Gene Expression Profiling in Neonatal Rat Myocardium in Response to Rosiglitazone

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Abstract

Rosiglitazone, widely used as insulin-senstitizing agents for the treatment of type II diabetes, is a high-affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). PPARγ is a central regulator of adipogenesis and has impact on both cellular and lipid metabolism. Studies have shown that rosiglitazone enhances insulin-mediated glucose uptake at the whole-body level. However, the fact that the results of several research studies and meta-analyses examining the relationship between rosiglitazone and cardiovascular death have so far been conflicting means that the safety of the drug is yet to be determined. The work presented here is the first step toward discovering the molecular events that lead to association of rosiglitazone and the increased risk of heart failure. To this end, we have employed Illumina's BeadArray^{TM} technology to screen the time course gene expression of ventricular myocytes in neonatal rats under the treatment of the drug and identified the differentially expressed genes and relevant over-expressed biological processes and pathways.

Gene expression data are analyzed by conservative statistical methods that controls false discovery rate. We have found 33 significantly up/down regulated genes and most of these genes are responsible for fatty acid and lipid metabolisms. Our analysis concludes that there is no significant evidence showing any short-term beneficial or negative effect the calcium signaling pathway, which plays important roles in the health of heart cells.

PPARγ and Rosiglitazone

PPAR γ

- A transcription factor belonging to the nuclear hormone receptor gene super-family (PPARs) [6].
- Dominant regulatore of gene expression involved in cellular and lipid metabolism and adipocytes development [5].
- Correlation between PPARγ and insulin sensitivity has come from studies of human populations.
- Disregulation of PPARγ has been associated with hyperlipidemia, obesity, severe insulin resistance, diabetes mellitus, and hypertension.

Rosiglitazone

- Avandia, manufatured by GlaxoSmithKline, is a family of thiazolidinediones (TZDs) known as ligands for PPARγ.
- TZDs activate PPARγ transcriptional activity to insulin sensitivity and glucose homeostasis [5].
- Recent population-based studies of older patients with diabetes report that TZD treatment might be associates with the increased risk of cardiovascular diseases such as myocardial infarction, heart attack, and congestive heart failure [7].

Materials and Methods

- Ventricular myocytes are collected from the 1-4 days old Harlan Sprague-Dawley Rats (*Rattus norvegicus*). They are then isolated and plated on fibronectin-coated dishes overnight to allow for recovery.
- DMSO is used as the drug carrier, and the DMSO treated samples without rosiglitazon are used as control groups.
- The bulk of cells extracted from approximately 100 neonatal rats are pooled into 24 samples.
- DMSO is added to 11 samples at times: 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 hours, prior to RNA extraction.
- Both DMSO and rosiglitazone treatments are added to another 11 samples at the 11 time courses prior to RNA extraction.
- The experiment is repeated twice using different sets of neonatal rats. So, the DMSO and DMSO+rosiglitazone treated samples at each time course have two biological replicates.

The gene expression profiles of DMSO+rosiglitazone treated samples are compared against that of DMSO treated samples. This comparison allows us to identify up/down regulated genes in response to rosiglitazone.

Microarray Data Analysis

- **Tool** R and Bioconductor packages [2] including `lumi`, `limma`, `GOstats`, and the annotation packages `lumiRatV1` and `GO`.
- ***p*-value** Calculated by linear models and moderated t-statistics, an empirical Bayes approach [1].
- **Adjusted *p*-value** Calculated by applying multiplicity correction using false discovery rate Benjamini-Hochberg method [4]. Here, we control the false discovery rate at 0.05, which implies that all the selected significant genes have adjusted p-value less than 0.05.
- **Testing GO terms** Over-representation of GO terms is tested by using `GOstats` which implements a hypergeometric test [3].

Conditional Hypergeometric Test

In addtional to identifying differenctially expressed genes, infering relevant biological processes and pathways from a set of interesting genes provides further insight into underlying biology.
The conditional Hypergeometric test is a generally accepted statistical significant test to assess whether the number of selected genes associated with the biological processes or pathways is sufficiently larger than expected. The top forty genes, whose q-value is less than 0.01 are selected as interesting genes. The GO terms or pathways are expected to be relavent or enriched if the gene set memebers are among the top-ranked genes obtained from the previous stage of the analysis. A sufficient small $p-$value, calculated by the Hypergeometric test, represented that the biological functions or pathways are enriched and relavent. The *size* value in the table below represents the number of genes associated with that particular GO term, and the *count* value represents the number of genes in a particular biological process that are significantly regulated. The cut-off p-value is 0.005.

	GOBPID	GOTerm	PValue	Count	Size
1	GO:0006629	lipid metabolic process	0.0000048	9	259
2	GO:0006631	fatty acid metabolic process	0.0001020	5	89
3	GO:0019882	antigen processing and presentation	0.0038227	2	17
4	GO:0006635	fatty acid beta-oxidation	0.0038227	2	17

Table 1: Over-represented GO biological processes (conditional test)

	Term	Genes
1	lipid metabolic process	Cte1, Srebf1, Cidea_predicted, Acot7, Acaa2, Acadvl, Ech1, Angptl4, Adfp
2	fatty acid metabolic process	Acot7, Acaa2, Cte1, Acadvl, Ech1
3	antigen processing and presentation	Tap1, Psmb9
4	fatty acid beta-oxidation	Acadvl, Ech1

Table 2: Differentially expressed genes associated with the GO term

Differentially Expressed Genes

In the table below, we list the top genes whose adjusted p-values are less than 0.05 from the 48 hour time course expression data. Of these, 16 were significantly up-regulated and 6 were significantly down-regulated.

	Symbol	GeneName	p-value	adj. p-val
△	Angptl4	angiopoietin-like 4	8.3894e-19	9.6588e-15
△	Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	4.2305e-15	2.4353e-11
△	Cte1	cytosolic acyl-CoA thioesterase 1	2.4467e-11	9.3899e-08
△	Vipr2	vasoactive intestinal peptide receptor 2	8.1550e-10	2.3472e-06
△	Ech1	enoyl coenzyme A hydratase 1, peroxisomal	4.1532e-09	9.5631e-06
△	Adfp	adipose differentiation related protein	3.0989e-08	5.9463e-05
△	Acadvl	acyl-Coenzyme A dehydrogenase, very long chain	8.8726e-08	0.0001
△	Retsat	all-trans-13,14-dihydroretinol saturase	2.5036e-07	0.0004
△	Ccl6	chemokine (C-C motif) ligand 6	3.1331e-07	0.0004
△	Aqp7	aquaporin 7	5.1416e-07	0.0005
△	Impa2	inositol (myo)-1(or 4)-monophosphatase 2	8.9320e-07	0.0009
△	Acaa2	acetyl-Coenzyme A acyltransferase 2	1.1935e-06	0.0011
▽	Ccl12*	chemokine (C-C motif) ligand 12	5.9597e-06	0.0043
△	Etfdh	electron-transferring-flavoprotein dehydrogenase	1.7875e-05	0.0114
△	Decr1	2,4-dienoyl CoA reductase 1, mitochondrial	2.3758e-05	0.0144
▽	RGD1564665*	similar to RIKEN cDNA 4930555G01	3.1766e-05	0.0183
▽	LOC501396	hypothetical protein LOC501396	3.3797e-05	0.0185
△	Olr472*	olfactory receptor 472	4.0457e-05	0.0207
▽	Arhgdib	Rho, GDP dissociation inhibitor (GDI) beta	4.5705e-05	0.0216
▽	Psmb9	proteosome subunit, beta type 9	4.6861e-0	0.0216
△	Acot7	acyl-CoA thioesterase 7	7.2723e-05	0.0322
▽	Tap1	ATP-binding cassette, sub-family B	9.0736e-05	0.0373

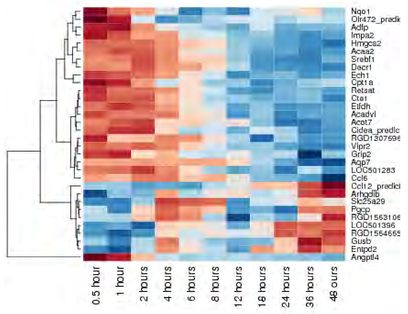
Angptl4 and Cardiac Lipoprotein Lipase (LPL)

- Postnatal cardiac myoctyes rely on the action of lipoprotein lipase (LPL) to hydrolyze triglycerides for energy uptake [8].
- Loss of LPL leads to impaired cardic functions and contractility including abdominal aortic constriction and perivascular fibrosis [9].
- Inhibition of cardiac LPL causes hypertriglyceridemia. Compensation is taken by increasing glucose uptake.
- Angiopoietin-like proteins (Angptl4) plays a role in metabolic regulation. During fasting, induction of Angptl4 switches metabolic patterns of tissues to ensure adequate energy partitioning that is essential for survival [8].
- Angpl4 is regulated by PPAR family of transcritpion factors.
- Angpl4 is acutely modulated in cardiac myocytes by the rosiglitazone treatment in this experiment.
- Overexpressed Angptl4 has shown to be poten inhibitors of LPL in heart [8].

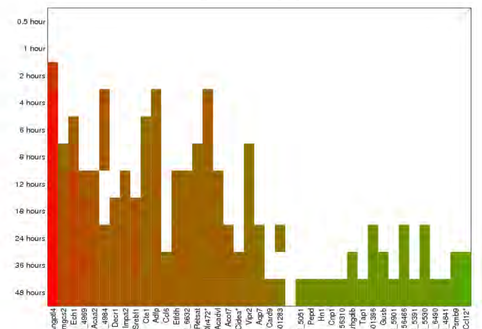
Term	Pvalue	Count	Size	Genes
Benzoate degradation via hydroxylation	0.00026	2	4	Acaa2, Ech1
PPAR signaling pathway	0.00179	3	38	Hmgcs2, Aqp7, Angptl4
Valine, leucine and isoleucine degradation	0.00922	2	22	Acaa2, Hmgcs2
Fatty acid metabolism	0.01476	2	28	Acaa2, Acadvl

Table 3: Over-represented KEGG Pathways (p-value <0.01)

Heatmap:40 top up/down regulated genes



Heat Diagram (adj. p-value < 0.1)



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