Investigating anti-proliferative effects of G_s-coupled receptors in primary human lung fibroblasts

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Pulmonary remodelling is implicated in idiopathic pulmonary fibrosis (IPF), where uncontrolled proliferation of lung fibroblasts (HLF) occurs. Elevation of the second messenger cAMP has been associated with anti-remodelling effects resulting in reduced proliferation (Lui *et al*, 2004) that may prove therapeutically beneficial in this disease. Here, we have investigated the remodelling phenotype of primary HLFs in response to proliferative agents LPA, PDGF and serum, and the ability of various agonists acting at G_s-coupled receptors to inhibit these responses.

Firstly, HLFs (Lonza) were seeded overnight at 3,000 cells/well in 96-well black clear bottom plates (Costar), starved for 24 hours in serum free medium, and incubated for 24 hours at 37 °C, 5 % CO_2 with pro-proliferative stimuli alone or concurrently with various agents that activate the G_s-cAMP pathway. Proliferation was assessed using BRDu incorporation (DELFIA, PerkinElmer). Secondly, HLFs were grown to confluency in 96-well ViewPlates and stimulated with test compounds in HBSS containing 5 mM HEPES, 0.1 % (w/v) BSA and 5µM rolipram for 2 hours at room temperature. AlphaScreen technology was used to measure agonist-stimulated cAMP accumulation. All data expressed as mean ± s.e. mean, n≥ 3.

LPA, PDGF and serum induced concentration-dependent increases in proliferation with EC_{50} equal to 1.42 ± 0.20 µM for LPA, 1.50 ± 0.27 % (v/v) for serum, and 1.28 ± 0.20 nM for PDGF. All G_s-activating ligands produced elevations in intracellular cAMP, with pEC_{50} ranging from 9.91 ± 0.06 M for salmeterol to 5.78 ± 0.10 M for forskolin. There was a good correlation between potency of cAMP accumulation and anti-proliferative responses against PDGF and serum (R^2= 0.97 and 0.88 respectively). However, when comparing the maximal response between the two assays, no correlation was observed. For some ligands the degree of cAMP accumulation closely mirrored the degree of inhibition of proliferation (MRE-269; 60.3 ± 3.77 % and 47.5 ± 3.11 %, respectively), for other ligands no correlation was apparent. The EP_4 agonist AGN205204 (Jiang *et al*, 2007) produced greater maximal inhibition of proliferation than cAMP accumulation (69.1 ± 9.73 and 49.6 ± 7.98 %, respectively), whereas the opposite was true for formoterol (37.0 ± 2.69 and 95.3 ± 12.1 %, respectively). In addition, differences were observed between anti-proliferative responses to different stimuli, for example the IP receptor agonist treprostinil produced 63.7 ± 6.39 % inhibition of PDGF-mediated proliferation, whereas such levels were not achievable against an equivalent stimulation with serum (35.6 ± 4.77 %).

In conclusion, we have demonstrated that agents that couple to the G_s-cAMP pathway have potential anti-remodelling properties, as evidenced by their inhibition of proliferation. However, our data indicate that there may be a more complex relationship between receptor activation and anti-proliferative effects than simply cAMP production. Future work will concentrate on investigating the role of cAMP compartmentalization (Zaccolo, 2011) and alternative receptor-mediated pathways in governing the inhibition of proliferation.

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