A Truncated Nucleus Isoform of FGFR1 Drives the Transformation of Leukemia Stem Cells in Leukemogenesis

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Background

Myeloid and lymphoid malignancies associated with chimeric FGFR1 kinases are the hallmark of stem cell leukemia and lymphoma syndrome (SCLL)[1]. In all cases, FGFR1 kinase is constitutively phosphoactivated in the cytoplasm as a result of chromosome translocations. Recently, we demonstrated that these chimeric kinases could be cleaved by granzyme B to generate a truncated derivative, tnFGFR1, which localized exclusively into the nucleus and was not phosphorylated [2].

Objective

In this study [3], we used the mouse bone marrow transduction and transplantation model to demonstrate the leukemic transformation ability of this tnFGFR1 and identified its critical downstream targets, FLT3 and KIT, in mouse and acute myeloid leukemia (AML) patient samples.

Materials & Methods

Stem cell transduction and transplantation in syngeneic mice was used to assess the transforming ability of tnFGFR1 in bone marrow stem cells, and RPPA and RNA-Seq were used to examine the related signaling pathways and regulated target genes.

Results

1. tnFGFR1 failed to transform BaF3 or primary BM cells in vitro.

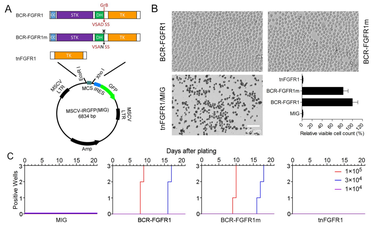


Fig 1. Assessment of the oncogenic transformation ability of tnFGFR1 in vitro. (A) Schematic of the retroviral constructs used in transformation assays. (B) Acute transformation potential of these three constructs, where the BCR-FGFR1 kinase and its mutant derivative (N = 3) can competently transform BaF3 cells to IL3 independence. (C) Plating assay using normal murine BM cells transduced with the different constructs, and tnFGFR1 failed to promote proliferation of BM cells.

2. tnFGFR1 transformed mouse HSC independently in vivo.

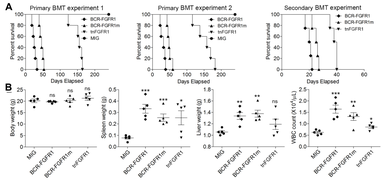


Fig 2. Assessment of the oncogenic transformation ability of tnFGFR1 in vivo. (A) Kaplan-Meier survival curves for cohorts of mice (N = 5) receiving transplants of BM stem cells transduced with virus carrying different constructs. (B) Scatter plots of indicated measurements for individual mice (N = 5) in the four subgroups. Data is presented as mean ± SEM.

3. tnFGFR1 leukemia cells show a mixed immunophenotype.

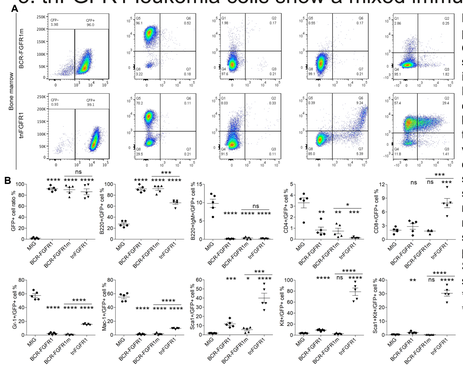


Fig 3. tnFGFR1 promotes leukemia development with a stem cell immunophenotype. (A) Representative flow cytometric profiles of the bone marrow cells from the mice transplanted with various derivative constructs at the time of sacrifice. (B) Quantitative scatter plots for each marker in each individual mouse within the subgroups. Data is presented as mean ± SEM, N=5. ns = not significant. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.

4. tnFGFR1 is frequently generated in human AML cells.

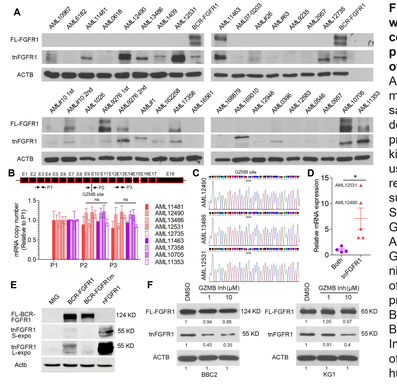


Fig 4. tnFGFR1 is expressed widely in primary leukemia cells from AML patients and is produced by GZMB cleavage of the full-length FGFR1. (A) Analysis of 29 primary AML bone marrow samples and 4 PDX samples from NSG mice demonstrates the extensive presence of the truncated kinase. (B) qRT-PCR analysis using primers targeting different regions of FGFR1 mRNA in a subset of nine AML samples. (C) Sanger sequencing across the GZMB cleavage site from three AML. (D) qRT-PCR analysis of GZMB mRNA levels in the same nine samples in (B). (E) Mutation of the GZMB recognition site prevents tnFGFR1 production in BaF3 cells transformed with BCR-FGFR1m. (F) GZMB Inhibitor II decreases production of tnFGFR1 in mouse BBC2 and human KG1 SCLL cells.

5. tnFGFR1 does not activate classic FGFR1 signaling pathways.

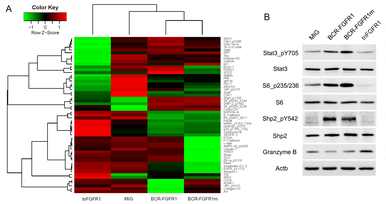


Fig 5. The truncated tnFGFR1 shows a distinct profile of oncogenic signaling compared with the full length chimeric kinases. (A) RPPA analysis of BaF3 cells transduced with either empty vector (MIG) or tnFGFR1 and maintained on IL3, or transformed with the BCR-FGFR1 chimeric kinase or its GZMB site mutated version. (B) Selected preferentially activated pathways identified by RPPA are further validated by western blotting.

6. tnFGFR1 directly activates FLT3 and KIT during leukemogenesis.

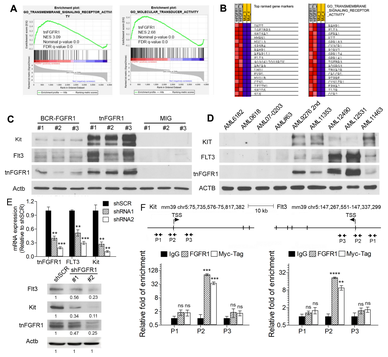


Fig 6. tnFGFR1 activates KIT and FLT3 in primary leukemia cells. (A) GSEA analysis of RNA-Seq data identifies significant enrichment for pathways involved in receptor signaling and molecular transduction in the tnFGFR1 transformed leukemia cells. (B) Heatmap of the top ranked genes and genes involved in transmembrane signaling. Western blot analysis revealed a correlation between tnFGFR1 and KIT and FLT3 expression in primary leukemia cells from both mouse model (C) and human AML samples (D). (E) KD of tnFGFR1 in primary tnFGFR1 transformed bone marrow cells leads to effective reduction of FLT3 and KIT at mRNA and protein level. (F) ChIP-qPCR analysis in primary tnFGFR1 leukemia cells using antibodies that are specific to tnFGFR1 and to the MYC-tag that was included in the transfection construct shows tnFGFR1 occupancy at the Kit and Flt3 gene promoter.

7. Targeting FLT3 can be a treatment for tnFGFR1 driven leukemia.

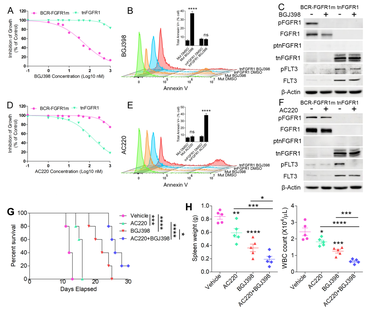


Fig 7. Targeting Flt3 can inhibit leukemia progression driven by tnFGFR1. (A) Only the primary cells from mice xenografted with BCR-FGFR1m, but not the tnFGFR1, showed the sensitivity to increasing concentrations of BGJ398, which was reflected in a significantly Increased cell apoptosis (B). (C) BGJ398 treatment specifically blocks phosphorylation of the full length fusion kinase, but has no effect on the tnFGFR1 and FLT3 signaling. (D) When the same cells were treated with the AC220 FLT3 inhibitor, the tnFGFR1 transformed cells showed a dose dependent suppression of growth. (E) Increased Annexin V staining in this case was observed in the tnFGFR1 cells treated with AC220. (F) AC220 treatment specifically targets the Flt3 signaling. (G) When mice (N = 5) inoculated with primary BCR-FGFR1 expressing leukemia cells were treated with BGJ398 (30 mg/Kg) and AC220 (10 mg/Kg), ANOVA statistical analysis revealed a significant increase in survival, which were reflected in the spleen weight and the WBC (H).

Summary

We now show that tnFGFR1 can independently lead to oncogenic transformation of hematopoietic stem cells. These leukemia cells show a mixed immunophenotype with a B-cell B220+Igm− profile in the majority of cells and Kit+ in virtually all cells, suggesting a stem cell disease. tnFGFR1 induces a distinct profile of altered gene expression with significant upregulation of transmembrane signaling receptors including FLT3 and KIT. De novo human AML also expresses tnFGFR1, which correlates with upregulation of FLT3 and KIT as in mouse leukemia cells. ChIP analysis demonstrates tnFGFR1 occupancy at the Flt3 and Kit promoters, suggesting a direct transcriptional regulation. Cells transformed with tnFGFR1 are insensitive to FGFR1 inhibitors but sensitive to the FLT3 inhibitor quizartinib (AC220), suggesting an alternative approach for the treatment of SCLL.

This study demonstrates a novel model for the transformation of hematopoietic stem cells by chimeric FGFR1 kinases with the combined effects of direct protein activation by the full-length kinases and transcriptional regulation by tnFGFR1. In human AML, tnFGFR1 activation leads to increased FLT3 and KIT expression, and higher FLT3 and GZMB expression levels are associated with inferior prognosis. These observations provide insights into the relative therapeutic value of targeting FGFR1 and FLT3 in treating AML with this characteristic gene expression profile.

References

1. Jackson CC, et al., *Hum. Pathol.* 2010; 41: 461-76.
2. Hu T, et al., *Leukemia* 2018; 32:2363-2373.
3. Cai B, et al., *Mol. Cancer* 2022;21:156.

Acknowledgements

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