299d Regulation of Cd47-Sirp□ Interactions by Post-Translational Modifications

Shyamsundar Subramanian, Eric T. Boder, and Dennis E. Discher

CD47 is a ubiquitously expressed receptor that interacts with SIRP□ on many cell types including phagocytes and endothelial cells. These interactions are implicated in many processes such as phagocytosis inhibition, neutrophil migration, and T-cell activation. As these cell types are not identical in their glycosylation machinery, it is possible that the nature of glycosylation on these glycosylated proteins regulates their interaction. To verify the role of glycosylation, the human versions of this protein were either genetically modified or produced under conditions that result in synthesis of aglycosylated proteins. These aglycosylated proteins were shown to still interact and surprisingly to a greater extent than the glycosylated counterparts. Furthermore, results also show that while removal of N-linked glycosylation in CD47 does not change binding quantitatively, removal or reduction of sugars in SIRP□ enhances the interaction. Experiments are currently underway to identify whether enhancement in binding seen with reduction in SIRP□ glycosylation is due to changes in receptor clustering or chnages in intermolecular binding affinity. In addition, the single N-linked glycosylation site on the binding domain (N-terminal Ig-domain) of human SIRP□ is predicted to be unoccupied. This suggests that sugars on the other Ig-domains may be responsible for observed glycosylation effects and SIRP□ variants are being prepared to confirm if this is the case.