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1. SELECTIVE INFLUENCE OF TCF-4 MEDIATED WNT SIGNALING ON INTESTINAL INNATE AND ADAPTIVE IMMUNITY OF ILEAL CROHN’S DISEASE

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Aim: Ileal Crohn’s disease (CD) is characterized by diminished antibacterial activity and a specific decrease of small intestinal Paneth cell u–defensins HD5 and 6. We previously reported a causal link between this decrease and the Wnt pathway transcription factor Tcf-4. Wnt signaling has an important function in intestinal epithelium renewal, regulating stem cell maintenance and their transition to Paneth-cells. The involvement of disturbed Wnt signaling, resulting in alleviated innate immunity, reveals a new mechanism for ileal CD pathogenesis. To further investigate the pathways influence in the disease, we aimed to assess expression of Tcf4 target and Wnt pathway genes other than HD5 and HD6.

Methods: RNA was isolated from ileal biopsies of healthy individuals (n=14) and CD patients (ileal CD n=28, colonic CD n=11). The mRNA levels of genes, encoding Wnt/Tcf-4 pathway factors as well as Tcf-4 target genes (β-catenin, CEBP, Hic-5, ICAT, NLK, Sox-9, p53, p100 Axin-1 and -2, CDX-1, c-jun, Claudin-1, c-myc, Cycl-1, CD44, EphB-8, Ephrin-B1 and -B2, MMP-7, PPP1R-5, APC, CUL-1, DKK-1 and -2, DVL-2 and -3, Fzd5, Fzd6, Gastrin, LEF-1, LRP-5, PGLYRP1, SFRP-3, Tcf-1) were quantified using real-time PCR with external standards. T arget genes exhibiting mRNA expression correlating with Tcf-4 were further investigated. In silico promoter scans for potential Tcf-4 binding sites (WWCAWWG) and gel shift assays for in vitro confirmation were performed. Total Protein from ileal biopsies of controls and ileal CD patients was extracted and analyzed via Western Blot.

Results: In addition to the known decrease of Tcf-4, we found reduced expression and protein of the Wnt pathway transcription factors Tcf-1 in ileal CD (p=0.0022), but not colonic CD. The mRNA levels of Tcf-1 correlated with Tcf4 (rs=-0.5206; p=0.0242), but not colonic CD. The mRNA levels of Wnt/Tcf-4 pathway factors as well as Tcf-4 target genes (β-catenin, CEBP, Hic-5, ICAT, NLK, Sox-9, p53, p100 Axin-1 and -2, CDX-1, c-jun, Claudin-1, c-myc, Cycl-1, CD44, EphB-8, Ephrin-B1 and -B2, MMP-7, PPP1R-5, APC, CUL-1, DKK-1 and -2, DVL-2 and -3, Fzd5, Fzd6, Gastrin, LEF-1, LRP-5, PGLYRP1, SFRP-3, Tcf-1) were quantified using real-time PCR with external standards. Target genes exhibiting mRNA expression correlating with Tcf-4 were further investigated. In silico promoter scans for potential Tcf-4 binding sites (WWCAWWG) and gel shift assays for in vitro confirmation were performed. Total Protein from ileal biopsies of controls and ileal CD patients was extracted and analyzed via Western Blot.

Conclusions: A selective influence of the Tcf-4 mediated Wnt signaling has an important function in intestinal epithelium renewal, regulating stem cell maintenance and their transition to Paneth-cells. The involvement of disturbed Wnt signaling, resulting in alleviated innate immunity, reveals a new mechanism for ileal CD pathogenesis. To further investigate the pathways influence in the disease, we aimed to assess expression of Tcf4 target and Wnt pathway genes other than HD5 and HD6. The mRNA levels of Tcf-1 correlated with expression and protein of the Wnt pathway transcription factors Tcf-1 in ileal CD (p=0.0022), but not colonic CD. The mRNA levels of Wnt/Tcf-4 pathway factors as well as Tcf-4 target genes (β-catenin, CEBP, Hic-5, ICAT, NLK, Sox-9, p53, p100 Axin-1 and -2, CDX-1, c-jun, Claudin-1, c-myc, Cycl-1, CD44, EphB-8, Ephrin-B1 and -B2, MMP-7, PPP1R-5, APC, CUL-1, DKK-1 and -2, DVL-2 and -3, Fzd5, Fzd6, Gastrin, LEF-1, LRP-5, PGLYRP1, SFRP-3, Tcf-1) were quantified using real-time PCR with external standards. Target genes exhibiting mRNA expression correlating with Tcf-4 were further investigated. In silico promoter scans for potential Tcf-4 binding sites (WWCAWWG) and gel shift assays for in vitro confirmation were performed. Total Protein from ileal biopsies of controls and ileal CD patients was extracted and analyzed via Western Blot.

2. EVIDENCE FOR DYSBIOSIS IN POUCHITIS USING 16S RNA SEQUENCING

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Introduction: Restoration proctocolectomy (RPC) is the operation of choice for ulcerative colitis (UC) and some with familial adenomatous polyposis (FAP). Pouchitis is the most common complication following RPC. A bacterial dysbiosis has been proposed as the cause of this inflammatory process. Bacterial phyla in the ileal pouch mucosa were identified using the highly sensitive 16s rRNA PCR sequencing technique.

Methods: Ileal pouch biopsies were taken from pouch patients as follows: ulcerative colitis (UC) non-inflamed 4, UC pouchitis 7, familial adenomatous polyposis (FAP) non-inflamed 6, FAP pouchitis 2. PCR was performed using universal 16s rRNA primers on the DNA extracted from the biopsies. Clone libraries were sequenced using high-throughput DNA sequencing. The Ribosomal database project(1) was used to identify bacterial phyla from the sequences.

Chi-squared was used to analyse the differences between the groups.

Results: See Table 1.

Table 1

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>UC Control</th>
<th>UC Pouchitis</th>
<th>FAP Control</th>
<th>FAP Pouchitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacteria</td>
<td>6%</td>
<td>2%</td>
<td>5%</td>
<td>2%</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>21%</td>
<td>18%</td>
<td>29%</td>
<td>43%</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>29%</td>
<td>72%</td>
<td>3%</td>
<td>27%</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>42%</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Total other bacteria</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Bacteria from the phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria phyla were identified in all samples. A small number of sequences were found in some patients from Delnecoccus-thermus, Verrucomicrob, Fusobacteria and Cyanobacteria phyla. UC pouchitis patients had a significantly higher proportion of Proteobacteria and significantly lower proportion of Bacteroidetes compared with the other groups. UC pouch controls had a significantly higher proportion of Proteobacteria and significantly lower proportion of Firmicutes compared with FAP pouch controls (p<0.0005).

Conclusions: This is the first study giving data on bacterial taxonomy to suggest that a bacterial dysbiosis may occur in pouchitis. Furthermore this study suggests that there is a significant difference in the composition of microflora within FAP and UC pouches. Further work is in progress to identify individual bacterial species in a larger patient group.

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