BMP2K as a signaling effector of Atypical chemokine receptor 3

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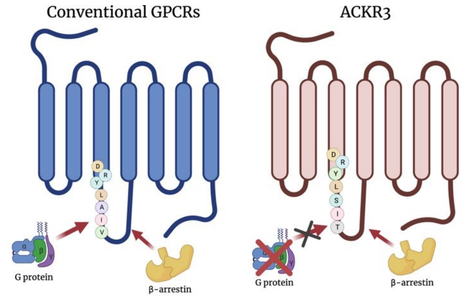
Background

G protein-coupled receptors (GPCRs) are seven-transmembrane receptors that are found everywhere in the body and are the largest family of cell-surface receptors. They mediate the majority of cellular responses and are the target of 1/3 of all FDA-approved drugs. GPCRs are known to signal through two main protein pathways, G-proteins and β-arrestins, the combination of which produces the cellular output.

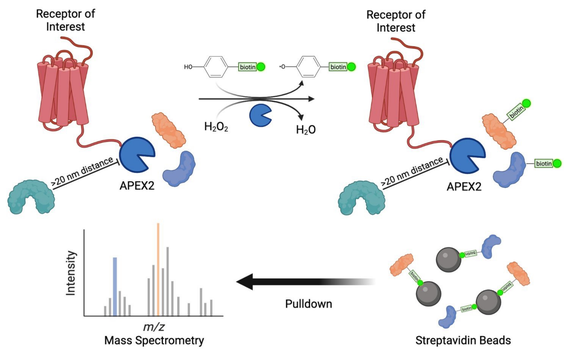
Atypical chemokine receptor 3 (**ACKR3**) is a GPCR that was found to signal non-canonically, as it does not couple to G-protein. ACKR3 was also demonstrated to signal independently of β-arrestin, making it a protein of interest to study GPCR signaling through alternative pathways.



Methods

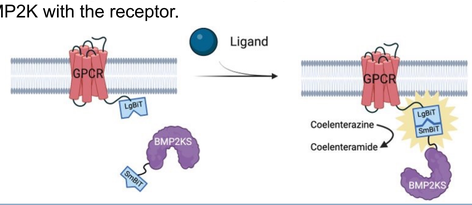
To investigate what other signaling effectors may be responsible for regulating ACKR3 signaling, we conducted **ACKR3-APEX2 proximity labeling** in a β-arrestin knockout cell line, with the endogenous ligand CXC12 and a vehicle control (HBSS buffer).

In this method of proximity labeling, APEX2 catalyzes the oxidation of biotin-phenol to a biotin-phenoxyl radical in the presence of hydrogen peroxide. Proteins less than 20 nm from APEX2 and our receptor of interest are tagged with biotin, which strongly binds to streptavidin beads. Then, mass spectroscopy is performed on these samples to identify proteins in proximity to the receptor of interest.



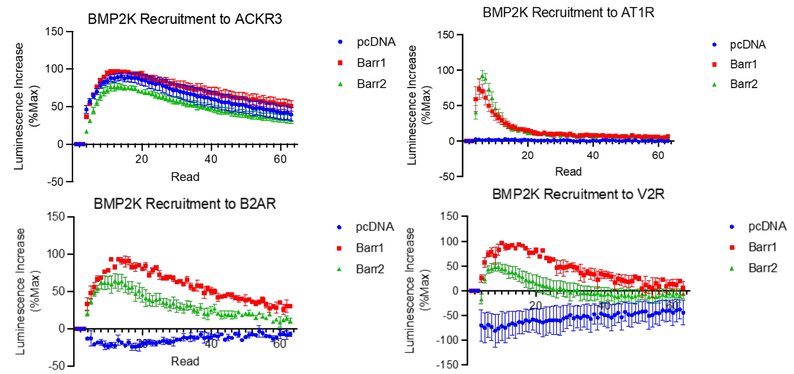
Approximately 6000 protein hits were identified with our ACKR3-APEX2 proximity labeling. We compared these protein hits to APEX2 proximity labeling at GPCRs in four other data sets. We found that the effector protein **BMP2K** is significantly upregulated with ACKR3 and is conserved across all four data sets.

To explore BMP2K recruitment to GPCRs, we used the protein-protein interaction assay: split-luciferase (NanoBiT). We tagged BMP2K with a SmBiT and the GPCR with a LgBiT. Upon recruitment of BMP2K to the GPCR, the SmBiT and LgBiT will undergo complementation and generate a luminescence signal that is tracked by a plate reader, and is a measure of the interaction of BMP2K with the receptor.



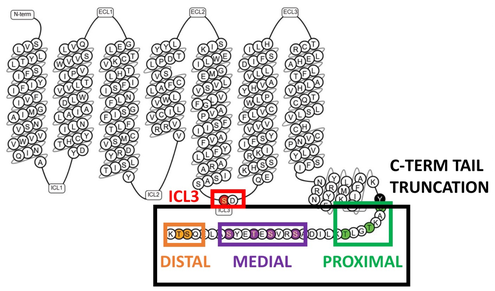
Results

Using the NanoBiT assay, we assessed BMP2K recruitment in β-arrestin knockout cells to four receptors: ACKR3, AT1R, B2AR, and V2R. We added back β-arrestin 1 and β-arrestin 2 (and used pcDNA as a control) to investigate how β-arrestin affects BMP2K recruitment. We measured the increase in luminescence signal for these interactions for around 60 reads on a plate reader.

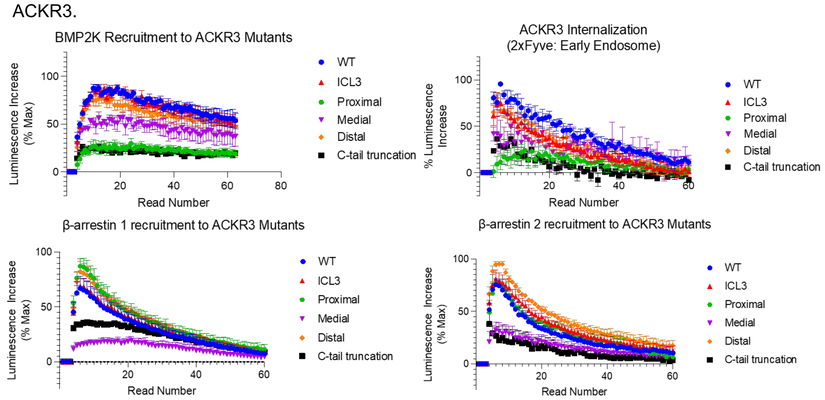


We found that BMP2K recruits to ACKR3, independent of the β-arrestins, while the other receptors tested (AT1R, B2AR, and V2R) recruit BMP2K with or through β-arrestin, as noted by the increase in luminescence signal with the β-arrestins compared to pcDNA. Additionally, B2AR and V2R seem to recruit BMP2K more with β-arrestin 1 compared to β-arrestin 2.

ACKR3 can be activated through phosphorylation. To investigate the sites on ACKR3 that may be involved with signaling, we created ACKR3 phosphodeficient mutants. Using site-directed mutagenesis, a method to make targeted changes to amino acids on a protein, we created five separate mutants on ACKR3's C-terminal tail: distal, medial, proximal, ICL3, and a C-terminal tail truncation.



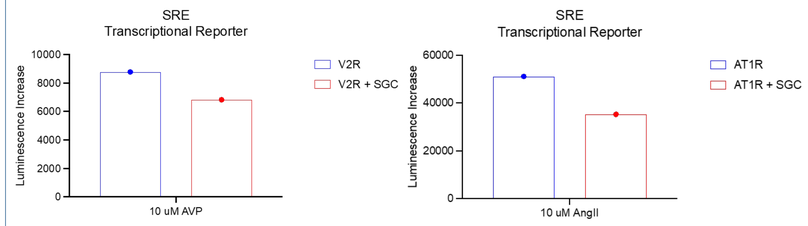
We wanted to test if different outputs of ACKR3 signaling rely on different phosphorylation sites. Thus, for each phosphodeficient mutant, we investigated BMP2K recruitment to ACKR3, ACKR3 internalization to the endosome, β-arrestin 1 recruitment to ACKR3, and β-arrestin 2 recruitment to ACKR3.



BMP2K recruitment to ACKR3 was significantly reduced with the proximal and C-tail truncation mutants. ACKR3 internalization was similarly reduced with the proximal and C-tail truncation mutants. Finally, β-arrestin 1 and 2 recruitment was reduced with the medial and C-tail truncation mutants.

Results

To investigate the importance of BMP2K in GPCR activation, we examined ligand-induced activation of the serum response element (SRE), a transcriptional reporter. With BMP2K inhibition (using the inhibitor SGC), we saw decreased levels of transcriptional activation for the receptors V2R and AT1R, compared to the control.



Conclusions

Using ACKR3-APEX2 proximity labeling, we found BMP2K to be a protein effector that is significantly upregulated with ACKR3. It is a conserved protein across other GPCR data sets.

BMP2K recruits to ACKR3 independent of β-arrestin, whereas AT1R, B2AR, and V2R recruit BMP2K with the help of β-arrestin.

Through site-directed mutagenesis of ACKR3, we determined that β-arrestin recruitment to ACKR3 does not correspond with ACKR3 internalization, however the pattern of BMP2K recruitment is similar to internalization. This may be due to two reasons: BMP2K recruitment promotes ACKR3 internalization, or that ACKR3 internalization is necessary for BMP2K recruitment.

This study is promising for further exploring non-canonical signaling of GPCRs and thus discovering and creating therapeutics with greater efficacy.

Future Directions:

- Perform further studies to uncover the functional significance of BMP2K in GPCR signaling
- Investigate other protein hits in our ACKR3-APEX2 proximity labeling data set

References and Acknowledgements

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