Host-microbial crosstalk in the pathogenesis of colon cancer in Primary Sclerosing Cholangitis

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Background

Primary sclerosing cholangitis (PSC) is an idiopathic chronic disorder of the liver associated with inflammatory bowel disease (IBD). Patients with PSC and IBD (PSC-IBD) often have mild colitis, but exhibit a five-fold increased risk of colorectal cancer compared to patients with ulcerative colitis (UC) only, and ten times higher than the general population.

We recently described distinct inflammatory responses in PSC-IBD versus UC, with increased Th1 and ILC infiltrates in these patients. Dysbiosis has been found in PSC supporting a role for the microbiome in the pathogenesis of disease.

In this study we aim to: 1) assess microbial functions in PSC-IBD using metatranscriptomic analysis, 2) evaluate whether PSC-IBD-associated pathways impact on epithelial transformation using patient-derived intestinal organoids.

Methods

Colonic mucosal brushings and biopsies were collected from patients with PSC-IBD, patients with UC, and from healthy controls (HC). RNA was extracted from the brushings and deep-RNA sequencing was performed. Colonic crypts were isolated using DTT and EDTA, seeded in basement membrane extract and cultured in media containing R-spondin 1, Noggin and Wnt3A to develop organoids. Organoids were stimulated with different cytokines for 24 hours and markers of cytokine downstream pathways and stemness/pluripotency were analysed by qPCR.

Results

Our pilot study shows that deep-sequencing of RNA obtained from brushings provides microbial reads to profile the functional activity of the adherent microbiome. We

successfully cultured primary intestinal organoids from both groups of patients and HC. As expected, stimulation with IFNγ resulted in *STAT1* induction in all the organoids. Interestingly, higher expression of *STAT3*, whose phosphorylated form is increased in colon cancer, was found in PSC-IBD-derived organoids. While the stemness-associated gene *NANOG* was upregulated in UC-derived organoids, OLFM4, a gene associated to pluripotency and early stages of neoplastic transformation, was higher in PSC-IBD-derived organoids.

Conclusion

RNA-sequencing of mucosal brushings allows functional profiling of the adherent mucosal communities, which in close proximity to the intestinal epithelium, shape the host immune response and may influence the development and/or progression of cancer. Organoids can be derived from colonic biopsies and provide an invaluable tool to evaluate the effect of immune and non-immune pathways on the intestinal epithelium. Our data show that genes associated with pluripotency and neoplastic transformation are differentially expressed in PSC-IBD- and UC-derived organoids, suggesting that distinct molecular pathways may be involved in cancer development in these patients.