Tet2-deficient HSPC enhance glycolysis promoting their expansion

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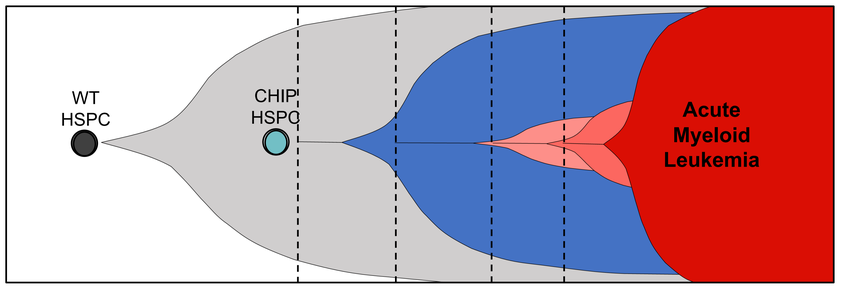
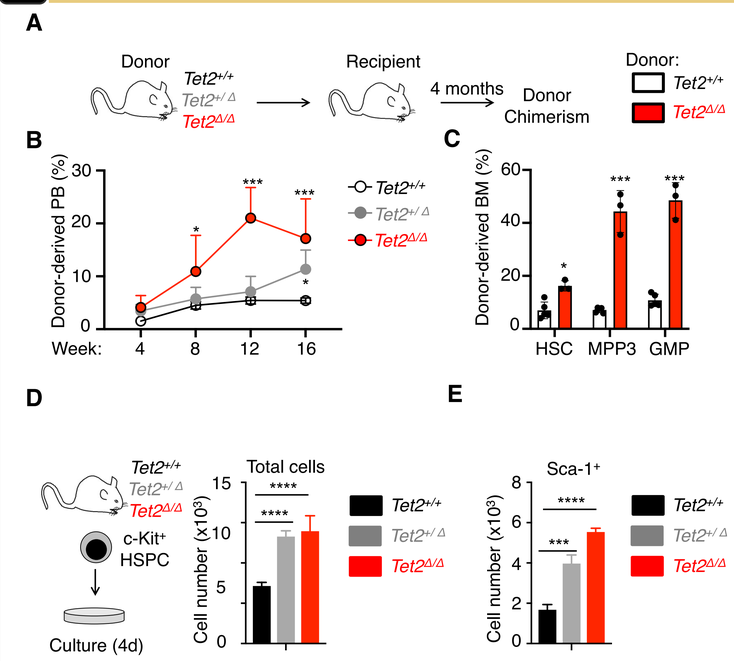
Introduction



- **Clonal Hematopoiesis is characterized by the expansion of distinct Hematopoietic Stem and Progenitor Cell (HSPC) clones that have mutations in frequently mutated genes such as: DNMT3A, TET2 and ASXL1.**
- **TET2 is the second most frequently mutated gene in CH and mutations to TET2 are frequently observed in hematological malignancies.**
- **TET2-driven CH is associated with hyperglycemia, atherosclerosis, and cardiovascular disease.**
- **TET2-deficient HSPC are conferred a survival advantage in the presence of inflammation.**
- ***We hypothesize inflammation promotes the expansion of TET2-deficient HSPC through undefined mechanisms.***
- ***We propose targeting these mechanisms may serve as a therapeutic strategy for stopping CH.***

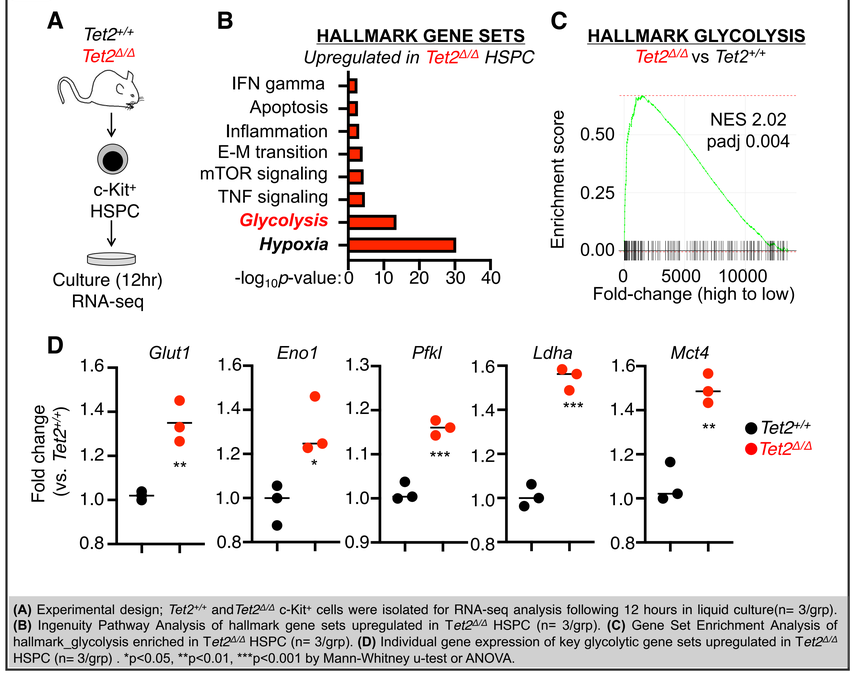
Results

1 Tet2-deficient HSPC preferentially expand in the blood and BM



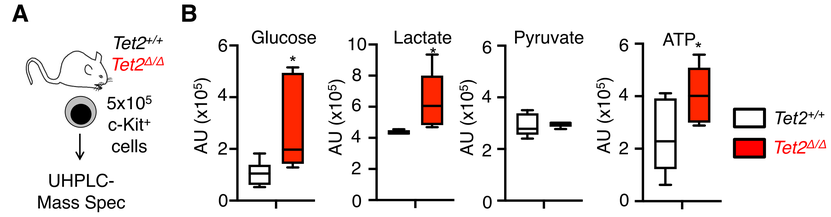
(A) Experimental design; 1.5x10^7 *Tet2*$^{+/+}$, *Tet2*$^{+/\Delta}$, and *Tet2*$^{\Delta/\Delta}$(CD45.2+) whole bone marrow cells were transplanted into *Tet2*$^{+/+}$ (CD45.1) non-conditioned mice. **(B)** Peripheral blood chimerism was checked once a month for 4 months (n= 3-5/grp). **(C)** Bone marrow chimerism was assessed after 4 months (n= 3-5/grp). 2x10^4 *Tet2*$^{+/+}$, *Tet2*$^{+/\Delta}$, and *Tet2*$^{\Delta/\Delta}$ c-Kit+ cells were cultured for 4 days and **(D)** total cell numbers or **(E)** Sca-1+ cells were analyzed (n= 3/grp). *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

2 Aberrant glycolysis gene expression in *Tet2*-deficient HSPC



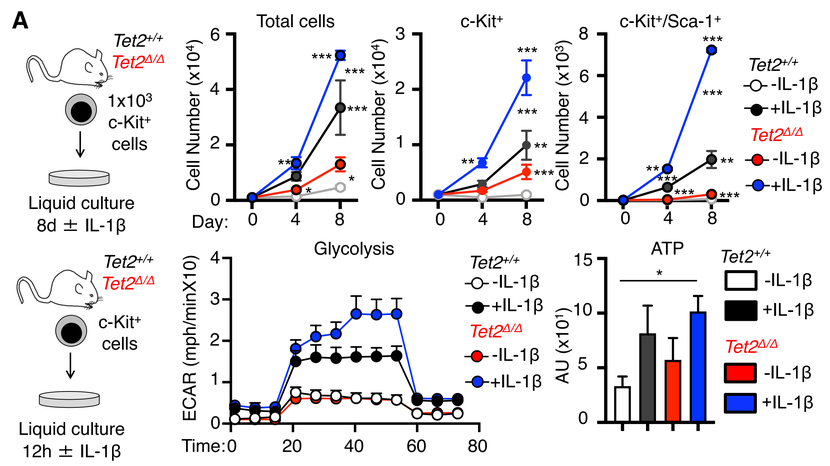
(A) Experimental design; *Tet2*$^{+/+}$ and *Tet2*$^{\Delta/\Delta}$ c-Kit+ cells were isolated for RNA-seq analysis following 12 hours in liquid culture(n= 3/grp). **(B)** Ingenuity Pathway Analysis of hallmark gene sets upregulated in *Tet2*$^{\Delta/\Delta}$ HSPC (n= 3/grp). **(C)** Gene Set Enrichment Analysis of hallmark_glycolysis enriched in *Tet2*$^{\Delta/\Delta}$ HSPC (n= 3/grp). **(D)** Individual gene expression of key glycolytic gene sets upregulated in *Tet2*$^{\Delta/\Delta}$ HSPC (n= 3/grp) . *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

3 Increased glycolytic metabolites and ATP in *Tet2*-deficient HSPC



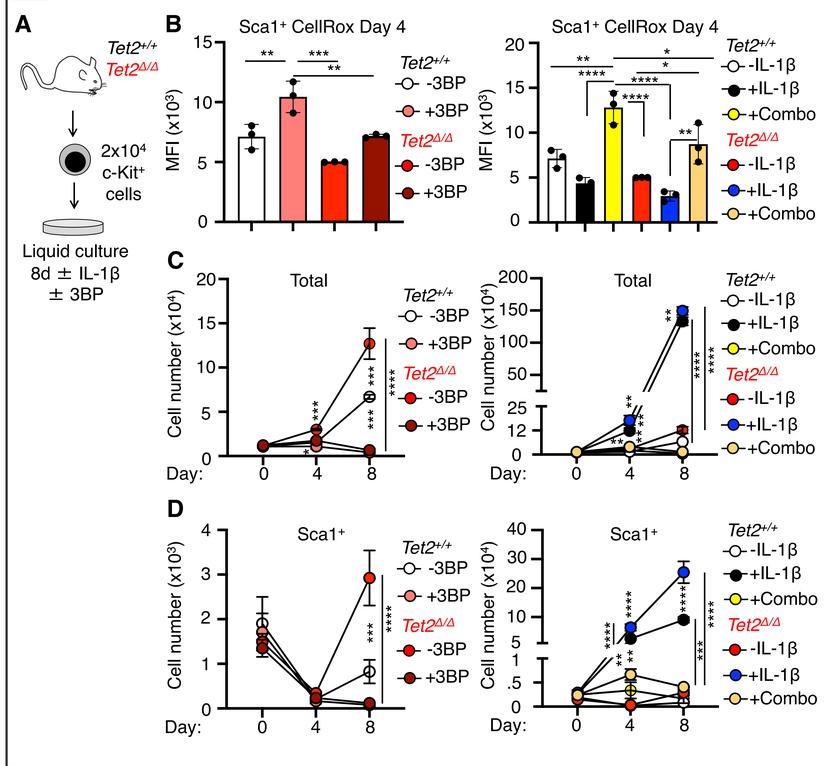
(E) Experimental design; 5x10^5 *Tet2*$^{+/+}$ and *Tet2*$^{\Delta/\Delta}$ c-Kit+ cells were isolated for metabolomics analysis using UHPLC-Mass Spec (n= 6/grp). **(F)** Glycolytic metabolites measured by UHPLC-Mass Spec (n= 6/grp). *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

4 IL-1 enhances *Tet2*-deficient HSPC expansion and glycolytic activity



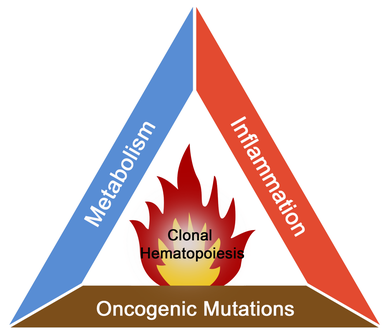
(A) Experimental design; 1x10^3 *Tet2*$^{+/+}$ and *Tet2*$^{\Delta/\Delta}$ c-Kit+ cells were isolated for 8 days of liquid culture and FACS analysis at days 0,4 and 8 (n= 3/grp). **(B)** Number of total cells or **(C)** c-Kit+ and c-Kit+/Sca-1+ HSPC at specified time points (n= 3/grp). **(D)** Experimental design; 2x10^5 *Tet2*$^{+/+}$ and *Tet2*$^{\Delta/\Delta}$ c-Kit+ cells were isolated for Seahorse analysis following 12 hours of liquid culture (n= 4/grp). **(E)** Glycolysis stress test and **(F)** UHPLC-Mass spec analysis of ATP in cells from (D) (n= 4/grp). *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

5 Glycolysis blockade abrogates *Tet2*-deficient HSPC expansion



(A) Experimental design; 2x10^4 *Tet2*$^{+/+}$ and *Tet2*$^{\Delta/\Delta}$ c-Kit+ cells were isolated for 8 days of liquid culture and FACS analysis at days 0,4 and 8 (n= 3/grp). **(B)** CellRox analysis of Sca-1+ HSPC at specified time points (n= 3/grp). **(C)** Number of total cells or **(D)** Sca-1+ cells comparing +/- 3BP treatment or +/- IL-1B by itself or in combination with 3BP. *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

Conclusions



- ***Human and mouse TET2-deficient HSPC expand in vivo and in vitro.***
- ***TET2-deficient HSPC upregulate glycolytic genes resulting in increased glycolytic metabolites.***
- ***IL-1 treatment accelerates TET2-deficient HSPC expansion and glycolytic response.***
- ***Inhibiting glycolysis in TET2-deficient HSPC minimized cell expansion and the effect was more pronounced in combination with the inflammatory cytokine IL-1.***
- ***Our data suggest a mechanism where glycolysis and inflammation accelerate the expansion of TET2-deficient HSPC.***
- ***We propose blockade of glycolysis and inflammation as a therapeutic strategy for targeting TET2-driven CH.***

Future Directions

- ***Determine the necessity of key glycolysis and hypoxia related genes SLC16A3, LDHA and HIF-1a in the expansion of TET2-deficient HSPC.***
- ***Determine the role of inflammation in the expansion of TET2-deficient HSPC.***
- ***Determine if these metabolic changes and/or inflammation can endow normal HSPC with a fitness advantage.***
- ***Determine a promising therapeutic target for TET2-driven CH.***

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