S1P-Induced Microvascular Remodeling is Mediated by Bone Marrow Cell Recruitment

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Statement of Purpose

All organ systems in the body have bone marrow derived stem or progenitor cells (BMCs) that play pivotal roles in maintenance of healthy tissues: combating infection and regenerating damaged tissue after injury[1]. Sphingosine 1-phosphate (S1P) is a pleiotropic, autocrine and paracrine signaling small lipid molecule that binds to a family of five high affinity G-coupled receptors ($S1P_1$-$S1P_5$) to direct a wide range of biological processes. Because strong coordination exists between recruitment and surveillance of hematopoietic cells and tissue regeneration local modulation of S1P receptor signaling may also play an important role in recruiting other critical BMCs from circulation. To this end, our laboratory seeks to develop new strategies in regenerative medicine using S1P receptor selective agonists and antagonists[2] to regulate the recruitment and activity of BMCs during tissue healing.

Materials and Methods

AMD3100 (Sigma), a CXCR4 antagonist, was delivered at 5mg/kg wt. subcutaneously and VPC01091 (UVa), an $S1P_1$ agonist/$S1P_3$ antagonist was administered at 10mg/kg weight intraperitonealy to mice before peripheral blood and bone marrow isolation. White blood cell, flow cytometric analysis was performed with CD44, CD105, Sca-1, CD11b, CD45 and SMA antibodies (AbCam, Biolegend, BD Bioscience). FTY720 (Cayman Chemicals), an S1P1/S1P3 agonist, was encapsulated at 1:200 (w/w) in 71kDa methyl ester-capped 50:50 poly(lactic-co-glycolic acid) (PLAGA) films and delivered in the dorsal skinfold window chamber model to wild type and $S1P_3^{-/-}$ bone marrow chimeric C57BL/6 mice.

Results

Concurrently antagonizing CXCR4 and $S1P_3$ produced significant increases in circulating CD105, CD44 and Sca-1 positive cells (Figure 1).

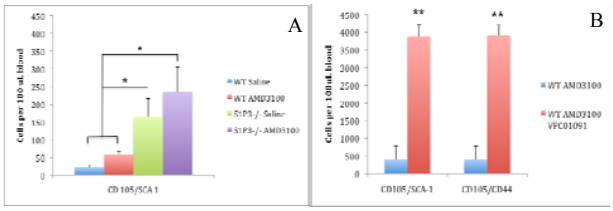

Fig. 1. Antagonizing CXCR4 and $S1P_3$ receptor leads to significant increase in circulating WBCs (* $p<0.05$ to WT Saline, ** $p<0.01$ to WT AMD3100).

The sustained release of FTY720 caused a significant increase in vascular length density (107%) and arteriolar diameter expansion (60%) 3 days after implantation in wild type animals. The increase in these metrics was severely attenuated in $S1P_3^{-/-}$ bone marrow chimeric mice (12% and 8% respectively) as shown in Figure 2.

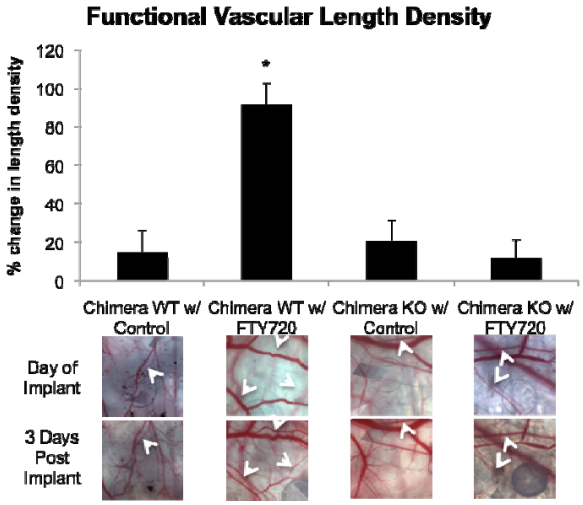

Fig. 2. Length Density 3 days after FTY720 treatment was significantly decreased in $S1P_3^{-/-}$ bone marrow chimeras (* $p<0.05$ to WT control)

Discussion and Conclusions

Our work establishes the importance of $S1P_3$ in the recruitment and functionality of BMCs from the bone marrow to sites of tissue regeneration. Ongoing studies will assess microvascular remodeling after the restoration of $S1P_3$ function in $S1P_3^{+/+}$ bone marrow chimeric $S1P_3^{-/-}$ mice. Our hope is to harness the body's innate regenerative systems for tissue engineering applications by use stem cell mobilizing agents to increase the supply of BMCs and S1P-receptor signaling to increase their demand locally.

References

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2. Sefcik L, Aronin C, Awojoodu A et al. Tissue Engineering Part A; In-press

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