Principal Investigator: Lan Zhou
Grant Title: Developing O-glycan Modified Notch Decoys to Mobilize Hematopoietic Stem Cells

Abstract

Objectives:

Conservative Notch-ligand adhesive interaction and its modification by *O*-fucosylation is critical for HSC quiescence and niche maintenance. This is supported by our previous findings including, 1) deficiency of *O*-fucosylation leads to decreased HSC quiescence, decreased HSC adhesion to bone marrow niche cells, and increased HSC egress from the marrow (Yao, *Blood*, 2011); 2) neutralizing DLL4 or Jag1 enhances HSC egress to the periphery; 3) blocking Notch2 signaling transiently down-regulates CXCR4 and acts synergistically with G-CSF and AMD3100 (CXCR4 antagonist) to mobilize HSPC with superior engraftment and hematopoietic recovery (Wang, Stem Cells, 2015; Wang, Haematologica, 2017). In this study, we will test the hypothesis that *O-glycan-modified recombinant Notch peptides function as decoys to promote HSPC mobilization.*

Methods used:

We first generated recombinant Notch1 and Notch2 peptide comprising the core binding region, with or without *O*-glycan modification. We used flow-based binding assay, MASS SPEC, and surface plasmon resonance (SPR) analysis to assess the binding affinity of these peptides with the Delta-like and Jagged ligands. We then used in vitro adhesion assay and 3D osteoblastic (OB) niche assay to compare the effectiveness of Notch decoys to block HSPC interaction with stroma and induce HSPC egress from 3D OB niche.

Results:

1. We generated 4 groups of recombinant mouse Notch ($mN1_{11\text{-}13}$, $mN1_{8\text{-}13}$, $mN2_{11\text{-}13}$, $mN2_{8\text{-}13}$) peptides in fucosylation-deficient Fc-tagged form, *O*-Fucose-extended form, and O-fucose and Lfng extended form ($mN1_{11\text{-}13}$+Fuc+Lfng).
2. We recently generated HIS-tagged Notch1 peptides in non-modified, *O*-Fucose-extended form, and O-fucose and Lfng extended form. The yields of of HIS-tagged peptides are better than Fc-tagged peptides.
3. We found that Notch1 core-binding EGF repeats bind Delta and JAG1 ligand with much higher affinity than corresponding Notch2 peptides.
4. Lfng-modified $mN1_{11\text{-}13}$ and Rfng-modified $mN1_{8\text{-}13}$ binds Delta and JAG1 with the strongest affinity, respectively.
5. mN1 “decoys” (both O-fucose extended form and Lfng-modified form) effectively block the adhesion of HSPC with stroma cells and induce the egress of HSPC from 3D OB niche.