A multi-omics study of the murine retinal and neural response to chronic low-dose irradiation

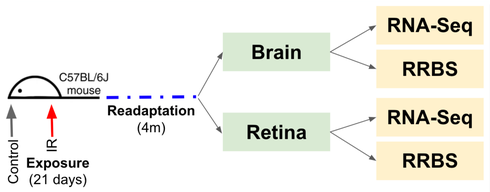
Prachi Kothiyal[1*], Greg Eley[1], Hari Ilangovan[2], Katherine A. Hoadley[3], S. Robin Elgart[4], Xiao W. Mao[5], Parastou Eslami[6]

[1]Scimentis LLC, [2]SAIC, [3]University of North Carolina at Chapel Hill, [4]University of Houston, [5]Loma Linda University, [6]Universal Artificial Intelligence

BACKGROUND

Spaceflight has been reported to have detrimental impact on the structure and function of brain and retina.

Objective: Characterize expression and methylation in the murine retina and brain with or without exposure to chronic low dose/low dose rate gamma radiation (0.04 Gy at 0.01 cGy/hr).



Experimental Data

- RRBS and RNA-Seq data obtained from GeneLab datasets GLDS-202/203
- N=9 (4 controls, 5 exposed to low-dose gamma radiation)
- RRBS reads processed with nf-core (methylSeq) and MethylKit
- RNA-Seq counts normalized with DESeq2.

OVERALL METHYLATION PATTERNS

- Brain and retina showed similar patterns for overall methylation in different gene regions (**Figure 1**).
- The patterns were consistent with those observed in other tissue types and species (Anastasiadi et al).
- Majority of promoter sites in CpG islands were unmethylated; the distribution became bimodal when CpG shores were included but was still skewed towards the unmethylated sites.
- CpG sites within exons and introns were skewed towards being methylated with a slight change in the distribution with irradiation.

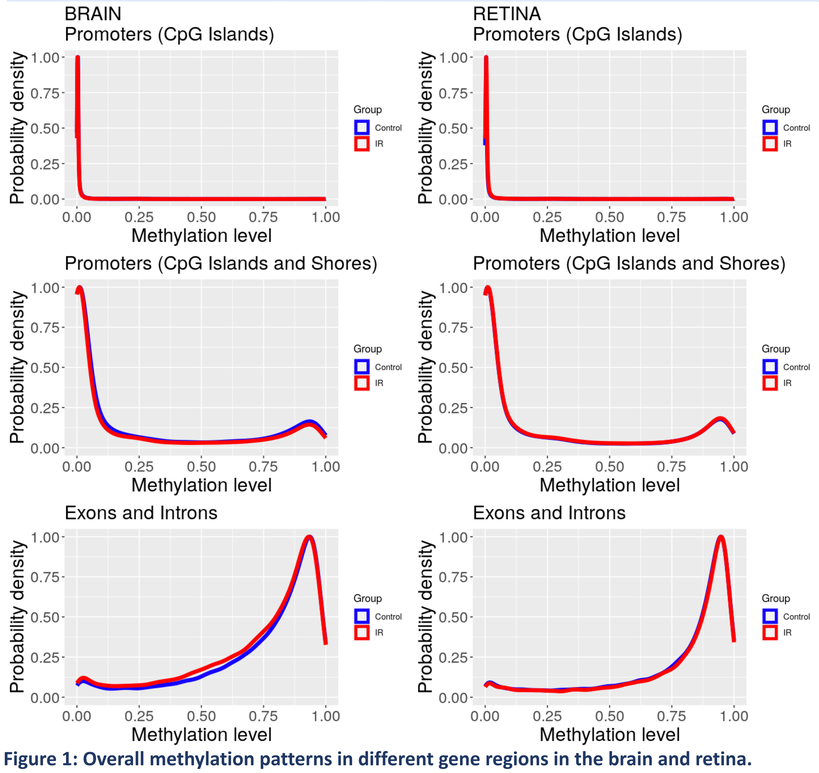


Figure 1: Overall methylation patterns in different gene regions in the brain and retina.

References

Anastasiadi D, et al. Consistent inverse correlation between DNA methylation of the first intron and gene expression across tissues and species. *Epigenetics Chromatin*. 2018

Overbey EG et al. Spaceflight influences gene expression, photoreceptor integrity, and oxidative stress-related damage in the murine retina. *Sci Rep*. 2019.

Rivera AL et al. MGMT promoter methylation is predictive of response to radiotherapy and prognostic in the absence of adjuvant alkylating chemotherapy for glioblastoma. *Neuro Oncol*. 2010

CORRELATED GENES WITHIN ASSAYS/TISSUE TYPES

- Gene-wise correlation was calculated in IR and controls for expression-methylation within a tissue, and brain-retina within an assay
- For each comparison group, more correlated genes were observed with the addition of radiation (**Figure 2**).

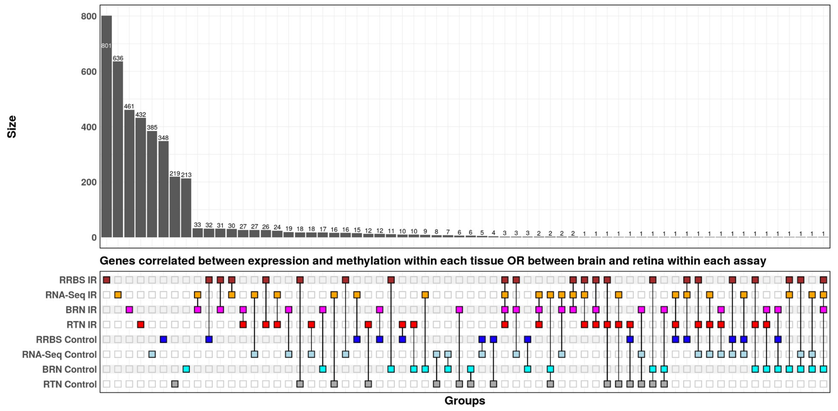


Figure 2: Upset plot with correlated genes in each group and their intersection across groups.

- 35 genes showed correlation in brain vs. retina expression and methylation only with IR:

Agbl2, Ahi1, Atp6v0d1, Begain, Bex2, Drd4, Ebf4, Elfn1, Eps8l1, Fam89b, Fbxl7, Gm28778, Grm8, Il4ra, Kalrn, Katnip, Kpna6, Ldlrad3, Mmel1, Mmp15, Nog, Nptx1, Pdgfra, Pigl, Pkdcc, Rasgrp4, Rmc1, Rpl8, Slc18b1, Smagp, Syt14, Taok1, Trmt2a, Vipr1, Ybx1

- 33 genes showed correlation in expression vs. methylation within brain and retina only with IR:

Arrdc3, Atad2b, Avpi1, Eln, Fbxo21, Fgf11, Fktn, G3bp2, Gabrg3, Il20ra, Lrrc28, Mfsd9, Mgmt, Mrc2, Napa, Ncs1, Nsmce1, Ppm1e, Rmc1, Rpl8, Sgsh, Stim1, Sufu, Tmem39b, Tufm, Uba3, Ulk3, Wrap53, Yipf5, Zfp703, Zfp787

- *Mgmt is* a methyltransferase involved in DNA repair with its promoter methylation predictive of response to radiotherapy (Rivera et al)
- 5 genes were correlated within both tissue types as well as at least one assay (*Eln, Fktn, Rmc1, Rpl8, Uba3)*

RADIATION-SPECIFIC PROCESSES AND GENES

- Top over-represented processes for genes correlated exclusively in IR groups included *synapse organization* and *Wnt signaling* (**Figure 3**).
- Genes involved in majority of these processes included *Fmr1* (implicated in synaptic plasticity), *Drd4* (up-regulated in the murine retina due to spaceflight; Overbey et al), *Kalrn* (linked to axonal development), *Syt4* (involved in neuronal dense dense core vesicles mobility, and *Nptn* (a neuroplastin acting at synapses).

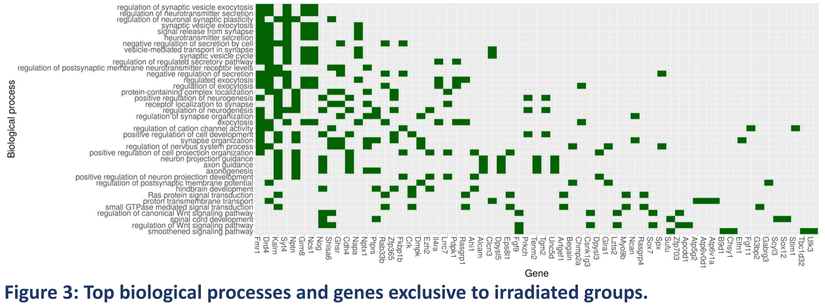


Figure 3: Top biological processes and genes exclusive to irradiated groups.

TISSUE-SPECIFIC GENES AND PROCESSES WITH IR

- Over-representation analysis was run for genes with correlated expression-methylation exclusively in one tissue type (462 and 433 genes exclusive to irradiated brain and retina, respectively).
- Retina showed more enriched processes than the brain (642 vs. 173 at a discovery FDR cutoff of 0.25; **Figure 4**).
- Tissue-specific processes included *cerebellar cortex development, meiotic processes*, and *nuclear division* in the brain and *ERK cascade, eye development,* and *ncRNA processing* in the retina.
- *Response to radiation* was enriched in the retina driven by 17 genes:

App, Babam1, Braf, Cdkn1a, Clk2, Crb1, Cry2, Eif2ak4, Fbxl17, Grin1, Jun, Mtor, Net1, Pde8b, Ppp1cc, Sdf4, Tlk2

- 29 processes were enriched in both tissue types but with distinct tissue-specific gene signatures and included *response to hypoxia*, *protein phosphorylation* and *hindbrain development*.

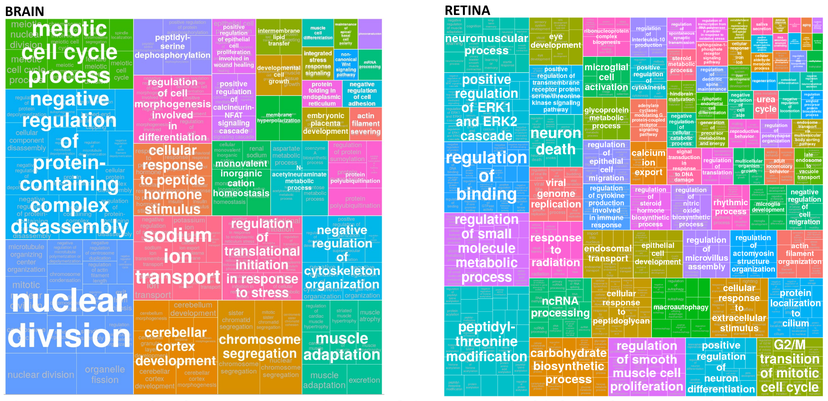


Figure 4: Non-redundant representative set of tissue-specific biological processes based on correlation between expression and methylation in each tissue type.

ENRICHED PROCESSES BASED ON GENE CORRELATION

Exposure	Correlation group	Total no. of processes (no. of processes driven by +ve or –ve gene correlation; top processes)
IR	Methylation Brain vs. Retina	626 (623 +ve, 23 –ve; *Wnt signaling, fat cell differentiation, organ morphogenesis*)
IR	Brain Methylation vs. expression	1 (1 –ve; hippo signaling)
Control	Expression Brain vs. Retina	24 (18 +ve, 6 -ve; *ECM organization, oxidative phosphorylation*)
Control	Retina Methylation vs. Expression	3 (1 +ve, 2 –ve; *monocyte differentiation, hypotonic response, Sertoli cell development*)

Table 1: Number of biological processes enriched in each group. Groups with no enriched processes are excluded from the table.

SIGNIFICANT FINDINGS AND FUTURE WORK

- Radiation causes correlated methylation patterns in brain and retina where the correlated genes are enriched in processes including *Wnt signaling* and *cell differentiation* (**Table 1, Figure 2**).
- 17 genes known to be involved in radiation response show correlation between expression and methylation exclusively in irradiated retina.
- Tissue-specific processes need to be interrogated for differences in response to radiation in brain vs. retina using a larger sample size.