Osteoblast and adipocyte differentiation are both attenuated by an antagonist of the Free Fatty Acid 4 receptor

Dysregulation of mesenchymal stem cell differentiation into either osteoblasts or adipocytes is a key characteristic of obesity and type 2 diabetes (T2D), contributing to the development of osteoporosis. An emerging role for the long chain free fatty acid-activated receptor, Free Fatty Acid 4 (FFA4) receptor , in the formation of osteoblasts and adipocytes has recently been described, although the exact mechanisms remain to be fully determined (1,2). Recently, Sparks et al reported the first chemically synthesised antagonist of FFA4 (compound 39) (3). Hence, this study aimed to examine the effect of compound 39 on osteoblast and adipocyte differentiation in order to further elucidate the role of FFA4 in the processes of bone formation and adipogenesis.

Pre-osteoblast MC3T3-E1 and pre-adipocyte C3H10T1/2 cells were differentiated in 10cm dishes using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with either AGD (50µg/ml Ascorbic acid, 10mM β-Glycerophosphate, and 10nM Dexamethasone) for 7 days or IID (100nM Insulin, 10nM Dexamethasome, and 500µM IBMX) for 5 days respectively in the presence/absence of vehicle (0.1% DMSO) or 10µM compound 39 (FFA4 antagonist). Changes in differentiation markers, PPARγ (adipocyte) and Runx2 (osteoblasts) were then measured using real time quantitative PCR and expressed relative to cyclophilin expression using the $2^{-\Delta\Delta Ct}$ method. Data is presented as the mean ± SE and statistical analysis carried out using unpaired 2-tailed Student's t-tests. Parallel studies using either Alizarin Red S (osteoblasts) or oil red O (adipocytes) staining were used to confirm changes in differentiation.

AGD treatment resulted in a two-fold (2.13±0.3, $p<0.05$; n=6) increase in Runx2 expression in MC3T3-E1 cells compared to non-treated cells. However, in the presence of compound 39, Runx2 induction by AGD was significantly attenuated (1.23±0.12 fold over non-differentiated control; significantly less than AGD treatment alone; $p<0.05$; n=6). Preliminary experiments also indicated a trend toward attenuated expression of the adipogenesis marker, PPARγ in differentiated C3H10T1/2 cells by compound 39. IID induced a 6.94±0.87 fold ($p<0.05$; n=3) increase in PPARγ expression over non-differentiated control levels, compared to 5±0.58 ($p<0.05$; n=3) in the presence of compound 39. Further experiments aim to address whether this effect of compound 39 on PPARγ induction is statistically significant. Importantly, Alizarin Red S staining in AGD-treated MC3T3-E1 cells and oil red O staining in IID-treated C3H10T1/2 cells were both strongly attenuated in the presence of compound 39, in agreement with the reported changes in Runx2 and PPARγ expression.

Hence, antagonism of FFA4 inhibited both adipogenesis and osteogenesis, as shown by attenuation of Runx2 and PPARγ induction and decreased detection of osteoblast and adipocyte formation by Alzirin red S and oil red O staining respectively. These data therefore support an important permissive role for FFA4 in the processes of both adipogenesis and bone formation and implicate a role for FFA4 in the treatment of obesity, T2D and osteoporosis.

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Ichimura et al (2012) *Nature* **483(7389)**:350–4.
Sparks et al (2014) *Bioorg Med Chem Lett* **24(14)**:3100–3.