The structural and pharmacological characterization of CXCR3 isoforms

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CXCR3 is a chemokine G protein-coupled receptor (GPCR) involved in various pathological conditions, including cancer and autoimmune diseases. Its primary role in the immune response is to recruit T-cells to the sites of inflammation, positioning it as a key target for modulating immune activity. In cancer, however, enhanced CXCR3-mediated T-cell recruitment has been associated with tumour progression and metastasis. Upon binding with proinflammatory chemokines—CXCL4, CXCL9, CXCL10, and CXCL11—CXCR3 undergoes activation, initiating both G protein-dependent and -independent signalling cascades. These chemokines exhibit unique expression profiles in different disease and biology states and exhibit a preference for distinct signalling pathways activated by CXCR3 which influences the receptor's functional outcomes. The existence of two splicing variants, CXCR3A and CXCR3B, further complicates its pharmacology, as these isoforms exhibit differential signalling profiles, contributing to the receptor's diverse roles in disease and therapeutic potential.

Despite extensive research, inconsistencies and knowledge gaps persist in understanding CXCR3 signalling profile, particularly for the CXCR3B variant. This study systematically characterized β-arrestin1 and β-arrestin2 recruitment by CXCR3 variants in response to chemokines using BRET biosensors in HEK293T cells. Additionally, G protein activation for CXCR3A and CXCR3B was quantified, examining Gαi (0, 1, 2, 3) and Gαq subtypes.

CXCR3A displayed β-arrestin1/2 recruitment in the order: CXCL11 > CXCL10 > CXCL9, with no response to CXCL4. CXCR3B only recruited β-arrestins in response to CXCL11, at a similar potency as CXCR3A, but not other ligands.

The inhibitory effects of CXCR3 antagonists, including mAbs (MAB160, Hu37) and small molecules (AMG487), were evaluated on both β-arrestin and G protein pathways. AMG487 showed the strongest inhibition across both receptor variants, at all effectors to all ligands. The mAbs, at all effectors to all ligands. The mAbs Hu37 and MAB160 were also able to inhibit ligand mediated effector recruitment at all effectors tested, but they showed a higher preference for inhibition of CXCL11 and CXCL10 mediated responses with only minor inhibition of CXCL9 mediated responses. This highlights the utility of mAbs as being able to tailor inhibition of specific ligand which may be beneficial therapeutically.

To further understand CXCR3 mediated inhibition by mAbs, my project aims to solve an inactive, Hu37 bound CryoEM structure. I have been able to express and purify CXCR3 with ICL3 substituted with an inactive state stabilising nanobody recognition sequence. The ultimate aim is to complex CXCR3 with Hu37 to investigate binding epitope and the conformational changes linked to pathway inhibition, elucidating the mechanisms behind Hu37's inhibitory effects. This research will therefore contribute to the development of selective therapeutic interventions targeting CXCR3 in immune-related diseases.