Modulation of Inflammatory Genes in Immune Cells by miR-150

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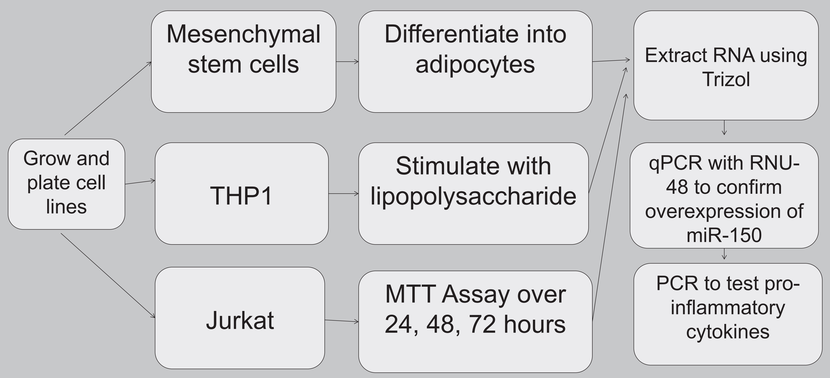
Introduction and Background

Obesity is an inflammatory disease associated with increased body fat, especially abdominal and visceral. Over 30% of the United States population is obese. Obesity can influence the development of several malignancies including gastric, colorectal, breast, and prostate cancer. A closer look at obesity may give an insight into the inflammatory response in other diseases. This project was aimed to correlate changes in body weight with inflammatory and metabolic parameters in obese female African American teenagers before and after diet intervention.

Participants in this study were obese and non-obese African American adolescents. Diet intervention was implemented for 12 weeks and included nutrition counseling, cooking demonstrations, calorie counting, and food measuring. Baseline and follow-up testing included a general exam for height, weight, BMI, serum inflammatory markers, and body composition (DXA), as well as a breathing test to measure lung inflammation (exhaled nitric oxide, eNO) and a complete laboratory metabolic panel (glucose, insulin, serum lipids). MicroRNAs were detected in serum by high throughout sequencing and confirmed by real-time PCR (rt-PCR). Overexpression of miRNA in Jurkat cells (T cells), THP-1 (pro-monocyte) and adipocytes was done using reagents and methods from Life Technologies.

Procedures and Methods



Results

Table 1. Diet Intervention modifies serum IL6 levels

Variable	Diet			
	N	Mean	Std Err	p-value
Body Composition				
Weight (pounds)	17	-0.5099	1.8942	0.7914
BMI	17	-0.0501	0.0156	0.0077
Waist Circumference	17	0.6327	1.2056	0.6082
Fat Mass (g)	17	-	477.320	0.5100
Lean Mass (g)		322.0600	0	
BMC (g)	17	65.0113	40.7173	0.1314
Metabolic				
Cholesterol	17	-3.4479	7.3665	0.6524
HDL	17	-1.9440	2.0262	0.3666
Triglycerides	17	-10.1994	4.8266	0.0747
LDL	17	-1.4953	4.3540	0.7409
HbA1C	17	-0.0216	0.0393	0.6008
Clinical				
Systolic Blood Pressure	17	-0.6588	2.7570	0.8143
Diastolic Blood Pressure	17	-0.8945	1.5129	0.5626
Inflammatory				
TNF-alpha	21	0.06597	0.190	0.7402
IL6	19	-2.7543	0.814	0.0076
IL8	21	1.7018	3.185	0.6096
Resistin	21	0.01401	0.039	0.7378
Adiponectin	21	-0.00198	0.039	0.9612
MCP_1	21	-23.79	18.530	0.2444
PAI_1_total	21	-0.05272	0.043	0.2626
CRP*	19	0.06338	0.106	0.5706
eNO	14	5.7585	5.2034	0.2941

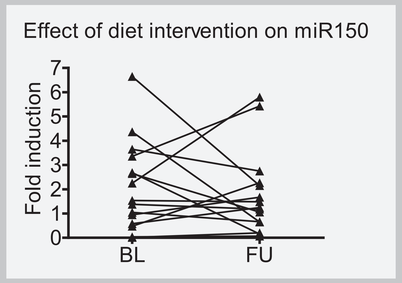
We found that diet intervention induced a significant reduction of BMI and Interleukin-6 (IL-6) levels, and a marginal reduction of circulating triglycerides.

Table 2. Changes in TNFα correlate with changes in miR-150

miR_name	PPEE	PPDE	PostFC BL/FU
hsa-miR-150-5p	0	1	0.59650751

Parameter	TNFα	HbA1C	PAI-1 (total)
Number of XY Pairs	13	13	13
Spearman r	-0.7253	-0.5333	-0.5055
95% confidence interval	-0.9149 to -0.2733	-0.8435 to 0.04347	-0.8321 to 0.08144
P value (two-tailed)	0.005	0.0605	0.078
P value summary	**	ns	ns
Is the correlation significant? (alpha=0.05)	Yes	No	No

A significant change in the circulating levels of miR-150 was observed after the intervention. Interestingly, changes in miR-150 correlated with changes in circulating TNFα, suggesting that this miRNA is associated with the modulation of inflammatory responses.'



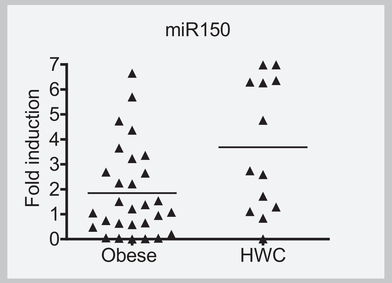


Figure 1. Comparison of circulating miR-150 after diet intervention (A) and increased levels of miR-150 in serum of healthy weight controls as compared to obese individuals (B)

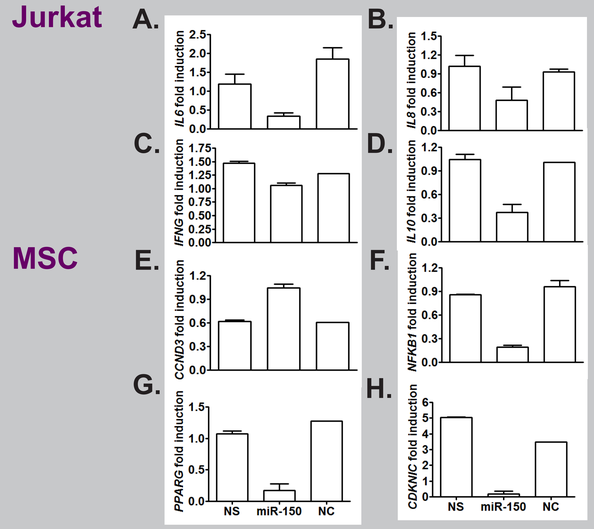


Figure 2. Gene modulation by miR150. Overexpression of miR-150 resulted in reduced mRNA levels ofIL6, IL8, and IL10 (A –D) in Jurkat cells while reduced NFKB1, PPARG and CDKNIC (F – H) and increased CCND3 was observed in MSC (E)

Conclusions and Future work

While our project was primarily exploratory, we made several observations about the inflammatory response in obesity. Our results showed that the overexpression of miR-150 induced a significant decrease in the expression of pro-inflammatory cytokines. This preliminary work forms the basis for further study of inflammatory mechanisms in obesity and other inflammatory diseases.

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