**Characterisation of the pro-atherosclerotic orphan G protein-coupled receptor, GPR146**

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Introduction. GPR146 is an orphan G protein-coupled receptor that has a convincing pro-atherosclerotic role through upregulation of the cholesterol biosynthesis pathway. Inhibition of this receptor may be particularly useful with treatment-refractory familial hypercholesterolaemia. However, the molecular pharmacology of this receptor remains understudied. Proinsulin C-peptide and foetal bovine serum (FBS) are proposed activators of GPR146, although the pairing with C-peptide has not yet been reproduced by an independent research groupand the active component in FBS has not yet been identified.

Aims. The aim of this study was to validate previously proposed ligands for GPR146.

Methods. C-peptide and FBS were tested using the following assays: reporter gene assays to investigate Gαs, Gαi/o, Gαq/11, and Gα12/13 signalling; a NanoBiT assay for β-arrestin recruitment; and Western blot or a BRET1-based biosensor for ERK1/2 phosphorylation (pERK1/2). A panel of 58 human sera was screened at GPR146 using Western blot probed for pERK1/2; the threshold for “hit” selection was set at ±2xSD. Human sera identified as “hits” were then further characterised using G protein- and arrestin-deficient HEK293A cells.

Results. Neither C-peptide nor FBS activated GPR146 in any assays tested (n=5); assay validity was confirmed by multiple positive controls. An overall increase in pERK1/2 was observed in response to human serum in GPR146-expressing cells compared to cells not expressing GPR146 (P<0.0001, paired t-test). 47/58 human serum samples elevated pERK1/2, with 5 surpassing the upper hit threshold indicating activation of GPR146.

Discussion. In this study, previously proposed ligands for GPR146 were not reproduced, indicating that C-peptide is not, and FBS does not contain, the endogenous ligand for GPR146. Instead, human serum was identified as an activator of GPR146. Future studies with human serum may identify the endogenous ligand for GPR146.

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