

Integrated miRNA and mRNA Expression Profiling for Ulcerative Colitis

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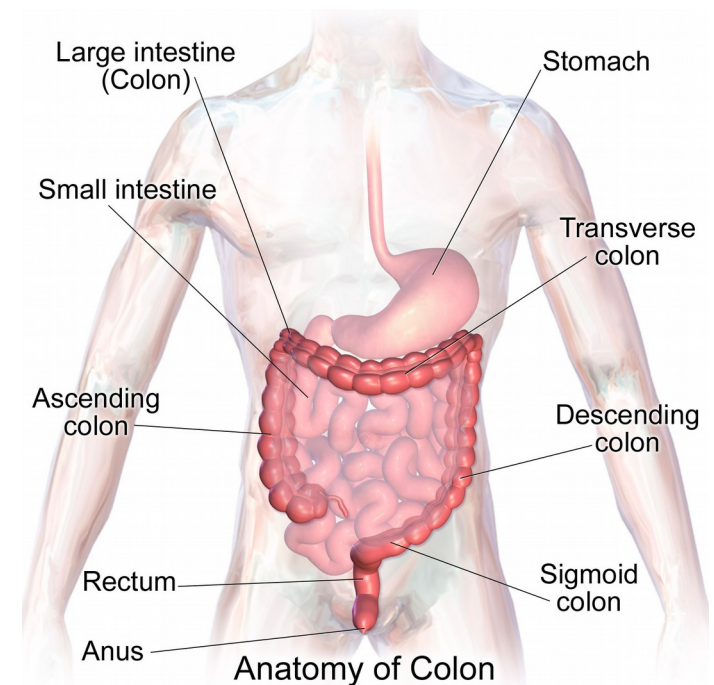
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Ulcerative Colitis

- Form of inflammatory bowel disease (IBD)
 - causes inflammation and ulcers in the colon
 - chronic and relapsing condition confined to the colon
 - separates from the other major IBD-type, Crohn's disease, which may affect the entire gastrointestinal tract in a segmental manner
 - autoimmune disease characterized by T-cells infiltrating the colon
- 12 regions of the genome may be linked to ulcerative colitis
 - no loci is consistently at fault, suggesting that the disorder arises from the combination of multiple genes



Motivation

- Recent studies demonstrated
 - ♦ Ulcerative Colitis (UC) is associated with differential colonic expression of several genes
 - ♦ that are mainly involved in immune response, cell adhesion, barrier integrity and tissue remodeling
- and miRNAs
 - ♦ important regulators of immune responses
 - ♦ expression levels change during inflammation
- Integration of mRNA and miRNA expression profiling may allow the identification of functional links between dysregulated miRNAs and mRNAs in Ulcerative Colitis

Study Design

- 78 colon samples from active (AUC), remission (REM) and control (CO) patients
- mRNA & miRNA expressions measured with
 - Affymetrix® Human Genome U219 Array
 - Affymetrix® miRNA 3.1 Array

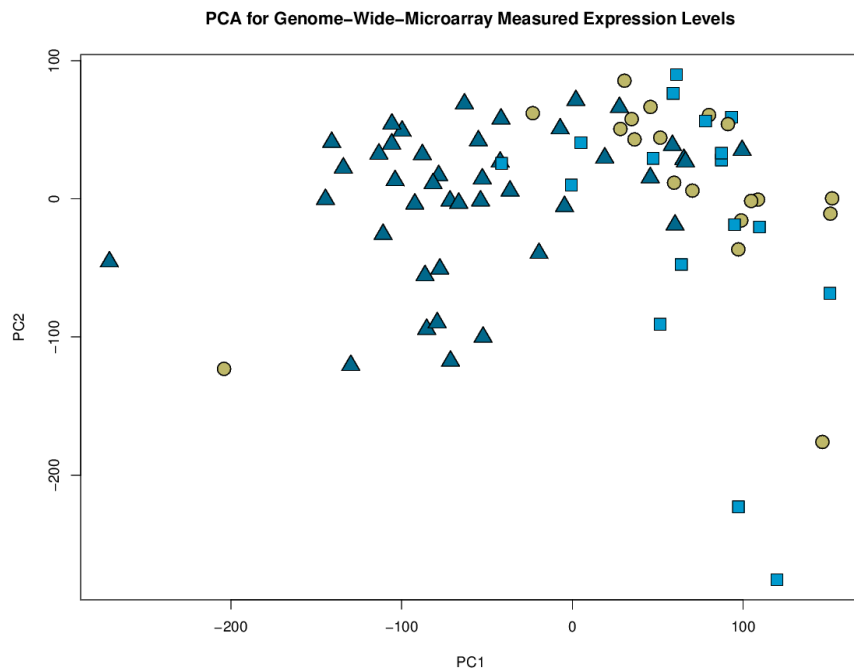
		Controls (CO)	Remission (REM)	Active UC (AUC)
No. of patients		19	17	42
Sex	(male/female)	7 / 12	10 / 7	17 / 25
Age (males)	(mean/range)	47.7 / 20 - 64	54.0 / 29 - 68	39.9 / 20 - 63
Age (females)	(mean/range)	48.7 / 23 - 68	38.7 / 27 - 63	37.1 / 18 - 67
Age	(mean/range)	48.3 / 20 - 68	47.7 / 27 - 68	38.2 / 18 - 67
Age at diagnosis	(<=25) / (>25)		4 / 13	16 / 26
Years with disease	(<=10) / (>10)		8 / 9	32 / 10
Geboes score	(mean/range)	0.63 / 0 - 4	1.7 / 0 - 7	13.4 / 1 - 22
Mayo score	(mean/range)	0.42 / 0 - 2	0.71 / 0 - 4	7.6 / 3 - 12
Smoking	(yes/no)	3/16	3/14	3/39

PCA Analysis

- The Affymetrix raw data was normalized using the robust multichip average (RMA) algorithm with Bioconductor* packages in R env.

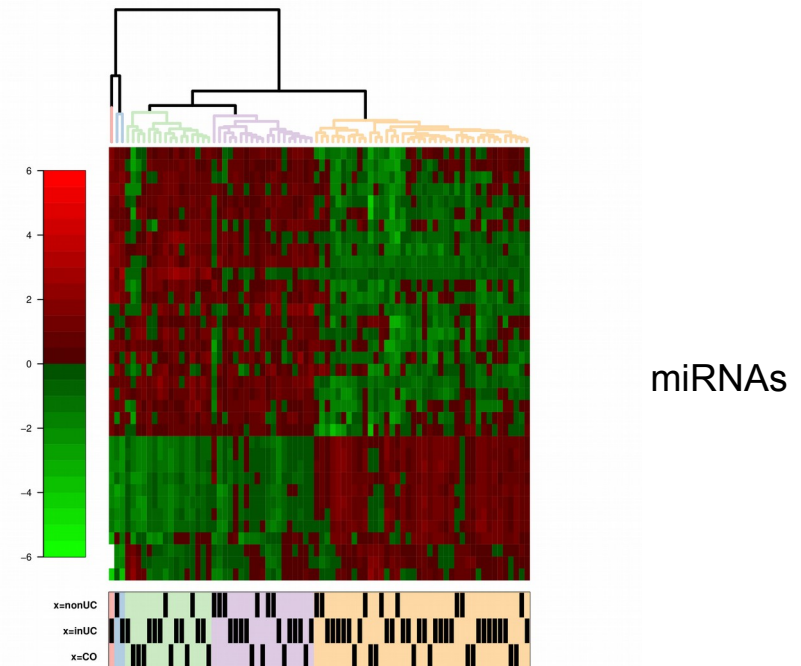
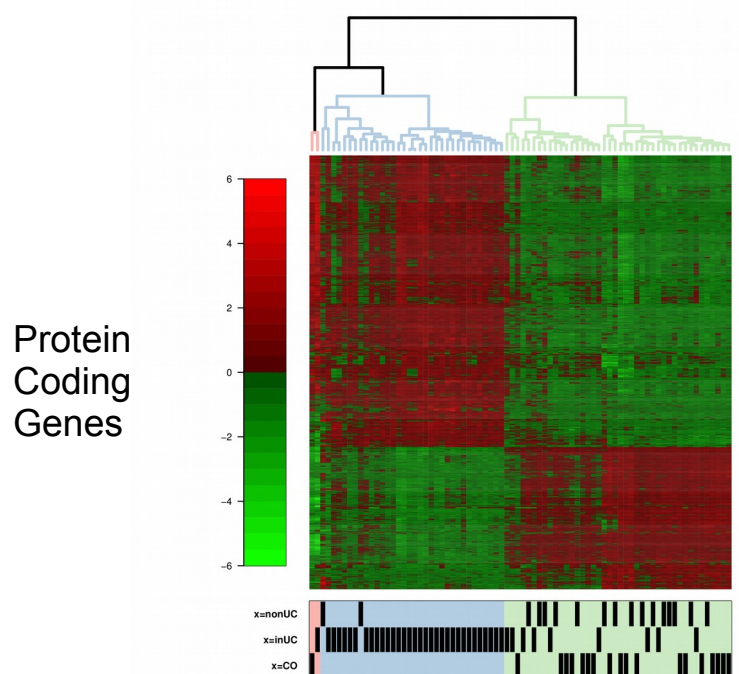
*(Huber et al., 2015)

- Principal component analysis shown below
 - ♦ Samples from same groups tend to cluster together



Differential Expression Analysis

- We used LIMMA* package to identify differentially expressed genes
*(Ritchie et al., 2015)
- DE thresholds are set as: > 2 -fold change & adj.p-value (FDR*) < 0.05
*(Benjamini Hochberg, 1995)
- 745 protein-coding genes and 14 miRNAs are identified as differentially expressed in both comparisons AUC vs CO and AUC vs REM

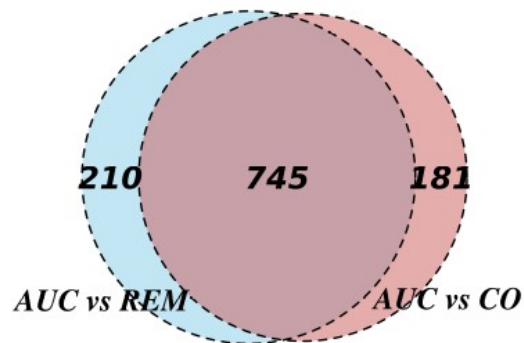


Differential Expression Analysis

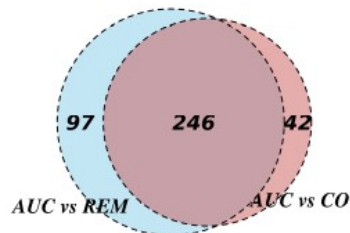
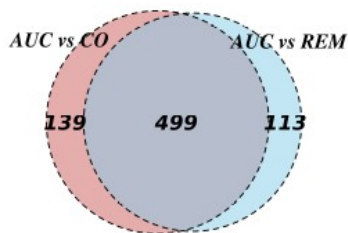
- Differential expression overlap

(Intersection set between AUC vs CO and AUC vs REM contrasts)

Dif. Exp. Protein Coding Genes

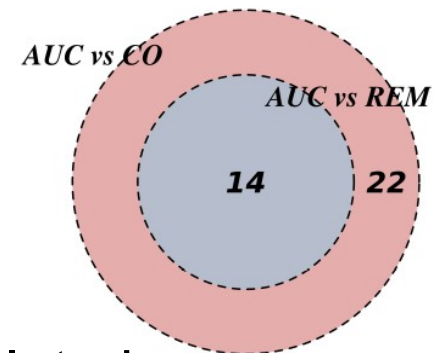


up-regulated

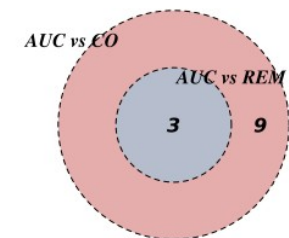
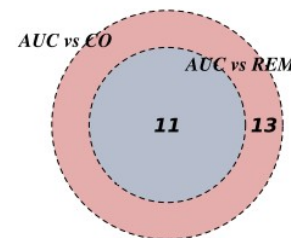


down-regulated

Differentially Expressed miRNAs



up-regulated



down-regulated

miRNA-mRNA interaction pairs

- We looked up for experimentally supported interactions in RAIN* database

*(Junge et al., in prep.)

- between 14 dif.exp. miRNAs and 745 dif.exp. protein coding genes

- RAIN interactions for

- 7 up-regulated miRNAs

hsa-mir-200b, hsa-miR-431, hsa-miR-409-5p, hsa-miR-212, hsa-miR-146b-5p, hsa-miR-31, hsa-miR-708

- 1 down-regulated miRNA

hsa-miR-375

Interaction Pairs			
		mRNA	
		down	up
miRNA	down (1)	19	65
	up (7)	<u>33</u>	110

Individual miRNA – mRNA pair example

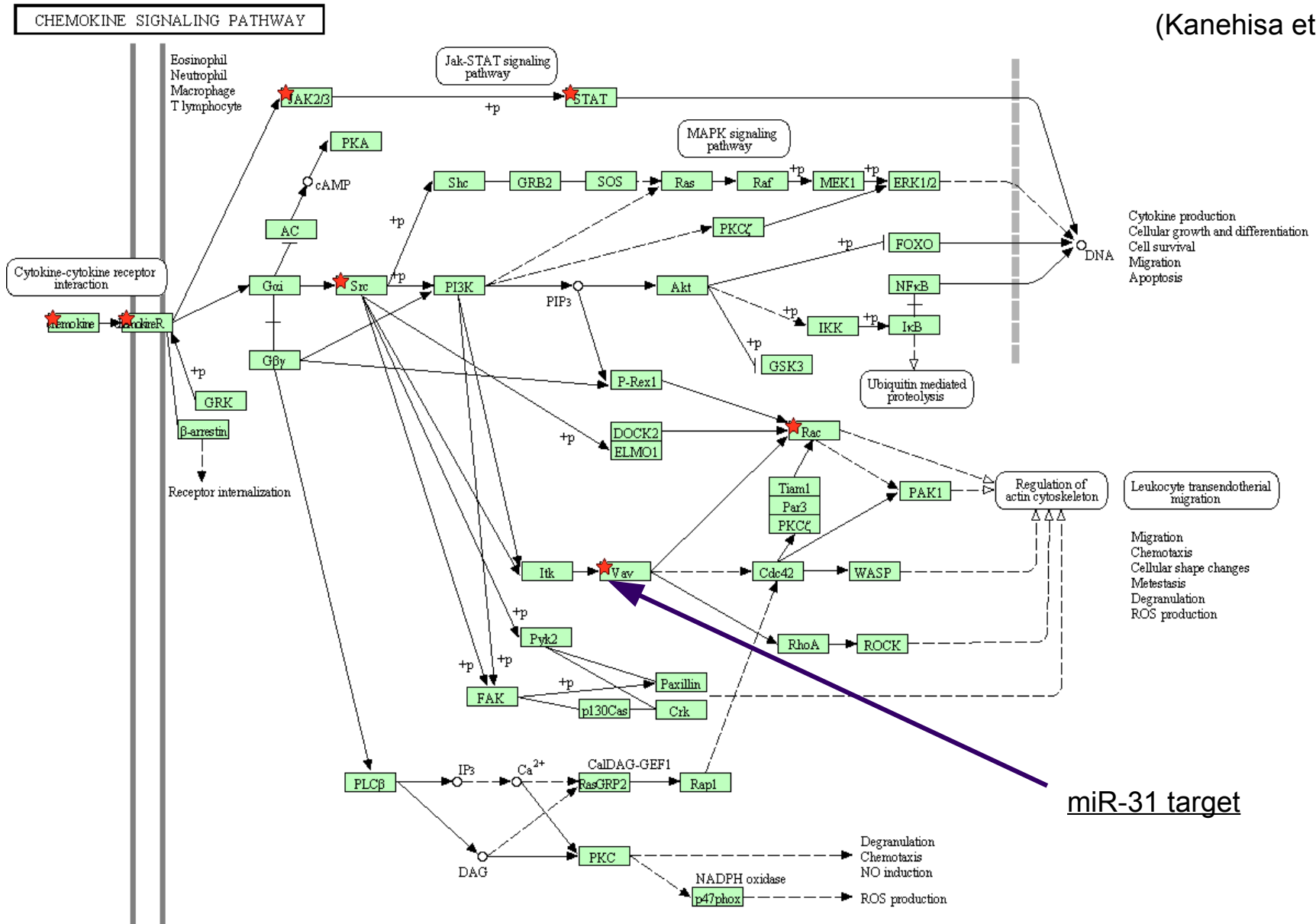
- We are now prioritizing candidate pairs for experimental validation and analyzing their effects in enriched pathways
- Interaction in RAIN database (retrieved from StarBase2.0)
 - ♦ hsa-miR-31-5p
 - logFC = 2.13
 - correlation coef. between miRNA expression and disease severity 0.59, $p\text{-value} < e\text{-}3$
 - ♦ VAV3 (Vav 3 Guanine Nucleotide Exchange Factor, activate pathways leading to actin cytoskeletal rearrangements)
 - logFC = -1.04
 - correlation coef. between mRNA expression with disease severity -0.56, $p\text{-value} < e\text{-}3$
 - ♦ Correlation coefficient between miRNA and mRNA expression -0.58, $p\text{-value} < e\text{-}3$

Enrichment Analysis for DE genes

Annotation Cluster	GO-TERM or KEGG pathway	Count	p-value	Adj. p-value (FDR)
1	Defense Response	96	8.4e-31	1.2e-27
	Inflammatory Response	69	4.4e-30	4.3e-27
	Response to wounding	86	1.0e-28	7.6e-26
2	Blood vessel development	37	1.8e-11	4.1e-9
	Angiogenesis	24	2.9e-8	2.9e-6
3	Innate immune response	28	7.9e-12	2.3e-9
	B-cell mediated immunity	15	3.4e-8	3.3e-6
	Activation of immune response	18	3.5e-8	3.2e-6
	Complement activation	10	1.4e-6	8.6e-5
	Complement and coagulation cascades (KEGG)	16	1.2e-6	1.6e-4
4	Cytokine activity	27	3.8e-8	3.1e-5
	Cytokine-cytokine receptor interaction (KEGG)	32	5.9e-6	4.2e-4
	Chemokine activity	12	1.1e-6	2.2e-4
	Chemokine signaling pathway (KEGG)	19	6.0e-3	1.0e-1

Integrating miRNAs to enriched pathways

(Kanehisa et al., 2000)



Ongoing & Future Work

- Analyzing enriched pathways and integrating experimentally supported miRNA – mRNA interactions into this pathways
- Adding miRNA – mRNA interactions from TarBase v7.0 and;
- Adding predicted miRNA interactions into our analysis
- Enrichment analysis for target genes of differentially expressed miRNAs
- Experimental validation of disease-driving miRNA – mRNA interactions

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