博士論文要旨

Muscle CD31(-) CD45(-) side population cells promote muscle regeneration by stimulating proliferation and migration of myoblasts

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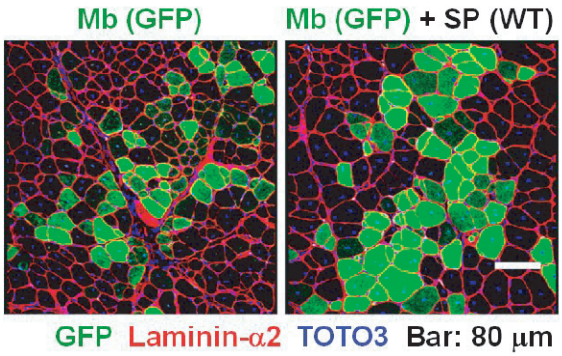
Introduction

Regeneration of skeletal muscle is a complex but well-organized process involving activation, proliferation, and differentiation of myogenic precursor cells, infiltration of macrophages to remove necrotic tissues, and remodeling of the extracellular matrix. Muscle satellite cells are myogenic precursor cells that are largely responsible for muscle fiber regeneration in adult muscle. In addition to muscle-derived satellite cells, skeletal muscle contains another type of stem cells referred to as side population (SP) cells. SP cells are defined as the cell fraction that efficiently effluxes Hoechst 33342 dye and therefore shows a unique pattern on FACS analysis. Previously reports showed that skeletal muscle-derived SP cell fraction are heterogeneous and contain at least three subpopulations. CD31-negative CD45-negative (CD31(-) CD45(-)) SP cells are a minor SP subfraction that have mesenchymal stem cell-like properties in uninjured skeletal muscle (Uezumi *et al.,* 2006). However, these cells actively expand in the early stages of muscle regeneration and return to normal levels when muscle regeneration is completed. Although CD31(-) CD45(-) SP cells are the only SP subset that exhibited the capacity to differentiate into myogenic, adipogenic, and osteogenic cells *in vitro,* their myogenic potential *in vivo* is limited compared with satellite cells. Therefore, I hypothesized that CD31(-) CD45(-) SP cells might play critical roles during muscle regeneration other than as myogenic stem cells.

Results and Discussions

1．Efficiency of myoblast transplantation is increased by co-transplantation of muscle CD31(-) CD45(-) SP cells in *NOD/scid* and *mdx* mice

To clarify the functions of CD31(-) CD45(-) SP cells during muscle regeneration, we isolated myoblasts from GFP-transgenic mice (GFP-Tg) and injected them (3 x 10^4 cells/muscle) with or without CD31 (-) CD45 (-) SP cells (2 x 10^4 cells/muscle) into TA muscles of immunodeficient *NOD/scid* mice or dystrophin-deficient *mdx* mice as a host. Two weeks after transplantation, co-transplantation of GFP (+) myoblasts with non-labeled CD31(-) CD45(-) SP cells produced a higher number of GFP (+) myofibers than transplantation of GFP (+) myoblasts alone. (Fig. 1) These results suggest that more myoblasts participated in myofiber formation after cotransplantation than after single transplantation, injected SP cells promoted growth of regenerating myofibers, or both.



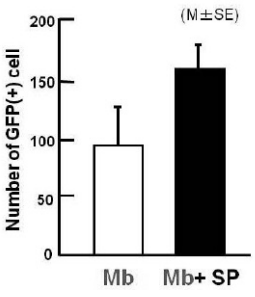


Fig. 1 Co-transplantation of myoblasts and CD31(-) CD45 (-) SP cells into skeletal muscle of NOD/scid mice promotes myofiber formation

2．CD31(-) CD45(-) SP cells promote proliferation and migration of myoblasts *in vivo*

To determine whether CD31(-) CD45(-) SP cells promote proliferation of implanted myoblasts, we dissected the muscles at 48 *hours* after transplantation, and stained the cross-sections with antiphosphorylatedhistone H3 antibody, a marker of the mitotic phase of the cell cycle. Co-transplantation of myoblasts

with CD31(-) CD45(-) SP cells significantly increased the percentage of mitotic GFP (+) cells compared with transplantation of myoblasts alone.

Further, as shown in Fig. 2, many more GFP (+) cells were detected in co-transplanted muscles than in myoblast-transplanted muscles 72 hours after transplantation. In addition, GFP (+) cells were more widely spread in the co-injected muscles than in muscles transplanted with myoblasts alone (Fig. 3).

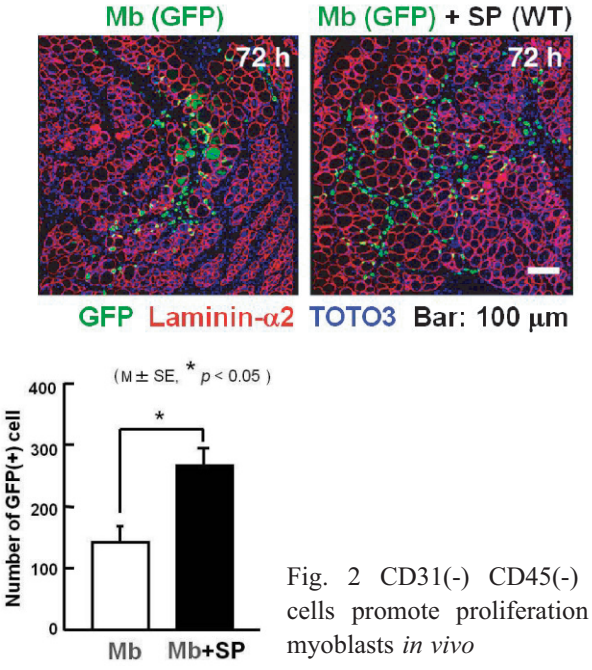


Fig. 2 CD31(-) CD45(-) SP cells promote proliferation of myoblasts *in vivo*

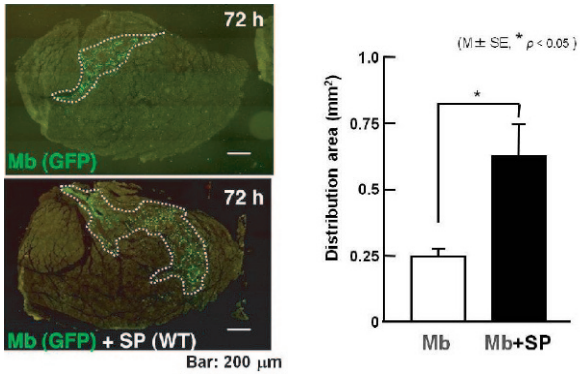


Fig. 3 CD31(-) CD45(-) SP cells promote migration of myoblasts *in vivo*

3. CD31(-) CD45(-) SP cell-derived MMP-2 promotes the migration of myoblasts

Genome-wide gene expression analysis revealed that CD31(-) CD45(-) SP cells highly express matrix metalloproteinases (MMPs). Among the MMPs upregulated in CD31(-) CD45(-) SP cells, we paid special attention to MMP-2. We confirmed that the mRNA level of MMP-2 and gelatinolytic activity were much higher in CD31(-) CD45(-) SP cells than in macrophages or myoblasts.

To directly investigate the effects of MMP-2 on the migration and proliferation of transplanted myoblasts, we injected GFP (+) myoblasts with CD31(-) CD45(-) SP cells prepared from wild-type mice or from MMP-2-null mice into TA muscles of *NOD/scid* mice. At 72 *hours* after transplantation, GFP (+) cells were more widely spread in the muscle co-injected with wild-type CD31(-) CD45(-) SP cells than in the muscles co-injected with MMP-2-deficient CD31(-) CD45(-) SP cells (Fig. 4). In contrast, there was no difference in the number of GFP (+) cells between two groups. These results strongly suggest that MMP-2 derived from CD31(-) CD45(-) SP cells significantly promotes migration of myoblasts, but does not influence the proliferation of myoblasts.

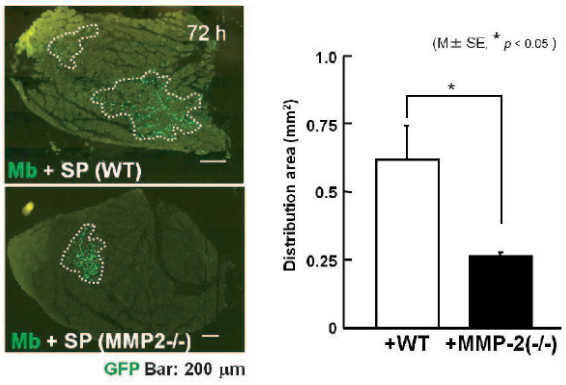


Fig. 4 MMP-2 derived from CD31(-) CD45(-) SP cells promotes the migration of myoblasts *in vivo*

Conclusion

Endogeneous CD31(-) CD45(-) SP cells promote muscle regeneration by supporting myoblasts proliferation and migration. These findings would provide us insights into the molecular and cellular mechanisms of muscle regeneration, and also help us develop cell therapy for muscular dystrophy.