# Bioinformatic discovery of miRNA target genes that drive inflammation in the leukemic bone marrow

*Linsey Zhang[1,3*], Theresa Menna[1*], Zhe Zhang[2], Ding-Wen Chen[1], Peter Kurre[1,4]*

[1]Comprehensive Bone Marrow Failure Center, Children's Hospital of Philadelphia, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [2]Department of Biomedical and Health Informatics, Children's Hospital of Philadelphia, Philadelphia, PA, USA; [3]Department of Biology, Swarthmore College, Swarthmore, PA, USA; [4]Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA

## Introduction & Significance

- Acute myeloid leukemia (AML) induces inflammation and suppresses hematopoietic stem/progenitor cells (HSPC) in the bone marrow (BM)
- miR155 is a proinflammatory miRNA enriched in AML-derived extracellular vesicles (EV). The role of miR-155 in inflammation in this context is unknown.

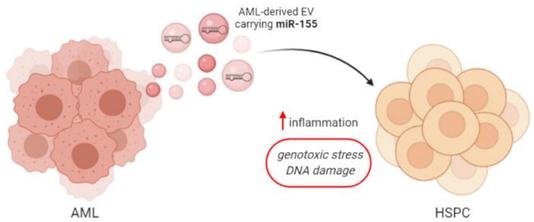


**Figure 1**. Schematic showing AML-derived EV enriched for miR-155 being delivered to BM HSPC. While miR-155 is known to be a proinflammatory microRNA, it's role in AML-induced inflammation and genotoxicity in HSPC is unknown.

- Here, we identify miR-155 targets expressed in HSPC and investigate the miR-155 targetome associated with inflammation and genotoxic stress
- Through this, we curated an HSPC gene expression profile collection on Gene Expression Explorer (GeEx)

## Methods

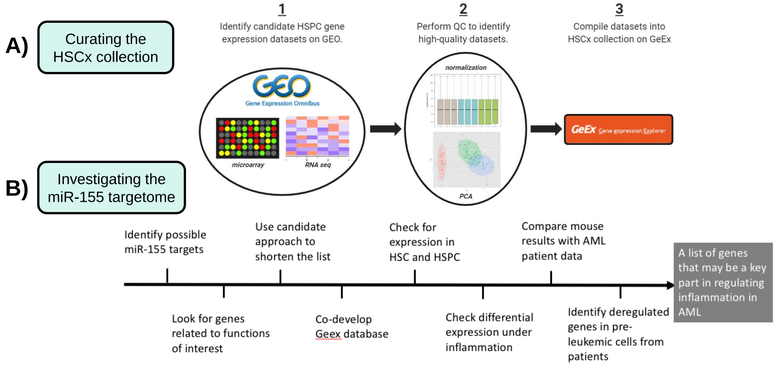


**Figure 2**. Schematic showing step-by-step bioinformatic approaches to curating the HSCx collection on GeEx, a gene expression explorer platform (A) and to developing a putative inflammation/genotoxic stress associated miR-155 targetome in HSPC (B). We identified homeostatic and inflammation-related murine and human HSPC datasets on the GEO database, reprocessed using uniform QC measures, and compiled online for efficient exploration and analysis. To identify potential miR-155 targets, we used miRDB, a prediction miRNA RISC targetome algorithm, to generate a list of genes (threshold > 60), selecting genes normally expressed in HSPC related to inflammation, genotoxic stress, and cell cycle regulation. Candidate genes were further narrowed by cross-referencing with related studies and publications.

## GeEx: The HSCx Collection

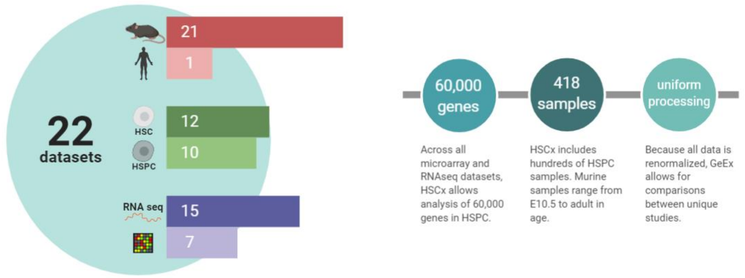


**Figure 3**. Infographic describing the contents of the HSCx collection, hosted on the Awsomics - GeEx platform. Included collections were downloaded from the GEO database and underwent renormalization and QC screening prior to being uploaded to GeEx. HSCx includes 22 datasets in total (21 murine, 1 human patient; 12 containing pure hematopoietic stem cell (HSC) samples, 10 containing HSPC; 15 RNA seq and 7 microarray), 60,000 genes, and 418 samples.

## Identifying & Validating miR-155 Targets

- We identified 8 putative miR-155 targets: *Wee1, E2f2, Csf1r, Rps6ka3, Rps6ka5, Ercc1, Rad51, Rad51b*
- HSCx shows all targets are expressed in HSC/HSPC under steady-state conditions
- In four inflammation-related datasets in HSCx, 5 out of 8 targets were dysregulated, with largest differences in HSC versus HSPC datasets.
- *Csfr1* consistently shows greatest dysregulation; it has also been recently identified as a potential therapeutic target for AML because of its antitumor ability

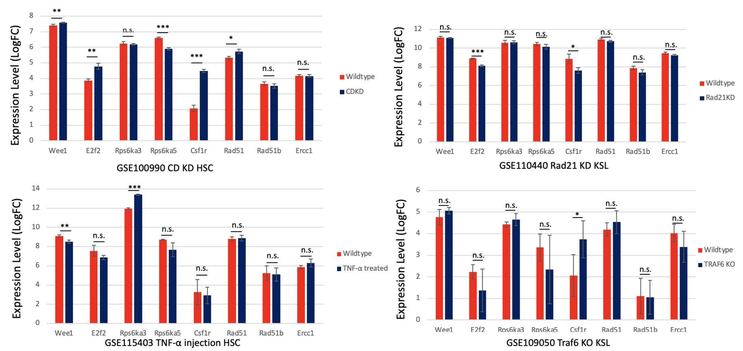


**Figure 4.** Expression of eight putative miR-155 targets in HSC or HSPC under different inflammatory conditions (RNA seq datasets). Log-fold change was compared between wildtype and inflammatory conditions using "differential expression" tools on GeEx. Statistical significance calculated by Student's t-test; * p<0.05; ** p<0.01, *** p<0.001.

## miR-155 Target Set Enrichment Analysis

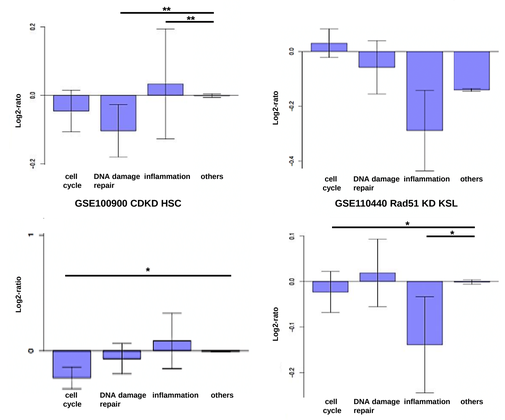


**Figure 5.** We generated miR-155 target gene sets for GO functions cell cycle, DNA damage, and inflammation that contain 115, 32, and 40 genes, respectively. Target set gene expression was compared to expression of all other genes in four inflammatory datasets. Statistical significance calculated by Student's t-test; * p<0.05; ** p<0.01, *** p<0.001.

- We expanded our miR-155 target list to include any genes found in at least 3 out of 4 algorithmic databases (TargetScan, miRDB, miRWalk, and miRanda) and built three targets sets based on gene ontology function.
- Some sets show upregulation patterns, suggesting possible non-RISC activity or peripheral impact of modifiers of miR-155 action under inflammatory conditions
- Dysregulation of miR-155 targets associated with cell cycle and DNA damage is not limited to the eight targets highlighted in Figure 4.

## Summary

- The HSCx collection on the GeEx platform is a valuable bioinformatic tool now available to the hematological malignancy research community.
- Overall, the results show that miR-155 targets expressed in HSC/HSPC are dysregulated under inflammatory conditions, suggesting miR-155 may play a key role in AML-derived inflammation and genotoxicity in leukemic BM.
- Ongoing *in vitro* and *in vivo* studies will selectively validate *in silico* dysregulated targets.

## Acknowledgements

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