ERG: from a fortuitous discovery to potential treatment for AML

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INTRODUCTION

- The ETS transcription factor ERG is essential for the maintenance of hematopoietic stem cells.
- ERG has also been implicated as an oncogene in the development of acute leukemia.
- Our studies and those of others have demonstrated that ERG directly contributes to the initiation and maintenance of lymphoid and myeloid acute leukemia subtypes.
- ERG co-factors critically involved in leukemogenesis remain largely uncharacterized.

OBJECTIVE

In this study, we report a critical role for the conserved amino acid proline at position 199 at the 3' end of the PNT domain of ERG in its leukemogenic activity.

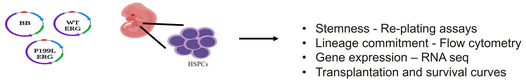
METHODS

- Functional, genetic, and biochemical studies were conducted to investigate the role of P199 in the leukemogenic pathway of ERG.
- A non-leukemogenic mutant form of human ERG in position P199 was used as a model (P199L-ERG).

1. Assessing P199L-ERG stability

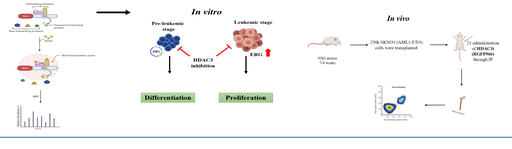


2. Characterizing P199L-ERG



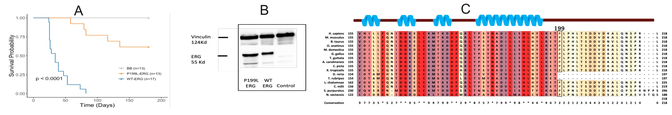
- Stemness - Re-plating assays
- Lineage commitment - Flow cytometry
- Gene expression – RNA seq
- Transplantation and survival curves

3. Proximity assay and targeting key interacting proteins



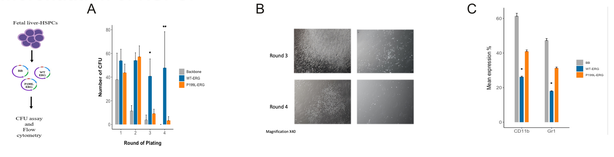
RESULTS

P199L severely disrupts ERG transforming capacity in-vivo.



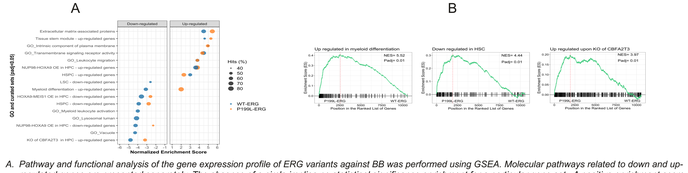
A. Survival analysis of C57B/6 mice transplanted with murine fetal liver derived HSPC following transduction with ERG variants (10^5 transduced cells/mouse). A log-rank test.
B. Immunoblot of HA tagged ERG to compare the levels of protein expression of WT and mutated ERG in the spleen of leukemic mice.
C. P199 is evolutionary conserved. Sequence alignment of ERG from different species in the region of the PNT domain.

P199L alleviates the effect of ERG forced expression on self-renewal and myeloid differentiation of HSPC.



A. Re-plating assay in semi-solid conditions of murine fetal liver derived HSPC transduced with ERG variants. The number of CFU per 10^4 cells plated is presented for each round of plating (n=4, Data are represented as mean ± SEM, Kruskal Wallis rank-sum test).
B. Microscopic view of colonies morphology between groups. MagnificationX40.
C. Summary bar graphs of flow cytometry analysis (n=3, Kruskal-Wallis rank sum test. Wilcoxon rank sum test was used for post-hoc analysis).

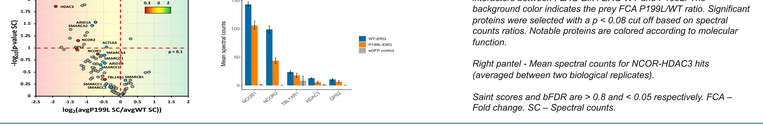
P199L impedes ERG-mediated transcriptional gene repression in HSPC.



A. Pathway and functional analysis of the gene expression profile of ERG variants against BB was performed using GSEA. Molecular pathways related to down and up-regulated genes are presented separately. The absence of a circle implies no statistical significance enrichment for a particular gene set. A positive enrichment score refers to up-regulated genes, and a negative score refers to down-regulated genes (ERG variants against the backbone). The percentage of hits ("tags" in leading-edge analysis) is displayed to indicate the percentage of genes contributing to the enrichment score for each set of genes.
B. GSEA of upregulated genes (P199L-ERG against WT-ERG).

HSPC – hematopoietic stem and progenitor cells, LSC – leukemic stem cells. NES – Normalized enrichment score. DEG – differentially expressed genes. BB – backbone.

P199L disrupts the interaction of ERG with chromatin modifiers.



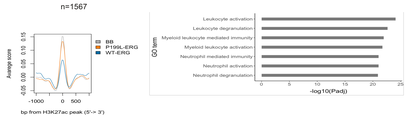
Left panel - Volcano plot comparing the spectral counts of identified interactors between FLAG-BirA-ERG WT and P199L. The background color indicates the prey FCA P199L/WT ratio. Significant proteins were selected with a p < 0.08 cut off based on spectral counts ratios. Notable proteins are colored according to molecular function.

Right pantel - Mean spectral counts for NCOR-HDAC3 hits (averaged between two biological replicates).

Saint scores and bFDR are > 0.8 and < 0.05 respectively. FCA – Fold change. SC – Spectral counts.

RESULTS

ERG affects chromatin modifications at myeloid related gene loci.



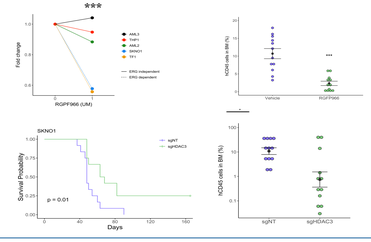
Left panel - Metagene plots showing H3K27ac peaks of down-regulated and hypoacetylated DEG following expression of WT-ERG force in Hoxb8 cells.

Right panel - GO analysis based on the nearest transcription start site to peaks identified in left panel. Adjusted p values are displayed.

DEG – differentially expressed genes.

ER-Hoxb8 cells (murine GMPs), *Wang GG. et al. Nat Methods. 2006.*

Pharmacologic and genetic HDAC3 inhibition results in reduced cell growth in high ERG expressing human AML cells *in vitro* and *in vivo*.



Left panel - HDAC3 inhibition using RGFP966 in low and high ERG expressing AML cell lines. Mean number of cells relative to RGFP966 concentration 0 after 48 hours in culture. T-tests, n=3 in each group.

Right panel - The effect of HDAC3 inhibition on SKNO1 cells in vivo. NSG mice were transplanted with 250K SKNO1 cells and treated with 15mg/kg RGFP966 once daily intraperitoneally for 21 days from day 2 after transplant. (n=12 in each group, t-test, mean and standard error). The bone marrow percentage of human CD45 cells was used to assess disease burden.

Left panel - Survival curve of NSG mice transplanted with SKNO1 cells after CRISPR-dCas9 targeting of HDAC3 (n=12 in each group, log-rank test, P=0.01).

Right panel - The percentage of bone marrow hCD45 cells as a measure of disease burden (Wilcoxon test, p=0.012).

CONCLUSIONS

- Our findings indicate that the aberrant overexpression of ERG maintains HSPCs in an undifferentiated state and promotes AML development
- The interaction between ERG and the NCoR-HDAC3 complex may play a significant role in the leukemogenic process
- HDAC3 inhibition could be beneficial in AML characterized by high ERG expression.

ACKNOWLEDGEMENTS

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