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Post-endocytic trafficking and sorting of FFA2 to very early endosomes

N. Caengprasath[1,2], A. Hanyaloglu[2]. [1]Medicine, Imperial College London, London, United Kingdom, [2]Surgery and Cancer, Imperial College London, London, United Kingdom.

Introduction: Free fatty acid receptor 2 (FFA2) is a G protein-coupled receptor (GPCR) activated by short-chain fatty acids acetate, propionate and butyrate produced by microbiota fermentation of dietary fibre, and with promising therapeutic potential for management of metabolic diseases (1). A fundamental mechanism controlling the signalling capacity of GPCRs is via receptor trafficking to diverse cellular compartments such as the early endosomes (EE), or the very early endosome (VEE) (2). A subpopulation of VEEs contains the adaptor protein APPL1, essential for driving receptor recycling from the VEE and regulating endosomal G protein signalling (3). Thus, endocytic trafficking not only regulates GPCR localisation, but also the spatial-temporal parameters of signalling. However, the membrane trafficking properties of FFA2 remain largely unknown.

Methods: HEK 293 cells expressing FLAG-tagged FFA2 were assessed for receptor internalization via confocal imaging and flow cytometry. Endosomal compartmentalization was assessed via anti-rabbit EEA1 or APPL1 antibodies. Real time receptor recycling was determined using TIRF microscopy and a pH-sensitive GFP super-ecliptic pHluorin (SEP) tagged at the receptor N-terminus. **Results:** FFA2 was localised not only at the cell surface but also intracellularly in untreated cells. In cells treated with either acetate, propionate or butyrate (1mM, 20 min) further internalization was observed. FFA2 internalized to endosomal structures of 300-500 nm in diameter, and were significantly smaller than endosomes containing the EE-localized beta2-adrenergic receptor (B2AR). Furthermore, there were significantly lower levels of FFA2 colocalisation with EEA1 (EE marker) compared to B2AR (36.26 ± 1.35% vs 67.47 ± 1.60%; respectively, $P < 0.0001$). FFA2 internalized into endosomes that were positive for APPL1 and to a similar level as a VEE-localised GPCR, the luteinizing hormone receptor (LHR) (31.13 ± 1.13% for FFA2 vs 34.67 ± 2.29% for LHR) (3). To determine the post-endocytic trafficking fate of FFA2, TIRF imaging of SEP-FFA2 revealed that recycling events were observed before stimulation, and increased following treatment with propionate (0.016157 events/min/μm^2 ± 0.02, unstimulated; 0.021587 events/min/μm^2 ± 0.01, propionate), suggesting that internalized FFA2 is sorted from VEEs to the recycling pathway.

Conclusion: Membrane trafficking and signalling of GPCRs are known to be a tightly integrated system, thus these findings that both constitutive and ligand-induced trafficking of FFA2 to the VEE may be vital in regulating FFA2 activity.

References:

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