Release Profile of Exogenous SDF-1 Differentially Affects Cortical SDF-1/CXCR4 Signaling *In Vivo*

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Statement of Purpose: Stromal cell-derived factor-1 (SDF-1) and its key receptor CXCR4 is implicated in several pathological/disease conditions such as, cancers[1] CNS diseases[2], autoimmune disorders[3], among others. SDF-1/CXCR4 signaling also plays a central role in directed CXCR4$^+$ stem cell recruitment that impart regenerative effects in damaged tissues of the brain[4], skin, heart, bone and others[5]. Efforts to modulate the SDF-1/CXCR4 signaling axis have thus gained considerable attention particularly for regenerative applications[6]. One approach includes delivery of SDF-1 via sustained release devices such as our previously published poly(D,L-lactic-*co*-glycolic) acid (PLGA) nanoparticles (NP) that release bioactive SDF-1α for 60 days[6]. However, the impact of SDF-1 sustained release on the endogenous SDF-1/CXCR4 signaling axis in the adult brain is largely unknown as auto-regulatory mechanisms typically dictate cytokine/receptor signaling (i.e. receptor downregulation, ligand sequestering, genetic regulation, etc.). We hypothesize that spatiotemporal presentation of exogenous SDF-1 is a key factor in achieving long-term manipulation of endogenous SDF-1/CXCR4 signaling. Here in the present study, we sought to probe our hypothesis using a transgenic mouse model to contrast the spatial activation of endogenous SDF-1 and CXCR4 in response to exogenous SDF-1 injected in bolus or controlled release form in the adult rodent cortex.

Methods: All protocols involving animals were approved by ASU IACUC. Critical tools employed include: 1) transgenic mice with an intracellular transcriptional reporter for CXCR4[7], 2) bioactive fluorophore-conjugated SDF-1 (AFSDF-1) and, 3) AFSDF-1 loaded PLGA nanoparticles[6]. Adult mice (n = 5 mice per group) were subjected to a 3.0uL intracortical injection of one of the following groups: 1) bolus AFSDF-1, 2) bolus vehicle, 3) AFSDF-1 NPs and, 4) NP vehicle. The dose of bolus AFSDF-1 (30ng) was based on the predicted protein payload released from the PLGA NPs within the first 24hrs (~30ng). The animals were sacrificed at 1, 3 or 7 days post-injection, transcardially perfused, then brains were extracted, cryofixed, and serially sectioned. A minimum of 3 sections per animal within the injection site was immunostained for total SDF-1 and cell nuclei (DAPI). Ipsi- and contralateral cortices were imaged using fluorescent microscopy and images were analyzed in ImageJ to determine spatiotemporal localization of CXCR4$^+$ cell bodies and percent area positive for total SDF-1 (exogenous + endogenous) and exogenous AFSDF-1 (Fig. 1A). Results are presented as mean ± standard deviation.

Results: Bolus administration of AFSDF-1 in the intact mouse cortex led to a localized, transient increase in CXCR4$^+$ cells at day 1 that subsequently returned to vehicle control levels by day 3 (Fig. 1B & C). Bolus AFSDF-1 did not significantly impact endogenous SDF-1 expression, particularly beyond the injection site (data not shown). Sustained release of AFSDF-1 significantly increased CXCR4$^+$ cell density relative to both bolus AFSDF-1 and vehicle NPs in regions most proximal and distal (>700μm) to the injection site (Fig. 1D & E). The spatially widespread increase in CXCR4$^+$ cells was however transient, decreasing significantly by day 3 and reaching vehicle NP levels by day 7 (Fig. 1E). Sustained release of AFSDF-1 also elicited complex patterns of total SDF-1 immunostaining across the ipsilateral cortex that persisted through day 7 (data not shown).

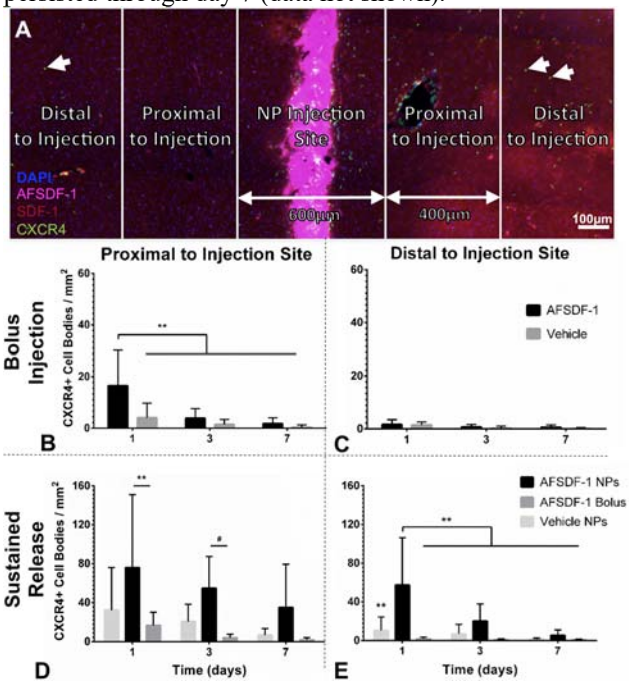

Figure 1. (A) Representative cortical image centered across implanted AFSDF-1 loaded PLGA NPs at 1-day post injection. White arrows represent CXCR4$^+$ cell bodies. (B, C) Bolus AFSDF-1 injections had localized and transient effects on CXCR4$^+$ cell density. (D, E) Sustained release also resulted in transient, but more spatially diffuse CXCR4 activation.

Conclusions: Our data suggests that the manner of AFSDF-1 presentation significantly affected activation of CXCR4 at day 1 (Fig. 1D, E) despite having similar protein payloads over the first 24 hrs (~30ng for both bolus and sustained release groups). Moreover, the transient nature of widespread CXCR4 activation for the AFSDF-1 NP groups (Fig. 1E) indicates controlled protein release does not necessarily translate to sustained biochemical effects (i.e. prolonged CXCR4$^+$ cell recruitment). Instead, cytokine/receptor auto-regulatory mechanisms may demand more complex release profiles (i.e. delayed and/or pulsed release) to achieve sustained cellular response. Further studies will include cell phenotype characterization of CXCR4$^+$ cells and evaluations in an injured cortical microenvironment.

References: [1] P. Bianco, et. al. *Cell Stem Cell*. 2008. [2] R. Würth, et. al. *Front. Cell. Neurosci*. 2014. [3] N. Karin. *J. Leukoc. Biol.* 2010. [4] J. Imitola, et. al. *Proc. Natl. Acad. Sci.* 2004. [5] R.C. Rennert et. al. *Regen Med*. 2012. [6] D. Dutta, et. al. *J Mater Chem B Mater Biol Med*. 2015. [7] P. B. Tran, et. al. *J. Comp. Neurol*. 2007.