Possible role of fatty acid GPCRs in endometrial cancer cell growth *in vitro*

K.W. Lee, F.J. Steyn and C. Chen, School of Biomedical Sciences (SBMS), University of Queensland, St. Lucia, QLD, 4072, Australia.

Endometrial cancer is one of the most common gynaecological tumours in developed countries, with a lifetime risk of 2-3%. Studies have revealed an association between obesity and the incidence of endometrial cancer. A high level of very low density lipoprotein (VLDL) is a common phenomenon in obesity along with high levels of free fatty acids (FFAs). GPR40 and GPR120, both G-protein coupled receptors, are functional receptors for medium- and long-chain FFAs, and implicated in the growth and development of different cell types. Gene analysis revealed that the human endometrial cancer cell lines used in this study, Ishikawa and HEC1A, predominantly expresses GPR120 and GPR40 respectively. In this study, we investigated the effects of long-chain fatty acids on Ishikawa and HEC-1A growth, as well as the contribution of GPR40 and GPR120 activation in this process. Using a tetrazolium-based proliferation assay we demonstrate that oleic acid (C18:1) significantly increased proliferation in both Ishikawa and HEC-1A cells within 72 hours in a dose dependent manner. A similar increase in Ishikawa cell proliferation occurs when cells are treated with the synthetic non-FFA GPR40 and GPR120 agonist, GW9508. This does not occur in the HEC-1A cells. To isolate the effects of the two receptors, Ishikawa cells were treated with a GPR40 antagonist, GW1100, in conjunction with GW9508. Results indicated that GW1100 had no effect on the stimulation of cell proliferation, suggesting all pro-proliferative effects of GW9508 are mediated through GPR120. These results suggest that GPR120 plays a more important role in the growth of endometrial cancer cell lines compared to GPR40. Further analysis of downstream pathways following GPR120 activation in Ishikawa cells using GW9508 along with antagonists for PI3K, PKC, PLC, and PKA were conducted. Results confirm a significant reduction in the pro-proliferative effects of GW9508 in cells treated with the PI3K, PKC, and PLC antagonists. Overall, results suggest that the pro-proliferative effects of OA may be mediated through pathways involving down-stream activation of PI3K, PKC, and PLC, following GPR120 activation.