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Expression and function of G_s-coupled GPCRs in human lung fibroblasts - activating the cAMP pathway for anti-proliferative effects

M. J. Roberts[1], T. C. Kent[2], S. J. Charlton[1], E. M. Rosethorne[1]. [1]Cell Signalling and Pharmacology, University of Nottingham, Nottingham, UNITED KINGDOM, [2]Novartis Institutes for Biomedical Research, Horsham, UNITED KINGDOM.

Introduction: Previous work targeting the β_2-adrenoceptor has shown that increasing levels of cAMP has anti-proliferative effects in human lung fibroblasts (HLFs)[(1)]. Fibroblasts express a variety of G_s-coupled receptors at different levels, therefore it would be beneficial to investigate the ability of these receptors to activate the cAMP pathway and inhibit HLF proliferation as a potential therapeutic approach in the treatment of idiopathic pulmonary fibrosis (IPF).

Method: Expression of G_s-coupled G protein-coupled receptors (GPCRs) was determined in HLFs using high density, 384-well GPCR TaqMan arrays as previously described[(2)]. cAMP levels were measured (HitHunter, DiscovRx) after 2 hr treatment with agonists or PDE inhibitor of confluent HLFs in 96-well ViewPlates. To measure proliferation, HLFs were seeded overnight at 4000 cells/well, serum starved for 24 hrs, then treated with a range of concentrations of agonists or PDE inhibitors in the presence of an EC_{80} concentration of serum. DNA synthesis was monitored after 24 hrs using BrdU incorporation (Delfia, PerkinElmer), and cell number monitored after 48 hrs by staining nuclei with Hoechst 33342 (1µM) and acquiring images using ImageXpress Micro (Plan Fluor 4X objective; excitation 400-418nm, emission 435-470nm). Data were normalized to serum response and expressed as mean±SEM, n≥3.

Results: The receptors expressed in HLFs from high expression to low expression were IP, EP_2, melanocortin-1, β_2-adrenoceptor, adenosine$_{2B}$, EP_4, dopamine 1 and adenosine$_{2A}$. Targeting the IP, EP_2 and EP_4 receptors with selective agonists resulted in cAMP accumulation and inhibition of proliferation. For example, EP_4 receptor agonist AGN205204 inhibited serum-driven proliferation with pEC_{50} of 7.25±1.10 and 7.20±0.61, and maximum inhibition of 48.3±10.4 % and 69.3±8.54 %, for the BrdU and cell count assays, respectively. Furthermore, MRE-269 activation of the IP receptor, which had ~10x higher expression than the EP_4 receptor, inhibited serum-driven proliferation with pEC_{50} of 6.79±0.43 and 5.60±0.37, and maximum inhibition of 79.2±4.93 % and 83.6±16.4 %, for the BrdU and cell count assays, respectively. While mRNA was detected for melanocortin-1, adenosine$_{2B}$, and adenosine$_{2A}$, they showed minimal/no cAMP and no anti-proliferative response.

Conclusion: Not all receptors expressed by HLFs were functionally active in generating a cAMP response and inhibiting HLF proliferation. However, targeting IP, EP_2, and EP_4 receptors on HLFs with selective agonists resulted in cAMP accumulation and inhibition of proliferation, and therefore these receptors may be potential targets in the treatment of IPF.

(1)-Broome R et al. (2012). *Proceedings of the British Pharmacological Society* at

http://www.pa2online.org/abstract/abstract.jsp?abid=30027&author=Broome&cat=-1&period=-1

(2)-Groot-Kormelink PJ et al. (2012) BMC *Immunol* **13**:57.