Pharmacological inhibition of Lymphangiogenesis Impairs Skeletal Muscle Repair

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INTRODUCTION: Establishing a pro-regenerative niche is essential for skeletal muscle regeneration. Lymphatic vessels—key regulators of interstitial homeostasis, metabolic clearance, and immune trafficking—has been reported participate in regenerative responses across different tissues. Nevertheless, lymphangiogenesis' role in skeletal muscle repair remains elusive. Herein, we demonstrate local lymphatic vessels expansion post-cardiotoxin injury, and pharmacological blockade of lymphangiogenesis compromises muscle regeneration in mice.

METHODS: Animals: All animal work was approved by the IACUC. To characterize the temporal dynamics of lymphatic expansion after muscle injury, we performed CTX injury in 9-week-old C57BL/6 mice (n=3 males and 3 females per time point). Mice were sacrificed at four time points (0-, 7-, 14-, and 21-days post-injury (d.p.i)) for analysis. To investigate the functional role of injury-induced lymphangiogenesis, 9-week-old C57BL/6 mice (n=5 males per group) were subjected to CTX injury, treated with vehicle or MAZ51 (an inhibitor of the tyrosine kinase of VEGFR-3) during the course of regeneration and analyzed at 7 d.p.i. To create the CTX-induced injury model, mice were anaesthetized with isoflurane, followed by intramuscular injection of 50 µl of 10 µM CTX into the TA muscle. Histology: TA muscles were dissected and embedded in OCT, frozen in pre-cooled isopentane and processed using a cryostat to obtain 10-µm cryosections. The cryosections were stained with WGA, anti-laminin and anti-LYVE1 antibody as well as secondary antibodies. The CSA of centrally nucleated fibers in laminin-antibody-stained sections, the percentage of lyve1-positive vessel area were quantified using cellpose and Image J.

RESULTS SECTION: Following CTX-induced muscle injury, we observed a dynamic change in lymphatic area, as quantified by LYVE1 immunostaining (**Fig.1a**). The representative image of different time points were shown in Figure 1. Lymphatic vessel area percentage was low at the time of injury (0 d.p.i.: <0.05%). It increased markedly, peaking around day 14 post-injury (~0.3%), and subsequently declined by day 21 (~0.2%). This pattern indicates a rapid injury-induced expansion of the lymphatic network that is followed by a resolution phase during later regeneration (**Fig.1b**). Administration of the lymphangiogenesis inhibitor MAZ51 resulted in a significantly smaller average CSA of centrally nucleated fibers at 7 d.p.i. compared to the vehicle control group ($p < 0.0001$) (**Fig.1c**).

DISCUSSION: Our study demonstrates that active lymphangiogenesis is a hallmark of the skeletal muscle regenerative response following CTX-induced injury. The temporal expansion and subsequent resolution of LYVE1$^+$ lymphatic vessels mirror the typical phases of muscle repair, suggesting a previously underappreciated role for the lymphatic system in coordinating regeneration. Crucially, the pharmacological inhibition of this process with MAZ51 significantly impaired the growth of regenerating myofibers, indicating that lymphangiogenesis is not merely a passive bystander but a functional contributor to successful muscle repair. The exclusive use of male mice for the intervention study was to control for the significant confounding effects of the female estrous cycle, whose fluctuating hormone levels are known to modulate inflammation and regeneration. This design enhances internal validity but means the findings require future validation in females. A key limitation is the pharmacological nature of MAZ51. While it implicates VEGFR-3 signaling, its potential off-target effects mean the observed regeneration deficits may not be solely due to inhibiting lymphangiogenesis. Thus, our findings, while significant, necessitate validation through more specific genetic models. In summary, we provide foundational evidence that lymphatic vessels are a vital part of the regenerative niche in muscle. Targeting their growth precisely may offer a novel therapeutic strategy for improving muscle recovery after injury.

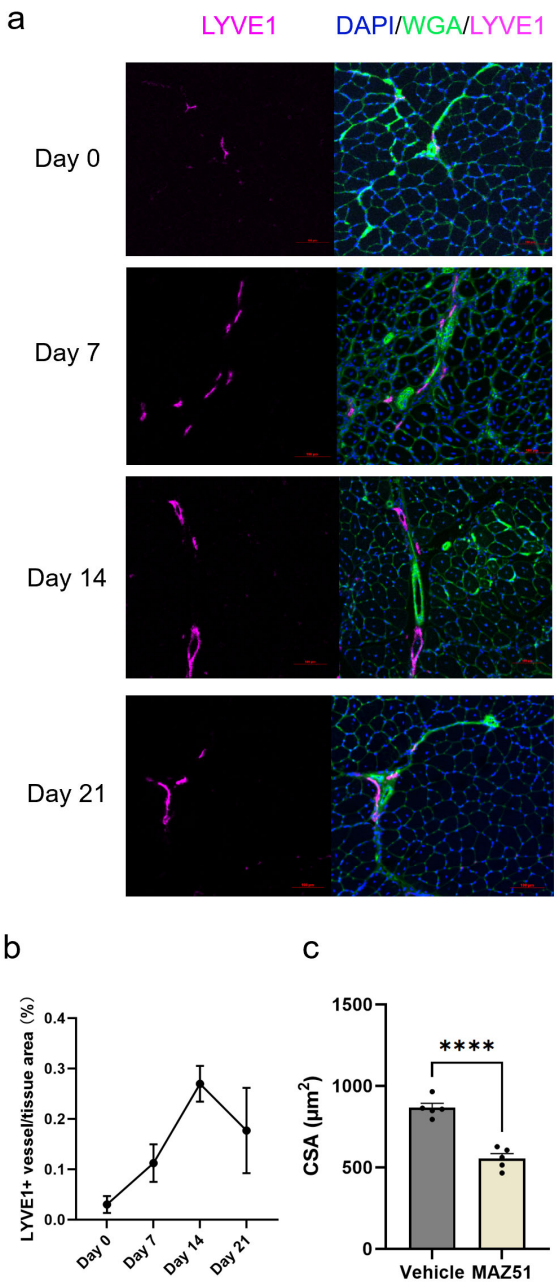

Fig.1 a-b, Representative image and quantification of lyve-1 positive vessel area in post-injury muscle at different time point. **c**, Quantification of average CSA of centrally nucleated fibers in vehicle- or MAZ51-treated 7 d.p.i. mice.

Significance/Clinical Relevance: The current study provides a compelling scientific foundation for the novel concept that therapeutic stimulation of lymphatic growth may represent a promising strategy to enhance regeneration in skeletal muscle diseases.