DEVELOPMENT OF DESIGNED BIVALENT LIGANDS FOR CXCR4 AND THEIR FUNCTION ON RECEPTOR BINDING

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The chemokine receptor CXCR4 is a membrane protein, which belongs to the G-protein coupled receptor family. Interaction of CXCR4 with its endogenous ligand stromal-cell derived factor-1 (SDF-1)/CXCL12 induces various physiological functions. Recently, a ligand-independent homodimerization of CXCR4 was revealed by BRET analysis. However, information obtained from BRET analysis is limited and not enough for elucidation of the native structure of CXCR4 dimers in living cells due to the mutation such as conformational change and functionalized effect. Furthermore, it is also difficult to estimate the precise distance between CXCR4s that form a dimer structure.

In this study, we designed and synthesized novel CXCR4 bivalent ligands that linked two FC131 analogues [cyclo(-D-Tyr-Arg-Arg-Nal-d-Cys-)], which is a highly potent CXCR4 antagonist, with a polyproline or PEGylated polyproline linker to sustain a constant distance (2 – 8 nm) between two ligands. We applied our bivalent ligands to estimate the distance between the binding sites of CXCR4 in dimer form by evaluating binding of these ligands with various lengths of linkers. The binding affinity was evaluated in a competitive binding assay against [125I]-SDF-1α. The results showed that the binding affinity of these bivalent ligands is clearly dependent on the linker lengths. It should be noted that the maximum increase in binding affinity was observed for both of the linker types of similar length (ca. 5.5-6.5 nm). Based on the increased binding affinity of linker-optimized bivalent ligands, ligands were applied as probes specific to CXCR4 on the cell surface because the receptors are overexpressed in several kinds of malignant cells. The dimer formation of the receptor should depend on the expression level, thus higher population of dimers should be observed on the surface of malignant cells. Accordingly, the ligand with the highest binding affinity was labeled with tetramethylrhodamine (TAMRA) and applied to the imaging of CXCR4.1 The CXCR4 recognition by the ligands showed clear dependence on the expression of CXCR4 on the cell surface. Thus, the ligand could distinguish cancer cells with high CXCR4 expression and normal cells such as endothelial cells.2 This information would be useful for the design of bivalent ligands of any GPCR which function is still not known.