P019 has been withdrawn.

P020

Regulation of intestinal epithelial homeostasis by the IBD risk gene CCNY

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Background: CCNY, encoding Cyclin Y, has previously been identified as a putative risk gene in Crohn's disease and ulcerative colitis; however, the function of CCNY in the gut is unknown. We have shown that Cyclin Y is a critical activator of the Wnt/β-catenin signalling pathway, which controls stemness and proliferation in intestinal epithelia. We thus investigated whether CCNY regulates epithelial homeostasis and wound repair in the gut.
Methods: To address the role of CCNY in intestinal epithelia, we used a RNA interference based loss-of-function approach in model cell lines. In addition, we generated transgenic mice with deletion of Ccny specifically in intestinal epithelial cells. These animals were subjected to the dextran sulphate sodium model of intestinal injury and repair, which mimics human inflammatory bowel diseases. We studied Wnt pathway activity in these models using reporter assays and pathway-specific antibodies, as well as functional in vitro assays. In addition, we determined colitis progression and epithelial homeostasis in mice using an established disease activity index and histopathological analyses.
Results: In contrast to non-intestinal epithelia, loss-of-function of CCNY did not reduce Wnt signalling activity in model intestinal cell lines. Accordingly, CCNY depletion did not impair epithelial proliferation or stemness in vitro. Moreover, markers of Wnt activity and cell proliferation were unchanged in Ccny mutant mice, and we observed no changes in disease activity during acute intestinal inflammation.
Conclusions: Our results thus far suggest that IBD risk gene CCNY is dispensable for intestinal epithelial homeostasis. The apparent uncoupling of Cyclin Y from Wnt signalling in the gut is the subject of ongoing investigation in our lab. In addition, we continue to investigate the possible contribution of CCNY to epithelial regeneration following colitis.

P021

An electrochemiluminescence (ECL) immunoassay for the detection of antidrug antibodies against anti-mucosal addressin cell adhesion molecule (MAdCAM) monoclonal antibody SHP647

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Background: Immunogenicity assessment is a regulatory requirement for biotherapeutic product (BTP) approval since antibodies that develop in response to a BTP may directly impact product safety and efficacy. A well-designed anti-drug antibody (ADA) immunoassay is critical for monitoring the immunogenicity profile of a BTP during its development. SHP647 is a fully human $IgG_2\kappa$ monoclonal antibody that targets human MAdCAM to reduce lymphocyte homing to the gut and gastrointestinal inflammation, and is in development for the treatment of Crohn's disease (CD) and ulcerative colitis (UC). A sensitive and specific ECL immunoassay for the detection of ADAs against SHP647 was developed and validated to support its use in clinical trials of SHP647.
Methods: SHP647 was either biotinylated as the capture agent, or labelled with ruthenium as the detection reagent. In the assay, human serum samples, positive controls and negative controls were diluted with assay buffer prior to co-incubation with both the capture and detection reagents overnight to form an antibody-drug complex. After incubation, each mixture was added to Streptavidin coated MSD plate to allow complexes to bind to the plate. In the presence of tripropylamine-containing read buffer, ruthenium produces a chemiluminescent signal that was triggered when voltage was applied. The resulting chemiluminescence was measured in relative units on a SECTOR Imager 6000™ instrument. Data are presented as endpoint log titers (log2) (the reciprocal of the serial dilution at which the sample response would be equal to the cut point of the assay).
Results: The assay precision (inter-run ≤4.0% and intra-run ≤3.4%) in normal human serum was demonstrated. Relative assay sensitivity was 3.25 ng/ml. The matrix specificity (recovery) ranged from 96.9% and 109.4% in 10 individual lots of normal, CD, or UC human serum. The assay achieved the detection of 300 ng/ml of ADA in the presence of 300 µg/ml of the drug. Interference was observed in the presence of 100 ng/ml soluble MAdCAM. The assay screening cut point factors and confirmatory assay cut points in normal, CD and UC populations were established.
Conclusions: The ECL immunoassay with sensitivity and high tolerance to both soluble MAdCAM and SHP647 for the detection of anti-SHP647 antibodies was successfully developed and validated in compliance with the regulatory requirements. The assay was used to support the Phase 2 OPERA II trial (NCT01298492) where the highest level of soluble MAdCAM in samples at Week 12 did not exceed 54 ng/ml and no samples had SHP647 level higher than 74.5 µg/ml. Therefore, the assay is considered suitable to support the OPERA II trial. However, the assay might not be able detect low levels of ADA when serum drug levels are high.

P022

Galectin-3, galectin-9, and galectin-3 binding protein in patients with inflammatory bowel diseases

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Background: Galectins are a family of lectins that bind β-galactosides. They effect variety of cellular and intracellular processes including inflammation, fibrosis, organogenesis, immunological response, and malignancy. Thus, galectins may be a therapeutic target for inflammatory diseases. Their role in inflammatory bowel diseases (IBD) has not been fully evaluated, yet. The study aimed to assess galectin-3,

galectin-9, and galectin-3-binding protein (M2BP) levels in patients with ulcerative colitis (UC) and Crohn's disease (CD), and to correlate it with inflammatory markers and the disease activity.

Methods: Consecutive patients, including 48 with UC, 77 with CD, and 30 healthy controls were included. The white blood cell count, haematocrit, platelet count, fibrinogen, C-reactive protein, galectin-3, galectin-9, M2BP levels in serum were measured and correlated with the disease activity.

Results: There were no significant differences in the median galectin-3 and galectin-9 levels between the UC group, CD group and the control group (Table 1). M2BP was significantly higher in the CD group vs. controls. The median M2BP level in the patients with active UC was significantly higher 72.74 (60.86–101.72) ng/ml than in the group with inactive disease 61.22 (39.31–72.60) ng/ml, $p = 0.006$. In the active CD group median M2BP level was higher than in the control group (79.854 (52.05–110.12) ng/ml, $p = 0.04$) In the UC group M2BP level correlated with CRP ($r = 0.304$, $p = 0.02$) and disease activity ($r = 0.298$, $p = 0.03$); galectin-3 correlated with galectin-9 ($r = 0.54$, $p < 0.001$). In the CD group, galectin-9 correlated with galectin-3 ($r = 0.549$, $p < 0.001$), and M2BP ($r = 0.4$, $p < 0.001$).

Conclusions: This is the first study to show that M2BP is increased in active IBD and in the UC its level is associated both with inflammatory markers and disease activity as well. In contrast, galectins 3 and 9 levels do not differ from healthy controls.

P023

A resting state fMRI study in patients with active Crohn's disease

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Background: Resting state functional magnetic resonance imaging (rsfMRI) measures spontaneous fluctuation in blood oxygen-level dependent (BOLD) signals in the brain at rest, generating neuroanatomically distinct functionally linked Resting State Networks (RSNs). Present RSN literature in CD is sparse, solely reporting in inactive disease and only focussed on specific RSNs. Here we use independent component analysis (ICA) to study changes across multiple RSNs in active CD.

Methods: 29 active CD patients and 27 age-, BMI- and gender-matched healthy controls (HC) were recruited. Active disease was defined as CRP > 5 mg/dl, or faecal calprotectin (FCP) >250 µg/g or through ileocolonoscopy or MRE. A hospital anxiety and depression (HAD) score was used as a patient-reported outcome. RsfMRI datasets were acquired on a 3T Philips Achieva scanner, with data corrected for physiological noise and motion. ICA analysis was carried out using MELODIC (FSL software). A multi-session temporal concatenation was used to generate 30 independent component (IC) maps of RSNs. A dual regression analysis with variance normalisation was performed to identify differences in RSN between HCs and CD patients. Anatomical T1-weighted images were collected to determine structural (grey matter volume (GMV)/cortical thickness) differences in CD (CAT, SPM software).

Results: CD participants had an age of (33 ± 14) years, Harvey–Bradshaw Index was (4 ± 1), CRP (9 ± 7) mg/dl and FCP (617 ± 554) µg/g. CD patients had significantly higher depression scores (CD: 3.0 ± 0.6, HC: 1.5 ± 0.5, $p < 0.05$). RSNs comprising the visual network, default mode network (DMN), salience network, dorsal attention network (DAN), frontal–parietal network (FPN), temporal and cerebellum networks were identified. Enhancement of activity and increased connectivity in DMN (posterior cingulate cortex (PCC)), the cerebellar network and thalamus, visual attention network, and FPN (postcentral cortex) was observed in CD. Atrophy (reduced GMV and cortical thickness (CT) in post-central gyrus and additional cortical thinning in right rostral middle-frontal cortex was seen in CD.

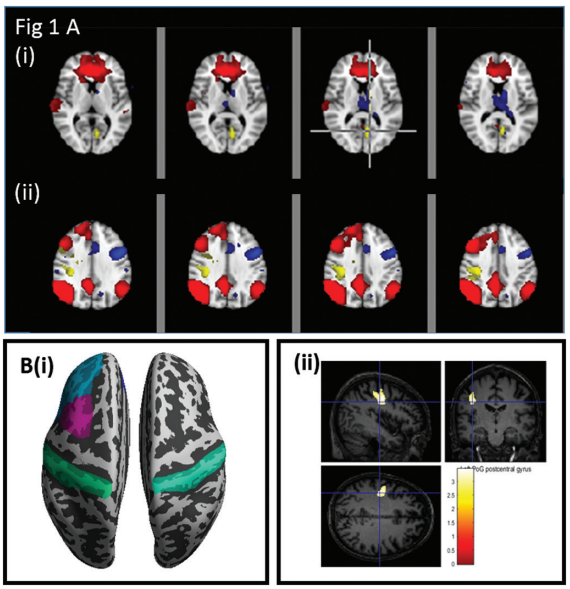

Figure 1. (A) RSNs (red = positive and blue = negative networks) areas of increased connectivity in CD>HC yellow in (i) DMN (posterior cingulate) and (ii) FPN (postcentral cortex). (B) Areas of atrophy in CD for (i) CT and (ii) GMV.

Conclusions: These data show abnormal increased connectivity in RSNs in CD patients in the DMN (PCC) and in FPN network (postcentral cortex which also showed associated atrophy). These changes may reflect neuroplasticity in response to chronic systemic inflammation and may relate to altered affective and cognitive self-referential processing.

P024

Plasma acetic acid, propanoic acid, and isobutyric acid are associated with treatment response in pouchitis patients treated with antibiotics

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