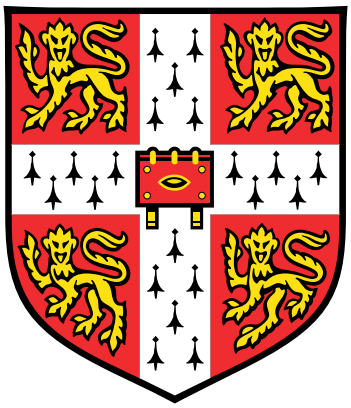
Extracellular receptor-ligand interaction screening using CRISPR activation



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To my family for their infinite patience and support.

DECLARATION

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others, except as specified in the text and Acknowledgements. This dissertation contains fewer than 60,000 words including appendices, bibliography, footnotes, and tables.

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ABSTRACT

Interactions between cell surface proteins mitigate multiple signalling and cell recognition events during development and disease. Previous methods to screen for extracellular protein interactions have relied on recapitulating these interactions using recombinant ectodomains of membrane-associated proteins. This approach is well suited for studying single pass transmembrane and glycophosphatidylinositol (GPI)-linked proteins, which tend to have single chain ectodomains. However, it has limited capacity for identifying interactions involving proteins with multiple transmembrane domains, which tend to have complex extracellular regions composed of more than one extracellular loops. Cell-based methods can help overcome this limitation by providing a native environment for the presentation of multipass membrane ectodomains. The success of cell microarrays transfected with cDNA libraries encoding plasma membrane proteins in identifying surface receptors demonstrates the feasibility of upregulating cell surface proteins for studying extracellular interactions, but requires significant cost to perform at scale.

Recently, advances in transcriptome editing using CRISPR/Cas9 have enabled highly efficient and specific gene activation on a genome-wide scale. By systematically upregulating plasma membrane proteins in human cell lines using CRISPR activation (CRISPRa), I developed a screening approach to identify novel receptor-ligand interactions in a convenient, single tube format. I show that this approach detects known interactions with a low false positive rate and apply it to identify ligands for the adhesion G-protein coupled receptors. I found that myelin-associated inhibitory proteins, the Nogo receptors, interact with Brain angiogenesis inhibitor 1 (ADGRB1) and show that the interaction is mediated by the first three thrombospondin repeat domains of ADGRB1. Together, this suggests that pooled CRISPRa screening presents a sensitive and convenient approach for genome-scale extracellular receptor-ligand identification, avoiding costly and technically challenging preparation of cDNA or recombinant protein libraries.

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