Fecal micro-RNAs indicate disease activity in ulcerative colitis

J. Verdier[1], I. Breunig[1], M.C. Ohse[1], S. Roy[1], S. Groner[1], S. Roubrocks[1], K. Streetz[2], C. Trautwein[1], C. Roderburg[1], G. Sellge[1]

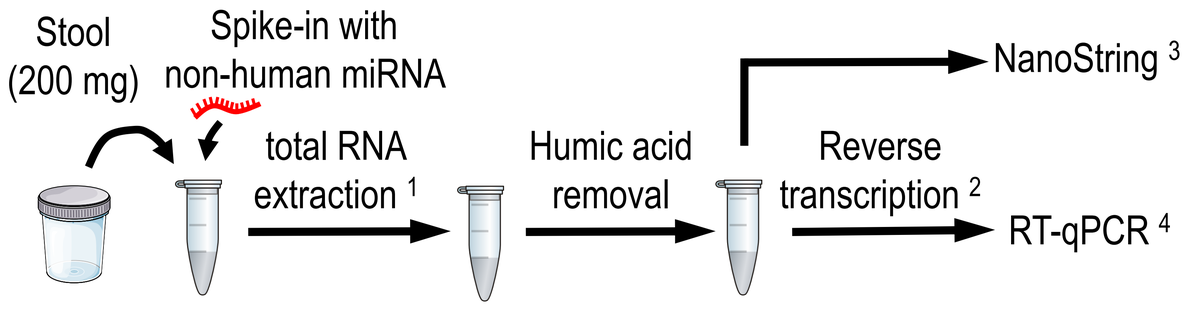
[1] University Hospital RWTH Aachen, Dep. of Internal Medicine III, Aachen, Germany

[2] Gemeinschaftsklinikum Mittelrhein - Kemperhof, Dep. of Gastroenterology, Kobelnz, Germany

Background and methods

Micro-RNAs (miRNAs) are promising biomarkers for personalised medicine owing to their tightly regulated expression and their stability in extracellular environments, suitable with non-invasive sampling methods. In this study, we investigated the expression of fecal miRNAs in Crohn's disease (CD), ulcerative colitis (UC) and Clostridium difficile infection (CDI).

A Nanostring screen for 800 different human miRNAs was applied to stool samples from 6 controls and 6 active CD patients. Levels of selected miRNAs were further measured by RT-qPCR in feces, colonic biopsies and sera from controls and CD, UC, and CDI patients.



[1] Stool RNA purification kit, Norgen Biotek; [2] miScript II RT kit, QIAGEN; [3] nCounter® human v2 miRNA Expression Assay, 800 miRNAs; [4] miScript primer assays, QIAGEN

Results

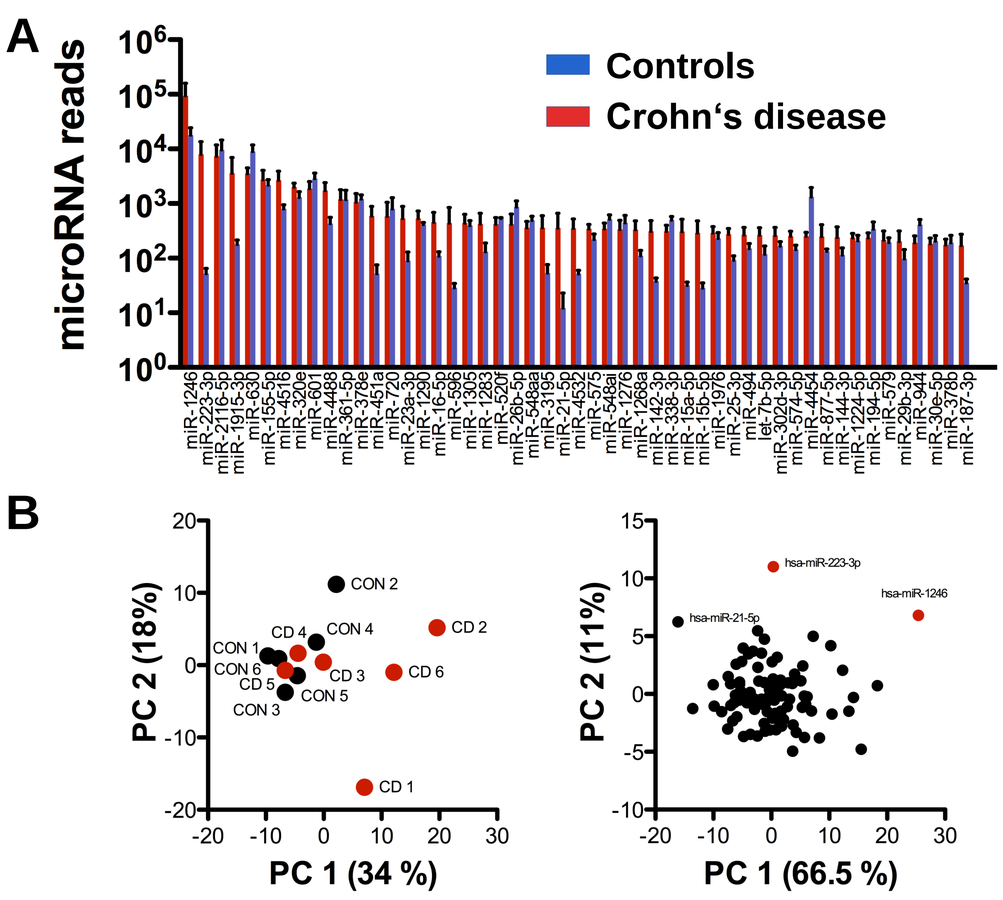


Figure 1. Screening for the presence fecal miRNA. **A.** Counts of the 50 most highly detected fecal miRNA in controls (blue) and in active Crohn's disease patients (red) **B.** Principal component analyses for individuals (left) and for the fecal miRNAs (right).

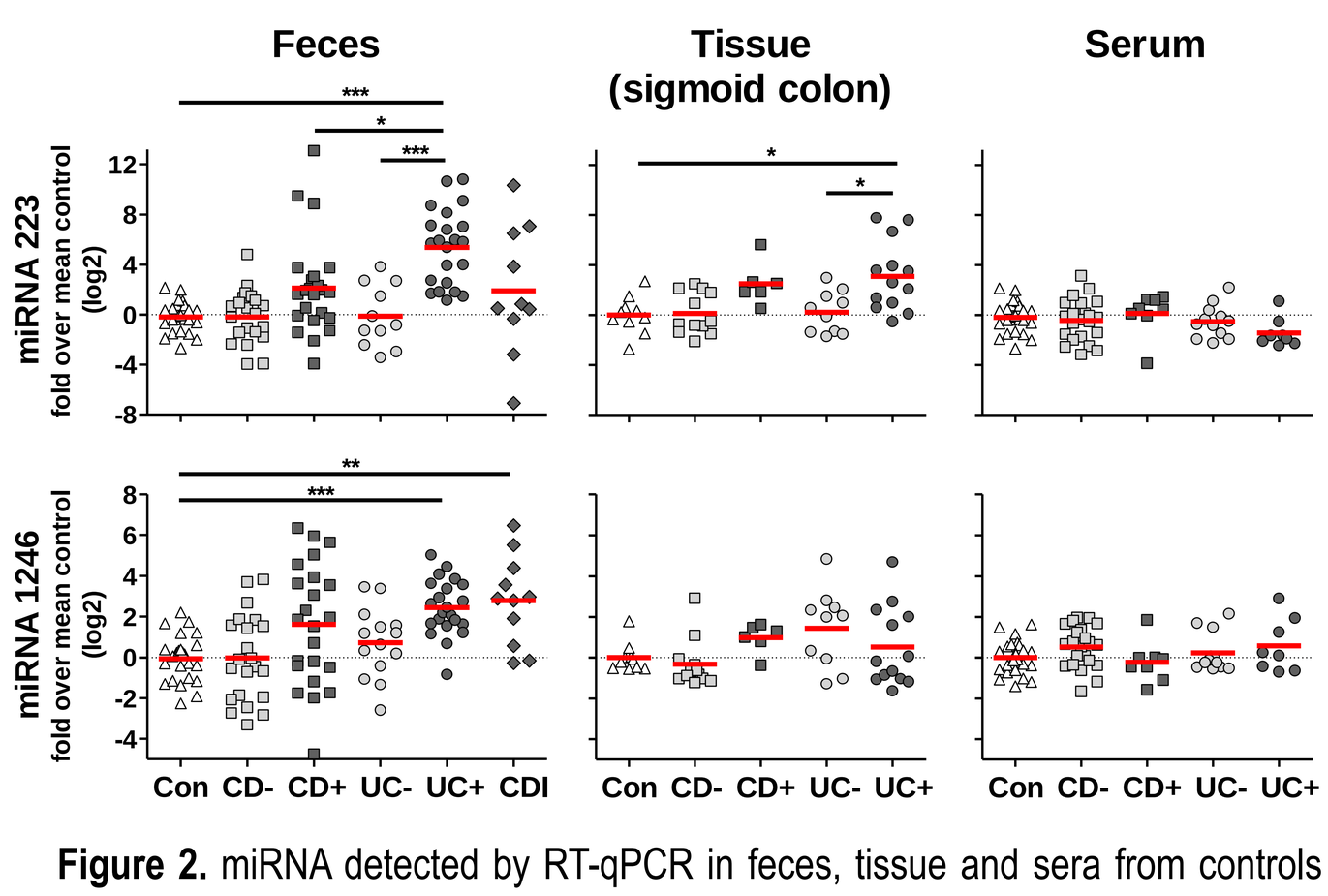
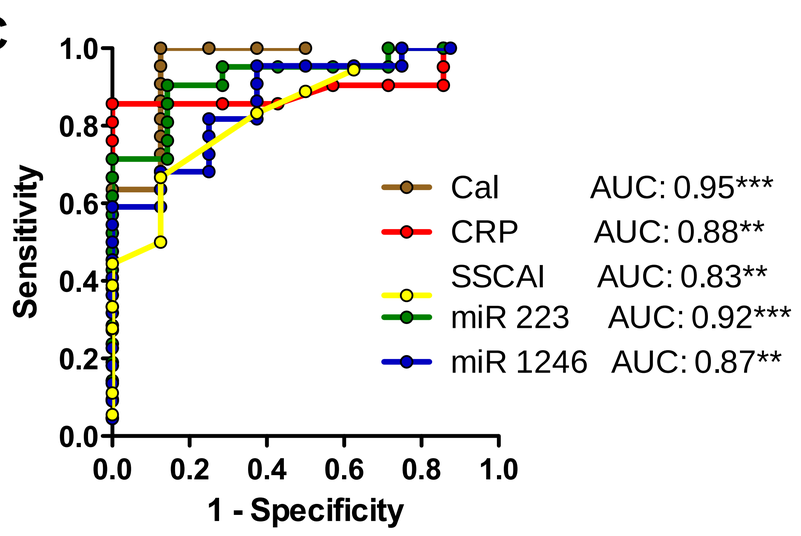


Figure 2. miRNA detected by RT-qPCR in feces, tissue and sera from controls (Con), and patients suffering from Crohn's disease (CD), ulcerative colitis (UC) and *Clostridium difficile* infection (CDI). CD-/UC-: fecal calprotectin < 250 mg/kg (feces/serum); without inflamation (tissue). CD+/UC+: fecal calprotectin > 250 mg/kg (feces/serum); with inflamation (tissue) *,p<0.05;**, p<0.01;***,p<0.001.

A

		miR223	miR1246	Mayo	Cal	CRP	SCCAI
miR223	r^s		0,48	0,49	0,61	0,46	0,28
	p		0,005	0,008	0,000	0,008	0,145
	n		33	28	33	33	29
miR1246	r^s	0,48		0,53	0,51	0,56	0,46
	p	0,005		0,002	0,002	0,000	0,009
	n	33		30	36	36	32
Mayo	r^s	0,49	0,53		0,72	0,46	0,42
	p	0,008	0,002		0,000	0,010	0,033
	n	28	30		30	30	26
Cal	r^s	0,61	0,51	0,72		0,53	0,49
	p	0,000	0,002	0,000		0,001	0,004
	n	33	36	30		36	32
CRP	r^s	0,46	0,56	0,46	0,53		0,61
	p	0,008	0,000	0,010	0,001		0,000
	n	33	36	30	36		32
SCCAI	r^s	0,28	0,46	0,42	0,49	0,61	
	p	0,145	0,009	0,033	0,004	0,000	
	n	29	32	26	32	32	



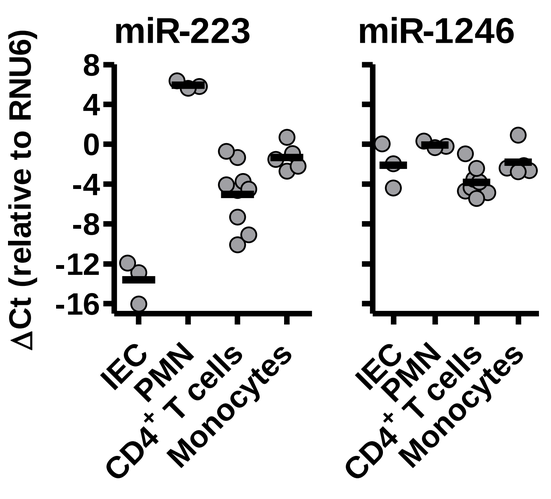


Figure 4. Relative expression of miR-223 and miR-1246 in intestinal epithelial cells (IEC), blood-derived neutrophils (PMN), CD4 T cells and monocytes.

B

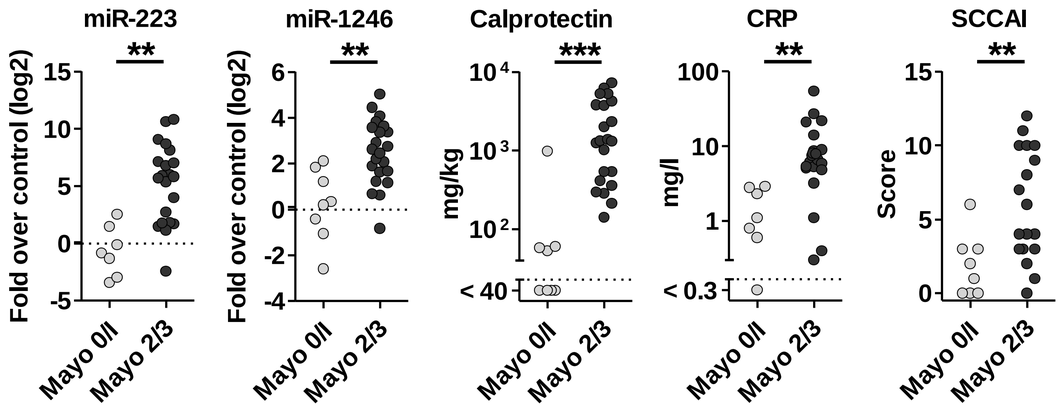


Figure 3. A. Correlations between clinical markers and fecal miR-223 and miR-1246 in UC. **B.** Fecal miRs, calprotectin, CRP and Simple Clinical Colitis Activity Score (SCCAI) in UC patients with an endoscopic Mayo score 0/1 vs 2/3. **C.** Receiver Operating Characteristic curve corresponding to the results shown in B. *,p<0.05;**, p<0.01;***,p<0.001.

Conclusions

1. About 100 different miRNAs are detectable in feces
2. Active IBD is associated with a distinct fecal miRNA profile
3. Fecal miR-1246 and miR-223 are markers for active UC
4. Fecal miRNA profiles may have the potential to differentiate between different forms of IBD and infectious colitis

Funding

DFG Deutsche Forschungsgemeinschaft

SEVENTH FRAMEWORK PROGRAMME

SysmedIBD