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Data in Brief

Disruption of O-GlcNAc cycling by deletion of O-GlcNAcase (Oga/Mgea5) changed gene expression pattern in mouse embryonic fibroblast (MEF) cells

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ABSTRACT

Adding a single O-GlcNAc moiety to a Ser/Thr molecule of a protein by O-GlcNAc transferase and transiently removing it by O-GlcNAcase is referred to as O-GlcNAc cycling (or O-GlcNAcylation). This O-GlcNAc modification is sensitive to nutrient availability and also shows cross talk with phosphorylation signaling, affecting downstream targets. A mouse model system was developed and evaluated to show genome wide transcriptional changes associated with disruption of O-GlcNAc cycling. Mouse embryonic fibroblast cells derived from O-GlcNAcase (*Oga*) knock out (KO), heterozygous (Het) and wild type (WT) embryos were used for an Affymetrix based microarray. Results are deposited in GEO dataset GSE52721. Data reveals that *Oga* KO MEFs had 2534 transcripts differentially expressed at 1.5 fold level while *Oga* heterozygous MEFs had 959 transcripts changed compared to WT MEFs. There were 1835 transcripts differentially expressed at 1.5 fold Het versus WT comparison group. Gene ontology analysis indicated differentially expressed genes enriched in metabolic, growth, and cell proliferation categories.
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Specifications	
Organism/cell line/tissue	*Mus musculus*/O-GlcNAcase (Oga/MGEA5) heterozygous mouse derived from Oga floxed male mice (genetic background C57BL/6) cross breed with MMTV-cre female mice (B6129-Tgn(MMTV-cre). Oga Het mice cross bred and collected MEF cells at E12.5 for the study.
Sex	Female
Sequencer or array type	Affymetrix mouse 430.2
Data format	Raw
Experimental factors	O-GlcNAcase (Mgea5) gene deleted MEF cell **Oga KO**, single Oga allele **Oga Het** normal **WT** (both Oga alleles present)
Experimental features	Gene expression of female MEF cells derived from Oga KO, Het and WT cells from the same litter
Consent	Not applicable
Sample source location	NIDDK, NIH, Bethesda)

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52721.

2. Experimental design, materials and methods

2.1. MEF cells

Transgenic mice bearing an O-GlcNAcase heterozygous allele were developed from *Oga* floxed mice cross breeding with *Cre* mice as described in [1]. The resultant *Oga* heterozygous mice were cross bred to derive mouse embryonic fibroblast (MEF) cells for this study. MEF cells were collected at embryonic day 12.5 following the protocol from the Fred Hutchinson Cancer Center (www.fhcrc.org/science/labs/fero/Protocols/MEFs.html). Cells were cultured in DMEM media added with 10% FBS and 100 U/ml penicillin and 100 μg/ml streptomycin. Gene and protein expression patterns of wild type, heterozygous and KO MEFs were evaluated using PCR and Western blotting. Genotypes were confirmed by PCR and increased O-GlcNAc modification in proteins observed in *Oga* KO and heterozygous cell lines compared to wild type reflecting *Oga* deletion (Fig. 1).

2.2. RNA extraction and microarray

Female MEF cells from wild type (WT), *Oga* heterozygous (Het) and *Oga* knock out (KO) genotypes, collected from a single litter were used for the microarray study. RNA extracted using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Total RNA digested with RNAse free DNAse I, followed by column purification (Qiagen Inc., CA). RNA quantified by NanoDrop and quality assessed by Bioanalyzer trace (Agilent Technologies, CA) and samples with RNA integrity number (RIN #) above 7.9 the were selected for the assay. The total RNA from each sample (5 μg) was used for cDNA synthesis and biotin labeling, in three technical replicates for each group. Samples were processed using Affymetrix kits according to the manufacturer's instructions at the NIDDK Genomic Core facility. Samples were hybridized

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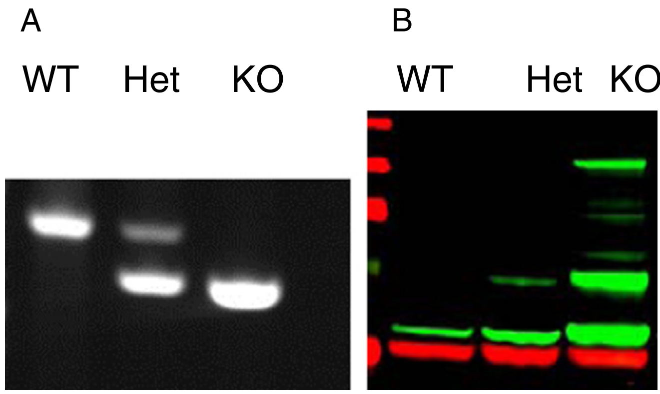

Fig. 1. Genotypes of the cell lines showing PCR bands for WT, Het and KO alleles (A) and a Western immunoblot showing O-GlcNAcylated protein expression (B), probed by anti O-GlcNAc antibody (RL2 — anti rabbit) followed by green fluorescence secondary antibody. Loading controls were shown by actin expression, detected by anti-actin followed by red fluorescence tagged secondary antibody (anti mouse).

to Affymetrix 430.2 arrays for 16 h at 45 °C rotating at 60 rpm. Array chips were washed, and stained using FS450 fluidic stations (protocol FS450-0004) using reagents from HWS kits (Affymetrix Inc.). The arrays were scanned using a 3000G Affymetrix scanner operated by GeneChip Operating Software (GCOS), which generated 9 data files. Data were normalized using the MAS5 algorithm.

2.3. Data analysis

Image files (.cel) were further analyzed by Partek software (Partek Inc., St. Louis, MO) to identify differentially expressed genes. Quality control features indicated all arrays were good and signal intensity box plots are shown in Fig. 2A. Principle component analysis (PCA) shows 66% of data could be described by genotype differences and well separated into 3 groups (Fig. 2B). The volcano plot from KO against WT comparison shows a large number of genes significantly affected even at 2 fold level (Fig. 2C). Data statistically analyzed using Partek ANOVA and *Oga* Het and KO groups were compared with WT and also with each other, and considered significant at $p < 0.05$ level. Gene lists were generated for 1.5 fold or 2 fold differentially expressed transcripts with multiple test correction for false discovery ratio (FDR) set at 0.05 level. Five transcripts interrogated *Oga* (*Mgea5*) gene were significantly reduced in KO group compared to WT and intermediate levels were seen in Het group. A total of 2534 transcripts from the KO group showed 1.5 fold change compared to WT with 1404 reduced transcripts and 1130 increased. Table in Fig. 3A shows the number of transcripts passed at 1.5 fold or (2 fold) level and unique or unknown transcripts for each comparison. The distribution of 1.5 fold significant transcripts in each comparison category is shown in a Venn diagram (Fig. 3B). These results indicate that deletion of both *Oga* alleles changed the gene expression significantly while partial deletion (Het) had limited effects on gene expression. Similar effect was also reflected by the other biological parameters observed in live animals [1]. A heat map from hierarchical clustering of differentially expressed genes in KO and WT is shown in Fig. 4 (y axis sample type, x axis genes, blue reduced, and red increased gene expression). The cluster map shows single row for each sample and 3 replicates from each genotypes were very similar and tightly clustered (raw colors). Columns of gene clusters in the heterozygous group (middle) have shown higher similarity with WT while few gene clusters are similar to the KO group. Gene enrichment analysis was performed using a Gene Ontology (GO) database (MM 2012-11-19 release) to understand the biological effects. The group of 2534 transcripts (KO vs WT) showed the highest enrichment in genes associated with metabolic, and proliferation processes (Fig. 4B). Het group compared to WT (959)

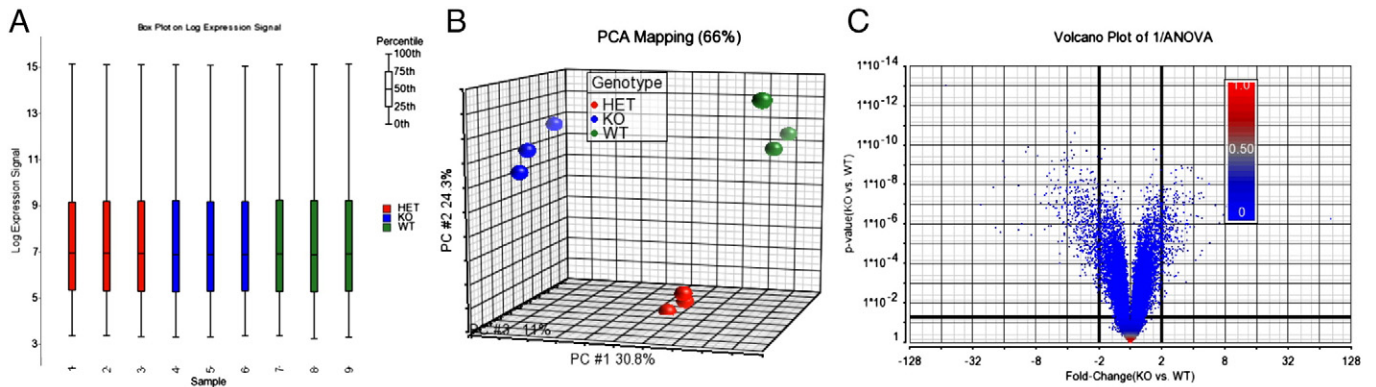

Fig. 2. Quality assessment of microarray data. (A) Boxplot, (B) PCA plot and (C) volcano plot showing significantly affected genes (solid lines at $p < 0.05$ and 2 fold level).

A

comparison	Total genes 1.5 FC (2FC)	Unique transcripts	unknown
KO Vs WT	2534(839)	1790 (619)	147 (36)
HET Vs WT	959(111)	686 (82)	183 (22)
HET Vs KO	1835(474)	1344(364)	83(13)

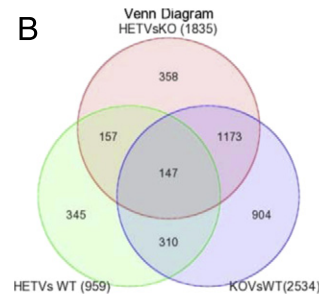

Fig. 3. (A) Total, unique and unknown transcripts associated with each comparison. (B) Venn diagram showing distribution of differentially expressed transcripts in all 3 comparisons at 1.5 fold level.

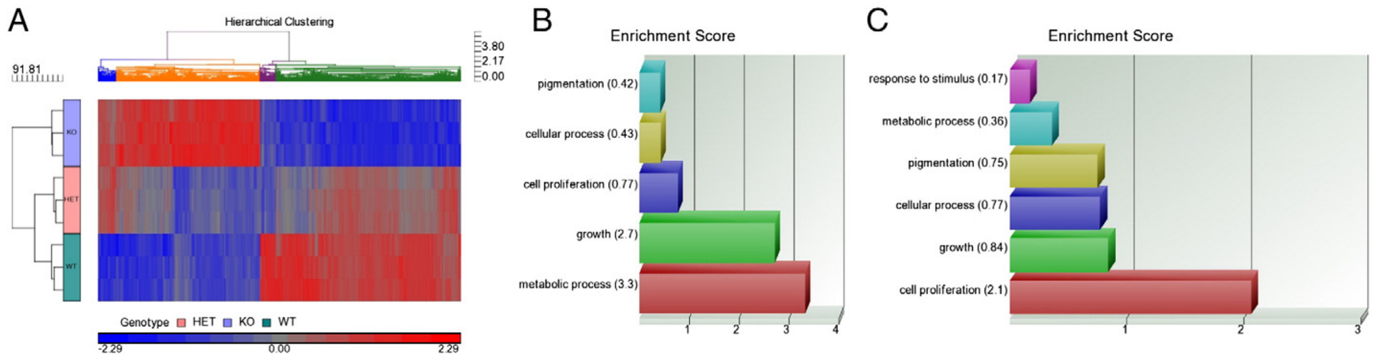

Fig. 4. (A) Hierarchical clustering of KO vs WT transcripts change at 1.5 fold ($p > 0.05$) showing 4 clusters (x axis) and GO enrichment categories in differentially expressed genes (1.5 fold) in (B) KO against WT and (C) Het against WT comparisons.

Table 1
Top 10 highly enriched pathways in 2 fold differentially expressed genes in KO vs WT group as analyzed by Genomatix software.

Pathway	*p* value	# of genes (observed)	# of genes (expected)	# of genes (total)
Spleen tyrosine kinase	4.88E−07	20	5.62506	102
Integrin	1.95E−06	26	9.48540	172
Toll like receptor	6.11E−06	23	8.27215	150
Interleukin 2	7.86E−05	19	7.16920	130
Guanine nucleotide exchange factor VAV	2.66E−04	15	5.40447	98
Angiotensin	2.98E−04	15	5.45962	99
Granulocyte colony stimulating factor receptor	4.20E−04	11	3.36401	61
Nuclear factor of activated t cells, cytoplasmic, calcineurin dependent	5.62E−04	16	6.39713	116
Chemokine (C–C motif) ligand 2	6.38E−04	12	4.08093	74

shows enrichment in proliferation and growth associated genes (Fig. 4C). The same data set is analyzed with Genomatix (Germany) software using gene ranker analysis also showing similar categories while highest probability in enrichment is associated with innate immunity genes (data not shown). Pathway analysis conducted using 839 (2 fold change) transcripts from KO versus WT comparison using GePS (Genomatix) and the highest 10 pathways listed are shown in Table 1. The highest number of genes are associated with the Integrin pathway and shown in Fig. 5.

3. Discussion

O-GlcNAc modification effects global cellular functions and details are emerging. The lack of a mammalian model system limits the understanding of O-GlcNAc signaling. Mouse *Ogt* deletion is embryonic lethal [3]. Deletion of the *Oga* enzyme which removes O-GlcNAc modification has also shown about 89% neonatal lethality in mice [1,2]. MEFs isolated from *Oga* KO, Het and WT embryos were used for the microarray to facilitate the understanding of global effects. *Oga* deletion in MEF cells is confirmed by gDNA PCR and as a result increased O-GlcNAc modification were observed in Het and KO cell types compared to WT. The present data shows O-GlcNAc modification affects not only cellular signaling but also gene expression. *Ogt and Oga* are known to associate with the transcriptome machinery [4,5]. Differentially expressed genes under *Oga* deletion indicate the effects in multiple cellular functions such as metabolism, cell proliferation, and growth. Perturbation of O-GlcNAc cycling affects metabolic pathways crucial for survival and corroborate with the observed neonatal lethal phenotype of *Oga* KO pups. Defects of cell proliferation and cytokinesis are also shown through other scientific evidence in *Oga* disrupted cells [2].

Tyrosine kinase pathways are involved in downstream signaling associated with immunoreceptors in B cell receptors. Many cytokine

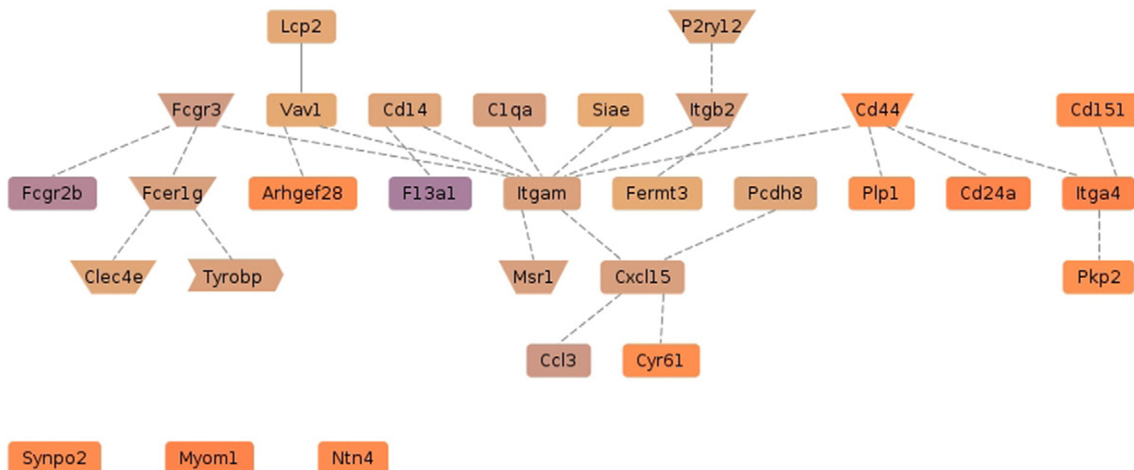

Fig. 5. Genes associated with Integrin pathway shown by genomatix pathway analysis.

and immunity associated genes were largely down regulated in KO MEFs compared to WT. Integrin pathway associated with extracellular matrix signaling and are significantly affected by *Oga* deletion. Observed gene regulation in *Oga* KO group could be explained by lack of *Oga* activity directly, or due to increased O-GlcNAc modification of proteins. Dysregulation of the O-GlcNAc cycling could also have effects on protein phosphorylation (Slowson and Hart [6]), ubiquitin pathway [7], transcription factor binding, or localization. The present microarray data provide insight into global effects on multiple cellular functions associated with disruption of O-GlcNAc modification.

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