Expression Profiling of Wnt Pathway Genes in Colon Biopsies of Patients with Ulcerative Colitis

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Background

There is an increasing demand of agents that can promote mucosal healing in Inflammatory Bowel Disease (IBD). Wnt/β-catenin signaling plays a critical role in epithelial regeneration and repair, and stimulating regeneration in the damaged epithelium by modulating Wnt signaling has been suggested as a potential treatment option for IBD. To guide development of Wnt modulating therapeutic molecules for IBD, an understanding of how Wnt signaling may be altered in IBD tissues is required. While earlier work showed altered Wnt pathway gene expression in UC tissues, these studies failed to consider disease conditions (moderate vs severe) and patient treatment history on expression of the Wnt family genes. These previous studies utilized RT-qPCR or microarray and did not reveal how Wnt pathway gene expression might be affected specifically in the epithelium and in the adjacent stromal stem cell niche. Here we report our work investigating expression patterns of Wnt pathway genes in UC biopsies from 12 patients with moderate and severe disease. Patients had either received no anti-TNF treatment or had gone through anti-TNF treatment and partially responded to the treatment.

Methods

Expression of a set of Wnt pathway genes was assessed in UC colon and rectum biopsies by RNAscope in situ hybridization and compared to expression patterns in normal control colon. The genes included the Wnt target genes *AXIN2*, *LGR5* and *RNF43*, Wnt ligands and the *Fzd5* and *Lrp6* receptors enriched in the intestinal epithelium as well as key Wnt signal modulators *RSPO1-4*.

Results

Table 1. Number of patients and biopsies involved in the study.

Condition	Number of patients and biopsies assayed
Moderate, anti-TNF naive	6 patients, 7 biopsies
Severe, anti-TNF naive	4 patients, 7 biopsies
Moderate, anti-TNF partial or non-responder	1 patient, 2 biopsies
Severe, anti-TNF partial or non-responder	2 patients, 5 biopsies

Total of 33 biopsies from 14 UC patients subjected to tissue quality check. 21 colon biopsies from 12 UC patients with varying disease conditions shown in the table were assayed for expression of Wnt pathway genes.

Table 2. Summary of expression of key Wnt pathways genes in UC colon biopsies.

Gene class	Genes	Expression in UC
Wnt target genes	*Axin2, Lgr5, RNF43*	mild reduction
RSPO	*RSPO1, RSPO2, RSPO3, RSPO4*	reduced
Fzd	*Fzd5, Fzd8*	mild reduction
Lrp	*Lrp5, Lrp6*	mild reduction
Wnt	*Wnt2b, Wnt4, Wnt5a*	Induced with altered patterns

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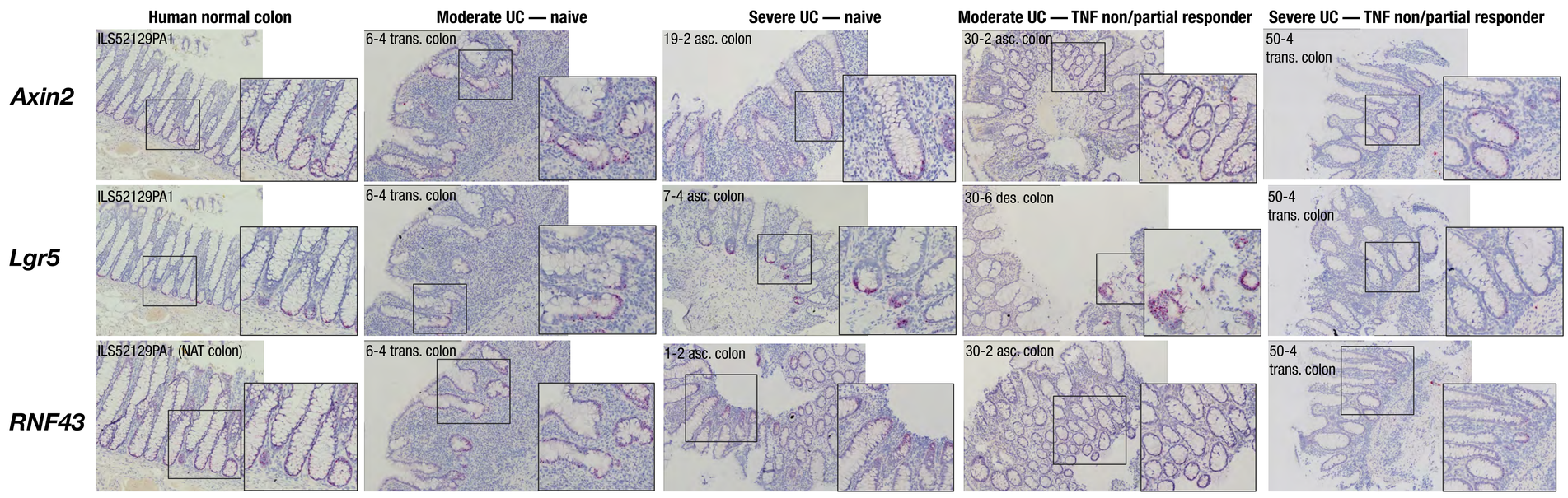
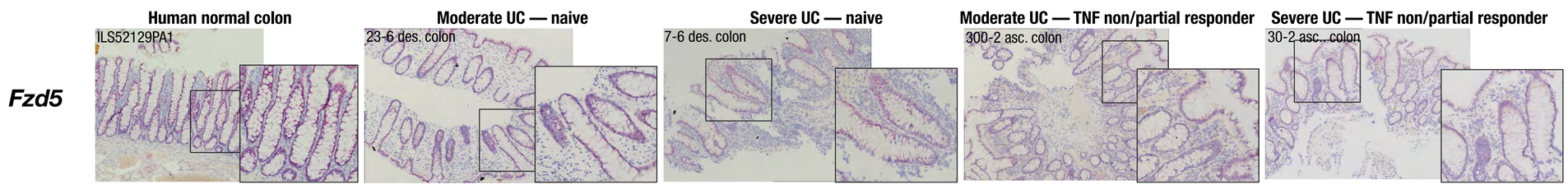
Results

Figure 1. Expression of Wnt target genes *Axin2*, *Lgr5* and *RNF43* in UC colon biopsies.



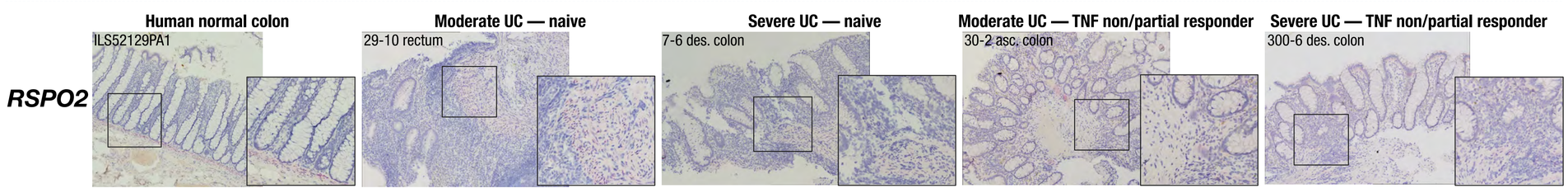
Expression of *Axin2* was unaffected or mildly reduced in moderate or severe UC colon and was mildly reduced in UC colon treated with anti-TNF. Expression of *Lgr5* was mildly reduced in UC colon that was naïve to anti-TNF and was reduced more obviously in severe UC colon treated with anti-TNF. Expression of *RNF43* was mildly reduced in UC colon and more so in UC colon treated with anti-TNF. Overall expression levels of Wnt pathway genes did not differ between moderate and severe UC colon and Wnt target gene expression was more affected in the anti-TNF treated colons, which may reflect more refractory disease.

Figure 2. Expression of Wnt receptors in UC colon biopsies.



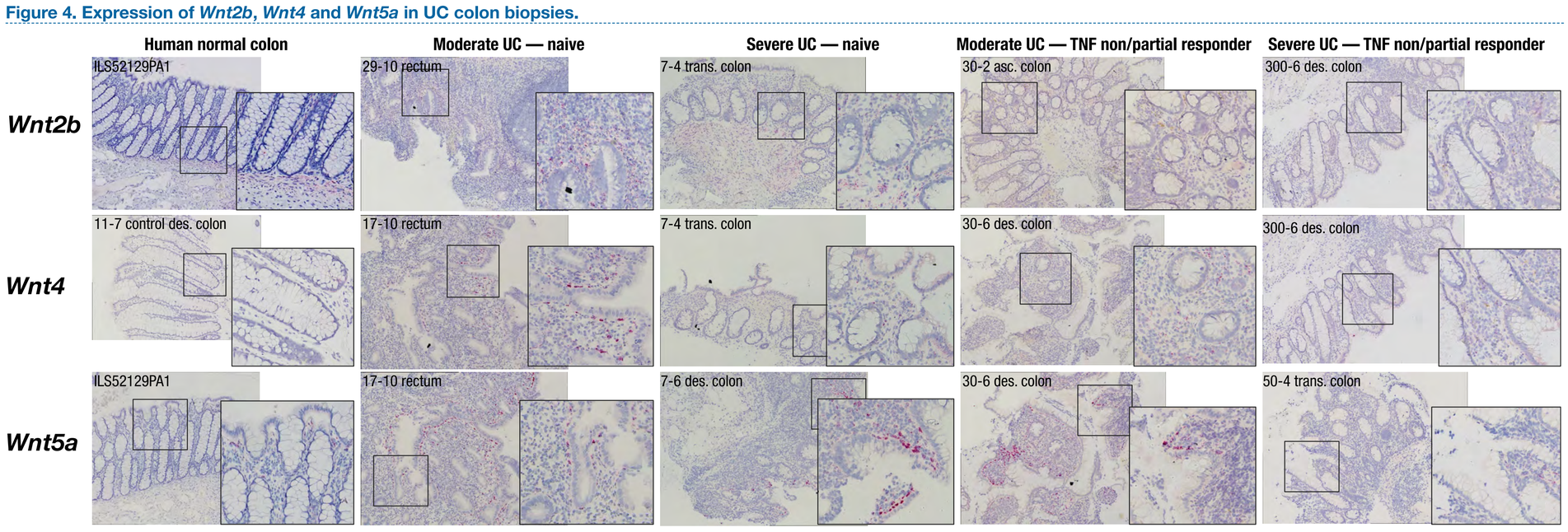
Expression of *Fzd5*, the highest expressed Fzd in the intestine, was mildly reduced in UC colon naïve to anti-TNF and was mildly reduced in UC colon treated with anti-TNF. Expression of *Fzd8*, *Lrp5* and *Lrp6* was also mildly reduced in UC colon biopsies (data not shown).

Figure 3. Expression of R-spondins in UC colon biopsies.



RSPO2 is the highest expressed R-spondin in the intestinal tissue. Expression of *RSPO2* was reduced in the UC colon. Effect on *RSPO2* expression was more noticeable in the severe UC samples. Expression of *RSPO3* was also reduced in UC colon biopsies (data not shown). Expression of *RSPO1* and *RSPO4* was very low in the colon and was hardly detected by *in situ*. *RSPOs* are normally expressed in the stromal cells next to the crypt bottom stem cell compartment but this expression pattern was disrupted in the UC colon as a result of immune cell infiltration.

Figure 4. Expression of *Wnt2b*, *Wnt4* and *Wnt5a* in UC colon biopsies.



Gross level of expression of *Wnt2b*, *Wnt4* and *Wnt5a* appear to be induced by the UC conditions, consistent with immune cell infiltration in the tissues as infiltrating macrophages were reported to secret Wnts. However, the location of Wnt expression was altered due to tissue damage and distortion, potentially making the Wnts less accessible to the intestinal stem cells which rely on Wnt signaling for maintenance and regeneration.

Conclusion

Reduced expression of Wnt receptors, RSPOs and Wnt target genes indicate insufficient Wnt signal induction in the damaged colon epithelium of UC patients. This suggests that repair of the damaged epithelium by Wnt agonist treatment may constitute a new mechanism of action and benefit patients with UC.

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