fat Mass (kg)
A	22	79	184	23.33	12.16	8.5	62.1
B	19	87	193	23.36	17	13.6	63.9
D	19	70	171	23.94	17.8	11.1	49.4
I	27	87.2	185	24.54	15.9	12.8	64.8
J	24	79.3	191	21.7	15.6	10.6	60.7
M	29	71.3	176	23.01	14.3	10.4	59.2

Table 2: GDF 15 blood levels in individuals before (pre) and after (post) an exercise on a bicycle ergometer at 60% VO₂ peak for 1-hr. Δ corresponds to the difference of GDF15 blood level, before and after the exercise.

GDF15 (pg/ml)			
Subject ID	Pre	Post	Δ (Post - Pre)
A	153,4	168,1	14,7
B	417,0	457,5	40,5
D	209,4	313,2	103,8
I	341,2	446,5	105,3
J	451,6	437,7	-14,0
M	263,8	329,5	65,6

Supplementary Figure Legends

Figure S1 :

(A) Representative images of Hemalun-Eosine stained muscles from uninjured mice (0 dpi) and during muscle regeneration (3 to 14 dpi). Regenerating fibers are detected with the presence of centronuclei. Scale bar 200 μm. (B) Expression of pro inflammatory cytokines at 1 dpi in injured muscles (Gly, CTX) compared to uninjured muscle (Ctrl). (C) Representative image of positive and negative gates which were set by analysing isotype or unstained control sample in each analysis. (D) Comparison of in vivo EdU incorporation in FAPs by flow cytometry after Gly damage at 1 dpi. (E) Split channels of

immunofluorescent staining performed in muscle at 1 dpi. FAPs are described as Podoplanin⁺/Sca-1⁺/CD45⁻, nuclei are stained with DAPI. White arrows point infiltrated ASCs. Bar scale 50 μ m. Results are expressed as mean $\pm$ SEM of 6 to 8 animals; *p<0.05, **p<0.01, ***p<0.001 vs Ctrl.

Figure S2 :

At 1 dpi with NaCl 0.9% injected in the quadriceps muscle, CD45⁺ cells (A) and FAPs (B) were quantified in the muscle by flow cytometry analyses. ASCs (C) or CD45⁺ (D) cells from the ScAT were quantified by flow cytometry analyses at 1 dpi with NaCl 0.9% injection in the quadriceps muscle. Time course of body weight (E) and glycemia (F) in uninjured (Ctrl) or muscle injured (Gly or CTX). Results are expressed as mean $\pm$ SEM of . Statistical significance is set at p $\leq$ 0.05.

Figure S3 :

(A) Representative image of grafted ScAT (delimited by the white dashed line) inserted in the host adipose tissue (left panel). Fluorescent image was performed to visualize functional revascularization with lectin staining (red) injected *in vivo* 7 days after the grafting (right panel). Scale bar : 1 mm. (B) Gating strategy of muscle-derived SVFs from CD34-GFP grafted animals which were glycerol injured (Inj-Gly) or not (Ctrl), here is presented a non-fluorescent animal as an internal cytometry control. (C-D) GFP genomic expression analysis of CD34⁺-GFP cells infiltrated in organs of grafted mice at 1 dpi. (E) Model of ScAT grafting from Rpsa26^{mT/mG} mouse into WT C57Bl/6 mice. (F) Flow cytometry analysis of the SVF from injured muscle of the grafted mice (1 dpi). Results are expressed as mean $\pm$ SEM of 3 to 6 animals. Statistical significance is set at p $\leq$ 0.05.

Figure S4 :

(A) Time course measurement of platelets number in blood from animals injected with anti-GPIIb/IIIa. (B) Time course expression of myogenic genes in quadriceps muscle from injured animals in the presence (Gly) or absence (Gly + α PL) of platelets. (C) Representative images of Hemalun-Eosine stained muscles from mice with muscle injury and platelet depletion. Scale bar : 200 μ m. Results are expressed as mean $\pm$ SEM of 3 to 11 animals. Statistical significance is set at p $\leq$ 0.05.

Figures

Figure 1

Figure 1

FAPs content increases within 24h after muscle injury. (A-C) Myofibre damage was induced by intramuscular injection of glycerol (Gly) or cardiotoxin (CTX) into the quadriceps and FAP number was quantified from 1 to 9 dpi by flow cytometry and compared between control (uninjured, Ctrl), Gly or CTX injected animals (A-B) as well as in controlateral non-injured quadriceps (C). Quadriceps-derived SVF were analysed for CD31, CD45, Sca1, CD34, CD140α and podoplanin expression by flow cytometry. (D)

Immunohistological analysis of injured quadriceps at 1 dpi and quantification of Sca1+/Podoplanin+/CD45- cells in situ. Bar scale 50 μ m. (E) Representative phase contrast images of Ctrl, Gly or CTX muscle-derived SVF cells under adipogenic culture conditions. (F) Cells were fixed at day 4 and stained with oil red O. (G) Expression of adipogenic genes in muscle-derived SVF cells from Ctrl, CTX or Gly animals. (H) Comparison of in vivo EdU incorporation in FAPs by flow cytometry after Gly damage. Results are expressed as mean $\pm$ SEM of 7 to 30 animals; * p <0.05, ** p <0.01, *** p <0.001 vs Ctrl.

Figure 2

ASCs content diminishes in ScAT within 24 hours after muscle injury. (A) Flow cytometry analyses of CD31, CD45, Sca1, CD34, CD140 α and podoplanin expression in ScAT derived SVF. (B) ASCs were quantified at 1 dpi in SVF from Gly or CTX injured animals and compared to uninjured animals (Ctrl). (C) Relative cell numbers in injured muscle and ScAT at 1 dpi. (D) ASCs and MSCs content in perigonadic adipose tissue (PGAT) and bone marrow (BM) respectively using flow cytometry at 1 dpi in Gly-injured animals. (E) Phase contrast images of adipogenic challenged ScAT-derived SVF from Ctrl, Gly and CTX - injured animals. Cells were fixed at day 4 of differentiation and stained with oil red O. (F) mRNA expression of adipogenic genes in ScAT-derived SVF from Ctrl, Gly and CTX-injured animals. (G) TUNEL staining of ScAT derived SVF from Gly- or CTX-injured, or non-injured animals (Ctrl) at 1 dpi. Results are expressed as mean $\pm$ SEM of 3 to 30 animals; * p <0.05, ** p <0.01, *** p <0.001 vs Ctrl.

Figure 3

ASCs leave the ScAT and infiltrate the injured muscle. (A) Time course evaluation of murine in vitro ASCs chemotaxis in response to plasma isolated from Ctrl, CTX and Gly animals at 1 dpi. (B) In vitro human ASCs chemotaxis in response to serum of individuals collected 1 hour after an acute bout of continuous exercise (60% VO₂ max). (C) Correlation of human ASCs chemotaxis with 1h-post exercise GDF15 blood levels. (D) Model of ScAT grafting from CD34-GFP mouse into WT C57Bl/6 mice (left panel).

Immunohistofluorescence image of the ScAT depot 7 days post-graft surgery (right panel). (E) Flow cytometry analysis of the SVF from injured muscle of the grafted mice (1 dpi). (F-H) Immunohistological analysis of injured quadriceps in grafted mice 1 dpi in situ (arrowheads point CD34-GFP+/Podoplanin+/CD45- or CD34-GFP+/CD140α+/CD31-/CD45- cells). Results are expressed as mean ± SEM; *p<0.05.

Figure 4

Figure 4

The interaction of blood platelets with ASCs determines their infiltration into the damaged muscle. (A) Cell surface podoplanin expression of ScAT-derived ASCs of injured or not animals by flow cytometry at 1dpi. (B) Representative phase contrast and fluorescent images of PKH26-stained platelets (yellow) co-incubated with ScAT-ASCs. (C) Quantification of PKH26-stained platelets with ASCs. (D) Model of platelet depletion. (E) Platelet numeration 1 day post platelet depletion (F) FAPs content by flow cytometry in injured muscle from control (+IgG) or platelet depleted animals (+αPL). Results are expressed as mean ± SEM from 3 to 20 animals; *p<0.05, **p<0.01 vs Ctrl, \$\$\$p<0.001 Injured vs Injured+α-Podoplanin, \$p<0.05 Injured vs Injured + αPL or vs Injured - ScAT.

Figure 5

Figure 5

The interaction of blood platelets with ASCs is crucial for effective muscle regeneration. (A) Time course of mRNA expression of myogenic genes in quadriceps muscle from injured animals. (B-C) Immunohistological-based quantification of regenerative (laminin centronucleated fibers) muscle fibers at 7 dpi. Scale bar : 200μm. (D) Size distribution of regenerating muscle fibers in control (black), platelet depleted (gray) and ASCs supplemented (red) animals at 7 dpi. (E) Time course of regeneration

(expressed in relative area) in the presence or not of platelets and the impact of ASCs injection. Insert : Linearized representation of regenerating fibers size distribution. (F) Model of ScAT lipectomy. (G) Time course of mRNA expression of myogenic genes in quadriceps muscle from injured animals containing (Inj) or not (Inj-ScAT) their ScAT. Results are expressed as mean $\pm$ SEM of 3 to 7 animals; * $p < 0.05$, ** $p < 0.01$ vs Ctrl, \$ $p < 0.05$ Injured vs Injured+ α -Platelets.

Supplementary Files

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