Understanding the molecular mechanisms of germline-dependent epigenetic inheritance: Computational analysis of multi-omics data

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# List of Abbreviations

# Abstract

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# 1 Dynamic chromatin accessibility in spermatogonial cells for transcriptional programmings from early postnatal to adult stages

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## 1.2 Abstract

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### 1.4.4 Differentially accessible chromatin regions associate with distinct epigenetic profiles

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# 2 Early life stress affects the miRNA cargo of epididymal extracellular vesicles in mouse

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## 2.1 Abstract

Sperm RNA can be modified by environmental factors and has been implicated in communicating signals about changes in a father’s environment to the offspring. The small RNA composition of sperm could be changed during its final stage of maturation in the epididymis by extracellular vesicles (EVs) released by epididymal cells. We studied the effect of exposure to stress in early postnatal life on the transcriptome of epididymal EVs using a mouse model of transgenerational transmission. We found that the small RNA signature of epididymal EVs, particularly miRNAs, is altered in adult males exposed to postnatal stress. In some cases, these miRNA changes correlate with differences in the expression of their target genes in sperm and zygotes generated from that sperm. These results suggest that stressful experiences in early life can have persistent biological effects on the male reproductive tract that may in part be responsible for the transmission of the effects of exposure to the offspring.

## 2.2 Summary sentence

miRNA cargo of extracellular vesicles in cauda epididymis is altered by paternal exposure to early life stress. This correlates with changes in the expression of target genes in sperm and in zygotes generated from that sperm.

## 2.3 Graphical Abstract

## 2.4 Key words

epigenetics, epididymis, epididymosomes, early life stress, extracellular vesicles, miRNAs, sperm.

## 2.5 Introduction

## 2.6 Results

### 2.6.1 Isolation of cauda epididymosomes confirmed by several methods

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### 2.8.11 Cholesterol measurements

### 2.8.12 Bioinformatics data analysis

## 2.9 Data availability

The datasets collected for this study are available as follows: - sRNA-seq dataset of cauda epididymosomes before and after sizeselection: NCBI GEO under accession number [GSE175976](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE175976). - Codes for bioinformatics analysis of RNA-sequencing datasets and all corresponding differential expression analyses: Github repository [https://github.com/mansuylab/alshanbayeva\_et\_al\_2021[https://github.com/mansuylab/alshanbayeva\_et\_al\_2021](https://github.com/mansuylab/alshanbayeva_et_al_2021%5Bhttps://github.com/mansuylab/alshanbayeva_et_al_2021)]. - Sperm and zygote sequencing datasets from previous publications can be found in ArrayExpress database at EMBL-EBI (<www.ebi.ac.uk/arrayexpress>) with the accession number [E-MTAB-5834](https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-5834/) (sperm) and [E-MTAB-6589](https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-6589/) (zygotes).

## 2.10 Authors’ contributions

AA and IMM conceived and designed the study. FM and MR performed the MSUS breeding and collected tissue samples. AA and DKT performed data analysis and generated figures. AA wrote the manuscript with input from DKT and IMM. AA performed all experiments for RNA sequencing and all molecular analyses. IMM supervised the project and raised funds.

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**Conflict of interest:** The authors declare no conflict of interest.

## 2.13 Supplementary Figures

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# Discussion

# Conclusion

# Appendix A

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# Appendix B

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# Appendix C

# References

Angel, E. (2000). *Interactive computer graphics : A top-down approach with OpenGL*. Boston, MA: Addison Wesley Longman.

Angel, E. (2001a). *Batch-file computer graphics : A bottom-up approach with QuickTime*. Boston, MA: Wesley Addison Longman.

Angel, E. (2001b). *Test second book by angel*. Boston, MA: Wesley Addison Longman.