Development of Macrocyclic Peptide Heterodimer as PPI Inhibitor against Immune Checkpoint CD47- SIRPα

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Tumor cell can disguise itself as a normal cell and thus escapes from the phagocytosis by macrophage through the protein-protein interaction (PPI) between immune checkpoint CD47-SIRPα. We recently developed a macrocyclic peptide D4-2 as a specific target binder to SIRPα by Random nonstandard Peptides Integrated Discovery (RaPID). The following *in vitro* and *in vivo* assays confirmed that D4-2 allosterically inhibited the CD47 from binding to SIRPα, raised the phagocytosis ratio and control the tumor size[1]. It has been shown that dimerization of macrocyclic peptides could be effective in strengthening macrocyclic peptides' binding affinity[2] and thus promote the curative effect. Here, we report the improvement of CD47-SIRPα PPI inhibitor D4-2 by hetero-dimerizing D4-2 with another binder. To get another SIRPα binding peptide, as the dimerization partner of D4-2, the RaPID selection against D4-2-SIRPα complex was performed. The discovered D4-2-SIRPα complex binders were then 50% mutated and adapted into SIRPα binders by additional RaPID selection. The new candidates' binding affinity was measured by surface plasma resonance (SPR), which revealed a new SIRPα binder mD2r3. Then mD2r3 was linked with D4-2 by a polyethylene glycol (PEG) linker (Fig. 1c). Compared with the D4-2 monomer, the SPR result of PEG_2, PEG_5, and PEG_{11} linker showed a considerable improvement in off-rate (k_{off}) and overall dissociation constant (K_D). The following *in vitro* CD47 inhibiting assay and phagocytosis assay confirmed the heterodimer linked with PEG_{11} had better activity inhibiting the PPI between CD47 and SIRPα and inducing macrophages' attack toward tumor cells than monomeric D4-2 peptide (Fig. 1d). In the presentation, due to the potential patent filing, the sequence identities will not be shown.

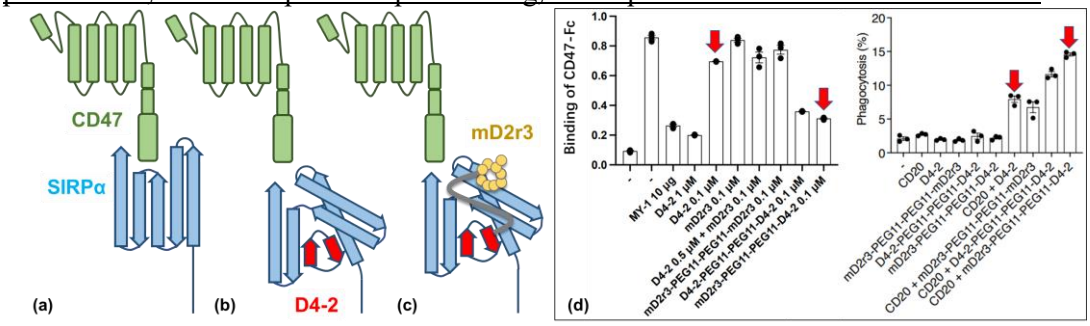

Figure 1 a) CD47-SIRPα complex. b) D4-2 as an allosteric inhibitor. c) D4-2-mD2r3 heterodimer. d) Dimerization with mD2r3 improved the function of D4-2 monomer.

1) Hazama, D., et al., *Cell Chemical Biology*, **2020**, 27, 1181–1191. 2) Bashiruddin, N., et al., *Bioconjugate Chemistry*, **2018**, 29(6), pp.1847-1851.