**B Meehan, JM Rhodes and BJ Campbell**

**TITLE**

**Mucosa-associated *E.coli* isolates from inflammatory bowel disease and colorectal cancer patients activate Wnt/β-catenin signalling *in vitro* and *in vivo*.**

**INTRODUCTION**

###### Increased numbers of adherent, invasive E.coli (AIEC) have been reported within intestinal epithelium of patients with Crohn’s disease (CD) and colorectal cancer (CRC)1. Genotoxicity and angiogenic activity of AIEC have been described by our group and others2-4. We hypothesised that a key contribution of cancer-promoting activity of AIEC may also be through their ability to activate Wnt/β-catenin signalling, and reported that Wnt target-genes were up-regulated in colonocytes at mRNA and protein level, including cyclooxygenase-2 (COX-2)5. Here, we further investigated the ability of AIEC to activate Wnt transcription and nuclear translocation of β-catenin. We sought also to confirm our findings in vivo using an AIEC mono-association mouse model.

**METHODS**

Activation of Wnt transcription activity in response to *E.coli* isolates1 (MOI: 10; for 4h) was assessed using a TCF/LEF HeLa cell luciferase reporter assay. Infected cells were also pre-treated with and without COX inhibitors. Nuclear translocation of β-catenin was assessed by immunofluorescence in CRC cell-lines SW480 and DLD1. Following 6-week mono-association of *Il10*-/- 129SvEv mice with CRC AIEC isolate HM44, intestinal tissue was fixed, Cox-2 and β-catenin expression assessed by immunohistochemistry and compared to germ-free controls.

**RESULTS**

Mucosal-associated *E.coli* isolated from CRC (HM44, HM358, HM545), Crohn’s disease (HM95, HM605), and ulcerative colitis (HM250, HM374) significantly increased Wnt TCF/LEF signalling, ranging from 1.56±0.11 to 2.60±0.06 fold above uninfected control HeLa cells (1.0±0.03); all p<0.001, Kruskal-Wallis. Infection of SW480 and DLD1 showed significant increases in *β*-catenin nuclear translocation as per prostaglandin E2 (1-10μM). Responses were blocked using COX inhibitors (diclofenac>indomethecin>aspirin; 1-100μM). Increased intestinal Cox-2 expression and Wnt signalling was observed *in vivo* in *Il10*-/- mice infected with AIEC strain HM44 (n=15) compared to germ-free mice (n=5); with Cox-2 elevated 2.04±0.10 fold, and nuclear localisation of β-catenin elevated 1.98±0.13 fold; both p<0.001; Mann-Whitney U.

**CONCLUSIONS**

###### IBD and CRC mucosa-associated E.coli activate intestinal Wnt-signalling in vitro and in vivo. The specific bacterial factors triggering early cancer-promoting signals such as elevated COX-2 and Wnt pathway activation are currently being investigated using a validated CRC E.coli fosmid-library screening approach3, with 12 confirmed positive clones currently undergoing sequence analysis.

**REFERENCES**

1. Martin HM *et al.* Gastroenterology 2004;127:80–93
2. Arthur JC *et al.* Science 2012;338:120-23
3. Prorok-Hamon M *et al.* Gut 2014;63:761-70
4. Buc E *et al.* PLoS One 2013;8(2):e56964
5. Meehan B *et al.* Gut 2015; 64:Suppl 1; A362-A363 (abstract)