

Annual Report for CCFC (2007).

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“Extracellular calcium-sensing receptor stimulates IL-11 secretion which repairs damaged intestinal barrier functions”.

The cells which line the intestine, absorptive epithelial cells, form attachments that provide a barrier from noxious substances in the gut lumen. These epithelial cells sit above another cell type, myofibroblasts, which can secrete growth factors and other molecules to increase the ability of the epithelial cells to work as a barrier. We have discovered that stimulating a receptor, the extracellular calcium-sensing receptor (CaSR) on the subepithelial myofibroblasts induces the synthesis and secretion of IL-11, a molecule which enhances the repair and recovery of damaged intestine. When we stimulate the CaSR on the intestinal epithelia we cause the production of the receptor for IL-11. This year we made the novel finding that CaSR stimulation of these myofibroblasts that produce IL-11 causes the synthesis and secretion of a different family of molecules called secreted Wnt antagonists. We have further discovered that one of these antagonists, Dkk-1, which turns off the genes required for cell proliferation in the gut, will also stimulate repair and recovery of a damaged intestine. We went on to show that CaSR stimulation of the intestine increased the production of the receptor for Dkk-1, called LRP6. This new axis of IL-11 and Dkk-1 produced from the sub-epithelia by CaSR activation and a choreographed production by the CaSR on the intestinal cells of the IL-11 receptor and LRP6 represents a new potentially therapeutic axis to repair damaged intestine. Our novel findings that we can induce the secretion of inhibitors of Wnt signaling such as Dkk-1 which will improve the barrier capacity of the intestine has revealed a new mechanism to regulate the intestinal stem cell niche micro-environment and promote intestinal differentiation. In parallel with these experiments we also discovered that CaSR activation of the intestinal epithelia alone stimulated the production of an unfolded protein called CHOP, which we discovered when it was complexed as a dimer or oligomer, could directly regulate absorptive epithelial differentiation.

Presentations Arising from this Project.

International:

1. MacLeod RJ, Pacheco II. Extracellular calcium-sensing receptor mediates Wnt5a secretion from colonic myofibroblasts to activate orphan tyrosine kinase receptor Ror2 to increase CDX2 and sucrase-isomaltase of intestinal epithelia. *Gastroenterology* 132: A101, 2007. **(Oral)**

2. Pacheco II, Hayes M, MacLeod RJ. Extracellular calcium-sensing receptor stimulates Wnt5a secretion which inhibits beta-catenin signaling of human intestinal epithelial cells. *Gastroenterology* 132: A302, 2007. (**Poster of Distinction**)
3. Pacheco II, Spencer C, MacLeod RJ. Extracellular calcium-sensing receptor stimulates IL-11 secretion from colonic myofibroblasts to decrease barrier permeability. *Gastroenterology* 132: A541, 2007. (**Poster of Distinction**)
4. MacLeod RJ, Peiris D, Pacheco II. Indian hedgehog (Ihh)- A new and potent agonist of the extracellular calcium-sensing receptor: evidence of a feed-forward cycle. *Gastroenterology* 132: A630, 2007. (Poster)
5. MacLeod RJ and Pacheco II. Secretion of Wnt5a and Dkk-1 stimulated by the extracellular calcium-sensing receptor (CaSR) is reciprocated by CaSR-mediated increases in Ror2, Kremen-1 and LRP6 on colonic epithelia. "Traditional Wnt Meeting" La Jolla, CA. June 2007. Sponsored by NIH, NCIC and Genentech. (Poster by Invitation)
6. Pacheco I, Foss A, Spencer C, MacLeod RJ. Extracellular calcium-sensing receptor stimulates Dkk-1 secretion from colonic subepithelial myofibroblasts to improve intestinal barrier permeability. "GI response to Injury" (Oral by Invitation) Sponsored by American Physiological Society and American Gastroenterological Association, Montebello PQ, Oct 2007.

Invited Lectures arising from this project (2007-06/2008).

1. "Novel Wnt paracrine signaling mediated by the intestinal extracellular calcium-sensing receptor (CaSR)"
Dept of Biochemistry, Queen's University, Kingston ON, June 4, 2008.
2. "Novel Wnt and Indian hedgehog (Ihh) paracrine signaling mediated by the intestinal and sub-epithelial extracellular calcium-sensing receptor (CaSR)"
The University of Chicago, Committee on Cell Physiology, March 7, 2008.
3. "New paracrine relationships in Wnt signaling mediated by the intestinal extracellular calcium-sensing receptor"
GIDRU International Symposium, Kingston, ON, Sept 26, 2007.
4. "Novel Wnt paracrine signaling mediated by the intestinal extracellular calcium-sensing receptor (CaSR)"
Mucosal Inflammation Program, Dept of Medicine, University of Colorado, Denver, USA, June 25, 2007.
5. "New Paracrine relationships mediated by the intestinal extracellular calcium-sensing receptor (CaSR) in health and disease."
Dept of Biology, Carleton University, Ottawa, Canada; March 30, 2007.

Peer-reviewed Papers arising from this project (2007- 06/2008)

1. Peiris D, Pacheco I, Spencer C, MacLeod RJ. The Extracellular Calcium-sensing Receptor (CaSR) reciprocally regulates secretion of BMP-2 and the BMP antagonist Noggin in colonic myofibroblasts. *Am. J. Physiol.* 292: G753-G766, 2007.
2. MacLeod RJ, Hayes M, Pacheco I. Wnt5a secretion stimulated by the extracellular calcium-sensing receptor (CaSR) inhibits defective Wnt signaling in colon cancer cells. *Am. J. Physiol.* 293: G403-G411, 2007.
3. Justinich CJ, Mak N, Pacheco I, Wells R, Blennerhassett M, MacLeod RJ. The extracellular calcium-sensing receptor (CaSR) on human esophagus and functional expression of the CaSR on an esophageal epithelial cell line (HET-1A). *Am. J. Physiol.* 294: G120-G129, 2008; Epub 25/10, 2007.
4. Singh V, Pacheco I, Uversky VN, Smith SP, MacLeod RJ, Jia Z. Intrinsically disordered human C/EBP homologous protein regulates biological activity of colon cancer cells under calcium stress. *Journal of Molecular Biology.* 380:313-326, 2008.
5. Pacheco I, MacLeod RJ. Extracellular calcium-sensing receptor (CaSR) mediates Wnt5a secretion from colonic myofibroblasts to activate the orphan tyrosine kinase receptor Ror2 to stimulate Cdx2 and sucrase-isomaltase of intestinal epithelial cells. *Am. J. Physiol.* (responding to first review).