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Data in Brief

Transcriptomic analyses of Onecut1 and Onecut2 deficient retinas

Jillian J. Goetz, Jeffrey M. Trimarchi *

Department of Genetics, Development and Cell Biology, 2114 Molecular Biology, Iowa State University, Ames, IA 50011, USA

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ABSTRACT

In this article, we further explore the data generated for the research article "Onecut1 and Onecut2 play critical roles in the development of the mouse retina". To better understand the functionality of the Onecut family of transcription factors in retinogenesis, we investigated the retinal transcriptomes of developing and mature mice to identify genes with differential expression. This data article reports the full transcriptomes resulting from these experiments and provides tables detailing the differentially expressed genes between wildtype and Onecut1 or 2 deficient retinas. The raw array data of our transcriptomes as generated using Affymetrix microarrays are available on the NCBI Gene Expression Omnibus (GEO) browser (Reference number GSE57917 and GSE57918).

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Specifications	
Organism/cell line/tissue	Mouse
Sex	N/A
Sequencer or array type	Affymetrix GeneChip Mouse Genome 430 2.0
Data format	Raw and analyzed
Experimental factors	Onecut1 WT and KO, Onecut 2 WT and KO
Experimental features	Transcriptomes from isolated retinas of Onecut1 or Onecut2 KO mice and age-matched littermates were compared to determine the effects of Onecut transcription factor deficiency during retinal development.
Consent	Level of consent allowed for reuse if applicable
Sample source location	Ames, Iowa, USA

Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57917.
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57918.

Experimental design, materials and methods, data

RNA isolation

To determine the retinal transcriptomic changes that result from Onecut1 and Onecut2 deficiencies, we isolated retinas from embryonic day (E)14.5 Onecut1-KO retinas and their WT littermates or adult Onecut2-KO and WT littermates [1]. TRI-reagent (Sigma) was employed to isolate RNA according to manufacturer's instructions. Briefly, the isolated retinas were deposited into 1 ml of TRI-reagent. At this point, we routinely freeze our samples in Tri-reagent at −80 °C and find that better RNA yields are achieved if you do freeze the samples. The samples are then thawed and pipetted repeatedly to homogenize. After letting the samples incubate at room temperature for 5 min, 100 ul of 1-bromo-3-chloropropane was added and the sample was vigorously shaken for 15 min. on a vortex. After standing for 10 min at room temperature, the samples were centrifuged for 15 min at 12,000 ×*g* at 4 °C. The aqueous phase was isolated and 500 ul of isopropanol was added and mixed by inversion (4–6 times). The samples were allowed to stand at room temperature for 10 min and then were centrifuged again for 15 min at 12,000 ×*g* at 4 °C. The samples were washed with 500 ul 75% ethanol, centrifuged further for 5 min at 12,000 ×*g* at 4 °C, and air-dried for 10 min. The RNA samples were then resuspended in 25 ul of nuclease-free water and stored at −80 °C. Concentrations were determined using a Nanodrop.

Microarray preparation

For reverse transcription, 400 ng of total RNA was added to the First Strand Enzyme Master Mix in the MessageAmp III RNA Amplification Kit (Ambion). This 10 ul reaction was mixed and then incubated at 42 °C for 2 h in a thermocycler. After incubation, 20 ul of Second Strand Master Mix was added to each sample and the samples were mixed. Second strand synthesis was performed for 1 h at 16 °C in a thermocycler. The samples were then heated to 65 °C. At this point,

* Corresponding author.

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the samples could be stored at −20 °C before the *in vitro* transcription reaction. A T7-based *in vitro* transcription reaction was performed by adding 30 ul of the T7 IVT Master Mix to the second strand synthesis samples and incubating the tubes in a thermocycler at 40 °C for 8 h. The samples were then stored at −20 °C overnight. Amplified RNA (aRNA) purification was then performed by first adding 40 ul of nuclease-free water to bring each sample to a total volume of 100 ul before adding 350 ul of aRNA binding buffer. This was immediately followed by the addition of 250 ul of ACS grade 100% ethanol to each sample. Samples were gently triturated three times to mix thoroughly before being applied to the center of the filter of an aRNA filter cartridge placed in an aRNA collection tube. After the flow-through was discarded, the aRNA filter cartridge was washed with 650 ul of wash buffer and spun at 10,000 ×*g*. The aRNA was then eluted into a fresh microcentrifuge tube using 100 ul of nuclease-free dH_2O that was preheated to 60 °C for 15 min. 10 ug of this aRNA sample was fragmented in a 40 ul reaction by heating to 94 °C for 35 min and then hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 arrays at Iowa State University's GeneChip Facility. Standard Affymetrix hybridization protocols were utilized.

R workflow for microarray normalization

Analysis of microarray data was performed using the Bioconductor Affy package for R [2]. Data was background adjusted and normalized using Mas5 and log(2) transformed.

```
> library("affy")
> raw<-ReadAffy()
> eset.mas5<-mas5(raw)
> exprSet.nologs<-exprs(eset.mas5)
> exprSet<-log(exprSet.nologs,2)
> write.table(exprSet, file="output.txt", quote=F, sep="\t")
```

Analyses of differential expression were limited to genes whose mean expression level among either n = 3 WT or KO retinas exceeded a log-transformed value of 7. Two-tailed t-tests resulting in p-values of less than 0.05 indicated significant differential expression.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.03.010.

References

[1] J.J. Goetz, et al., Onecut1 and Onecut2 play critical roles in the development of the mouse retina. PLoS ONE 9 (10) (2014) e110194.
[2] L. Gautier, et al., affy–analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20 (3) (2004) 307–315.