Understanding the role of CXCR7 in adrenomedullin signaling during lymphatic vascular development

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Project Summary and Aims

Adrenomedullin (AM) is a circulating peptide that has a critical role in vascular and lymphatic development. AM induces intracellular signals by binding to the canonical GPCR CLR and its modifying protein RAMP2. However, recent and historical studies of the chemokine receptor CXCR7 have led us to consider CXCR7 as a putative AM receptor. CXCR7 null mice (KO) are postnatally lethal due to cardiac malformations, including cardiac hyperplasia. Interestingly, we also observe cardiac hyperplasia in mice that overexpress AM. Based on this similar cardiac phenotype, historical literature, and other data, we hypothesize that CXCR7 associates with AM and affects the delicate balance of AM necessary for proper lymphatic and cardiac development. We propose that CXCR7 functions as a decoy receptor (a molecular sink) for AM. As a decoy, we hypothesize that CXCR7 binds to AM but does not induce a downstream signal. My project this summer centered around understanding this interaction.

**Objective 1** - Because CXCR7 KO mice exhibit similar cardiac abnormalities as mice which overexpress AM, we hypothesize that knocking out CXCR7 leads to enhanced AM signaling. We propose that CXCR7 null mice that are haploinsufficient for AM will resolve this enhanced AM signaling and rescue the cardiac phenotype. To test this we will perform timed matings between mice heterozygous for both AM and CXCR7. We can predict genotype ratios of offspring using classical Mendelian genetics. Once genotypes of progeny are determined, we can compare the viability of mice that are haploinsufficient for AM to mice with the same CXCR7 genotype but wildtype for AM. We hypothesize that AM^+/−;CXCR7^−/− mice will have a greater survival rate than AM^+/+;CXCR7^−/− mice due to their reduced AM levels.

**Objective 2** - In order to affect lymphatic vascular development, CXCR7 must be expressed on the surface of lymphatic endothelial cells (LECs). We will stain tissue sections of mice at various stages of embryonic development using immunohistochemistry (IHC) techniques to determine where CXCR7 is expressed. LECs will be stained by the antibody podoplanin. CXCR7 expression is knocked down by disrupting the CXCR7 gene using a GFP reporter allele. We can then use a GFP antibody to determine CXCR7 expression. GFP fluorescence in mice with the CXCR7 null allele will therefore indicate where CXCR7 would normally be expressed in CXCR7 wild-type (WT) mice.
Results

Objective 1- Using Mendelian principles of inheritance, we were able to predict the genotype ratios of offspring. Interestingly, the observed genotype ratios of offspring did not reflect the predicted outcome. We originally proposed that AM haploinsufficiency would resolve the embryonic lethality observed in CXCR7 null mice. However, we observed half as many AM<sup>+/−</sup>;CXCR7<sup>−/−</sup> mice as expected. These data suggest that mice with this genotype may be dying in utero. While these data do not agree with our original hypothesis, it indicates that changing the AM dosage affects survival of CXCR7 null mice; this supports our hypothesis that AM and CXCR7 interact in vivo. Future experiments will focus on determining whether AM<sup>+/−</sup>;CXCR7<sup>−/−</sup> mice are embryonic lethal and what leads to their lethality in utero.

Objective 2

Lymphatic endothelial cells (LECs) were stained with a podoplanin antibody to identify the lymphatic sac (LS) in mice (A,C). Utilizing a GFP antibody, mice LS were also stained for the GFP reporter allele to determine CXCR7 expression. As expected, the LS of CXCR7 WT mice do not stain positive for GFP because WT mice do not have a mutant allele that expresses the GFP reporter allele (B,C). LECs of CXR7 KO mice express both GFP and podoplanin (D-F). A higher magnification of the lymph sac confirms GFP expression by podoplanin positive LECs (G-I). These data indicate CXCR7 is expressed on the surface of LECs at e13.5, supporting the hypothesis that CXCR7 plays a role in lymph sac formation. We also plan to stain lymph sacs at e13.5 for AM to determine if CXCR7 and AM are localized to same area at this stage in development.