Nanobodies targeting and modulating G protein-coupled receptors outside in and inside out.

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G protein-coupled receptors (GPCRs) belong to the largest class of membrane proteins. Their prominent role in many (patho)physiological processes make GPCRs important drug targets. While many approved drugs act on GPCRs, only a small fraction of all 850 family members are currently being targeted, leaving an enormous potential for drug development.

Aims

Nanobodies have appeared to be ideal ligands to study, modulate, and exploit GPCRs. Their unique structure allows the binding to non-linear, conformational epitopes, such as those formed by the intra- or extracellular loops of GPCRs. Their small size also aids phage-library generation and phage expression. Here, we show examples of nanobodies targeting intra- or extracellular parts of chemokine receptors and their utilization in GPCR research and therapy.

Methods

Llamas were immunized with DNA encoding for the human chemokine receptors CXCR4, CXCR7 or the viral chemokine receptor US28, followed by boost immunizations with either cells or lipoparticles. VHH-phage libraries were constructed and used for three rounds of panning.

Results / Conclusions

For all three receptors, in outside-binding nanobodies antagonized ligand binding. Moreover, as bivalent constructs, the nanobodies displayed inverse agonistic activities on constitutively active receptors. Furthermore, intrabodies targeting US28 recognized and stabilized different receptor conformations and could completely block G protein signaling. The best binding nanobodies were further functionalized via the addtion of different effector domains. Nanobody-Fc constructs induced antibody-dependent cellular cytotoxicity (ADCC) of CXCR4-overexpressing leukemic cells. Nanobody-photosensitizer conjugates allowed the specific killing of CXCR4-overexpressing HEK293 cells or US28 expressing glioblastoma cells via photodynamic therapy. These studies show the feasibility of developing nanobodies targeting GPCRs and illustrate their potential in fundamental GPCR research and as targeting moieties for diverse therapeutic conjugates.