Mesalamine modulates several genes related to carcinogenesis, inflammation, and cell cycle pathways

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INTRODUCTION:

Since its development in 1942 by Nana Svartz, sulfasalazine (SASP) and its active metabolite 5-Aminosalicylate (mesalamine or 5-ASA 5-ASA(5-Aminosalicylic Acid)) have been the first tier drugs of choice in the management of Ulcerative Colitis (UC). Epidemiological studies recently have suggested that SASP as well as 5-ASA may reduce the risk of Colorectal Cancer (CRC) in UC patients, with a meta-analysis showing an odds ratio of developing CRC in UC patients on 5-ASA treatment to be 0.51. At dosages greater than 1.2 g/day, the meta-analysis indicates that the odds ratio of developing CRC dropped to 0.23.

Despite several decades of research, a consensus has not been reached regarding the mode of action of 5-ASA either as an anti-inflammatory agent for chronic maintenance in UC or as a potential chemo-preventative agent.

The appeal of 5-ASA as a cancer preventing, as well as anti-inflammatory medication is understandable, as it has been demonstrated to be clinically very safe. Azobonded sulfasalazine (SASP) bypasses small bowel absorption to be cleaved by the bacterial azo-reductase enzyme in the colon, allowing the 5-ASA moiety to achieve very high intraluminal concentrations in the colon. Therefore, the anti-neoplastic and anti-inflammatory effect of 5-ASA is expected to be localized to the colonic epithelium.

To our knowledge, no group of investigators has yet to date undertaken a mirroarray analysis of the intracellular epigenetic effects of 5ASA to elucidate the gene and gene families that this potent therapy may modulate.

OBJECTIVE:

1) **What are the genes or gene families that mesalamine (5-ASA) modulates?**

1) **Do these genetic targets correspond to any well described carcinogenic, cell cycle, or inflammatory pathways?**

MATERIALS AND METHODS:

DRUG TREATMENT

- LS180 colon cancer cells were incubated with 2mM of 5-ASA for 0hr, 4hrs and 24 hrs.

SAMPLE PREPERATION

- RNA was extracted at each of these 3 time points using the *Qiagen RNeasy Mini Kit* . cDNA was prepared using *Advantage RT-for-PCR kit* (Clontech).

MICROARRAY ANALYSIS

- Microarray analysis was conducted on Affymetrix 133A chips. All experiments were performed in duplicate.
- Informatics analysis were completed using *Ingenuity Pathways Analysis*.

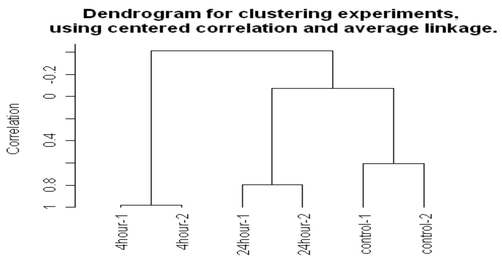
RESULTS:

Function	Gene	Fold changes 4hrs	Fold changes 24hrs
Anti-Inflamatory			
Cytokine	**IL-8**	**-3.6**	**1.3**
	IL17RB	**-2.6**	**1.1**
Chemokine	**CCL14**	**-4.2**	**1.3**
	CXCL10	**-5.6**	**1.3**
	CXCL11	**-6.3**	**1.4**
FK	**FK506BP11**	**2.5**	**-2**
mTOR	**CALR**	**-2.4**	**1**
Host defense	**Defensin 6**	**-33.3**	**1.1**
Pro-Inflamatory			
Cytokines	**IL-18**	**2.2**	**1.1**
	CD44	**2.3**	**1.1**
	IL1RII	**-16.2**	**2.1**
Anti-carcinogenic			
Oncogenes	**CEACAM1/2**	**-2.6**	**1.2**
	RAB3B (RAS)	**-4**	**1.4**
	ECT2	**-2.8**	**1.1**
	PIM1	**-2.6**	**1**
	EREG	**-2**	**1.1**
	FAS(FAIM)	**-5**	**1.1**
	Ki-67	**-4.3**	**1.3**
Cellcycle regulatory			
G2/M transition	**Cyclin G2**	**-3.3**	**1.1**
	CHK1	**-2.6**	**1.3**
G1/S transition	**CDK2**	**-2.5**	**1.1**
	E2F	**-3.6**	**1.2**

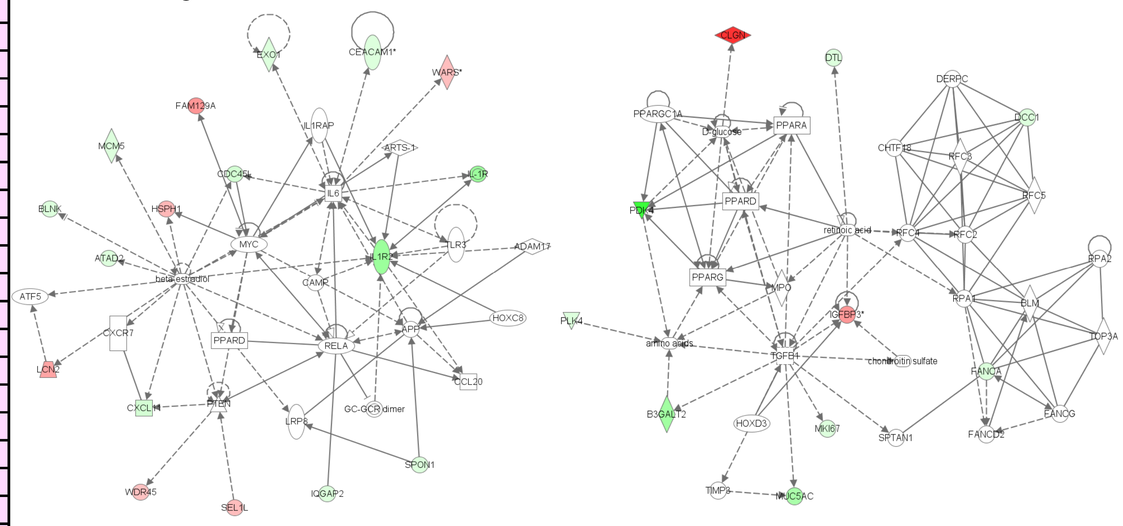
Microarray analysis: LS180 colon cancer cells treated with 5ASA for 4hrs had significant (p<0.02-0.00002) modulation of the the above listed genes against control (untreated) cells.

Informatics analysis of the microarray data, limiting results to fold changes >2 and $p<10^{-5}$ indicated two important findings:

1) *Clustering analysis*: Treatment with 5ASA at 4hrs was notably different compared with control (untreated) cells. However, clustering analysis showed that there was not a significant difference in gene expression between cells treated with 5ASA for 24hrs and control cells, as shown here. This suggests that the modulation of genes mediated by 5ASA is largely reversible following a single treatment.

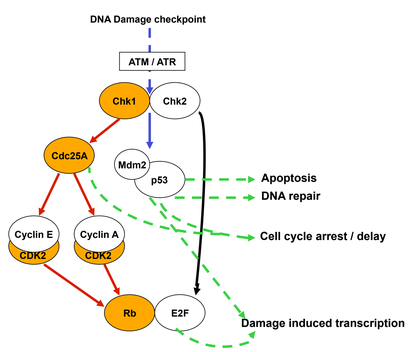


2) ***Ingenuity Pathway Analysis:*** revealed that 5ASA primarily affected pathways associated with two disorders: gastrointestinal disease and cancer.



Gene Networks Modulated by 5-ASA

Disease Process	Targets	p
Gastro-intestinal Disease	7	p<0.04
Cancer	11	p<0.02



Proposed pathway of Mesalamine action : Following their activation, Chk1 and Chk2 phosphorylate downstream effectors that further propagate the checkpoint signaling. Depending on the type of stress, velocity of DNA damage, and cellular context, this leads to (1) stress-induced transcription program (E2F1, p53), (2) direct or indirect initiation of DNA repair (p53), (3) acute delay (degradation of Cdc25A) and/or sustained arrest (p53) of cell cycle progression, and (4) apoptosis (p53, E2F). The known target sites of Chk1 (red) Chk2 (black) and both Chk1 and Chk2 (blue) on the individual substrates are shown. Are genes altered in our arrays.

SUMMARY & CONCLUSIONS:

We describe the first report of microarray analysis on the intracellular effects 5-ASA in a novel *in vitro* cell culture model. While the anti-inflammatory and anti-neoplastic effect of 5-ASA in UC has been demonstrated clinically, our study describes *in vitro* evidence of corresponding intracellular molecular pathways. The return of various genes to pretreatment levels and suggests a need for continuity of treatment. Future studies of the interaction of the various pathways modulated by 5-ASA will not only provide a better understanding of its clinically demonstrated efficacy, but also novel targets for future therapies.