Dual lineage inhibition of ETV1 and KIT disrupts the ETV1-KIT feed forward circuit and potentiates Imatinib antitumor effect in GIST

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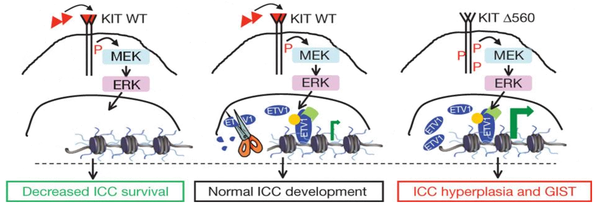
ABSTRACT

Gastrointestinal stromal tumor (GIST) is characterized by activating mutations of the KIT or PDGFRA receptor tyrosine kinases and originates from the interstitial cells of Cajal (ICCs), where KIT regulates its normal development and lineage specification. Despite the initial clinical success of imatinib, the majority of patients with advanced GIST develop imatinib resistance and die of their disease. The development of novel therapeutics that can improve the efficacy of first-line imatinib therapy and/or overcome imatinib resistance is imperative. We have previously identified ETV1, an ETS family transcription factor, as a lineage-specific survival master regulator for GIST and its precursor ICCs. Mutant KIT cooperates with ETV1 in GIST oncogenesis, in part by stabilizing the ETV1 protein through active MAP kinase signaling. Here, we demonstrate that ETV1 is required for GIST initiation and maintenance in vivo using compound genetically engineered mouse models (GEMMs). We have uncovered that ETV1 enhances KIT expression through direct binding to the KIT enhancers. Hence, ETV1 and mutant KIT form a positive feed-forward circuit and dual lineage dependence in GIST pathogenesis, where the ETV1 protein is stabilized by active KIT signaling and stabilized ETV1 augments KIT expression. Further, we demonstrate that inhibition of neither KIT (by imatinib) nor its downstream MAP kinase signaling (by MEK162, a MEK inhibitor) alone is sufficient to durably destabilize the ETV1 protein. Interestingly, the combined targeting of the dual lineage dependence of KIT by imatinib and ETV1 by MEK162 results in durable inhibition of the ETV1 protein and leads to significantly more growth suppression in vitro and complete tumor regression in vivo than either single agent. Our observations demonstrate that ETV1 is a novel therapeutic target in GIST. Importantly, the dual lineage targeting of ETV1 and KIT by the combination therapy may provide a more effective therapeutic strategy than imatinib alone in GIST clinical management.

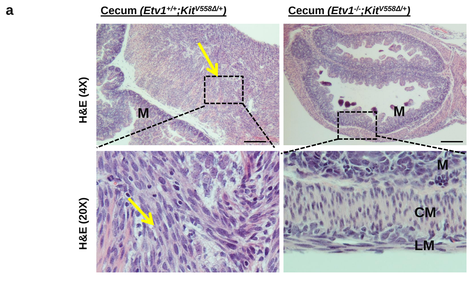


METHODS

Genetic Engineered mouse modeling, tumor cell xenograft, Chromatin IP, Western blotting, Immunofluorescent, Immunohistochemistry.

RESULTS

Figure 1: *Etv1* is required for the initiation of GIST development *in vivo*



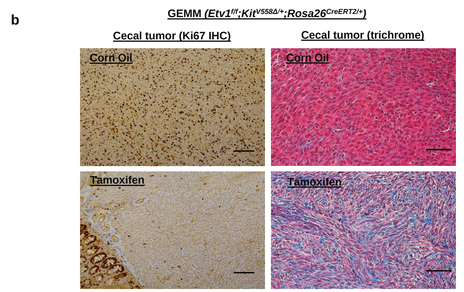


Figure1 a) Representative H&E staining of the cecal masses and the cecums of *Etv1*$^{+/+}$*;Kit*$^{V558\Delta/+}$ and *Etv1*$^{-/-}$*;Kit*$^{V558\Delta/+}$ mice. M: mucosa; CM: circular muscle; LM: longitudinal muscle. Scale bars: 100 μm. **b)** Representative Ki67 IHC (proliferation) and tricrome staining(fibrosis)of the cecal tumors of 8-9 weeks old *Etv1*$^{flox/flox}$*; Kit*$^{V558V/+}$*; Rosa26*$^{CreERT2/+}$ mice treated with either corn oil or tamoxifen. Scale bars: 50μm.

Figure 2: ETV1 regulate KIT expression through binding to KIT enhancers

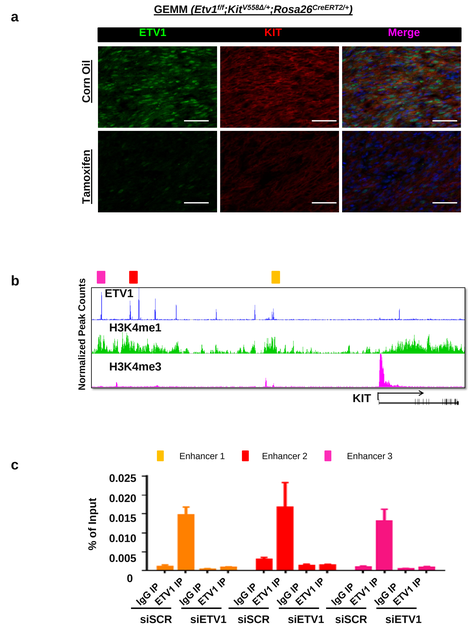


Figure2 a) Representative immunofluorescence (IF) images of Etv1 (green) and Kit (red) protein in cecal tumors from *Etv1*$^{flox/flox}$*;Kit*$^{V558\Delta/+}$*; Rosa26*$^{CreERT2/+}$ mice treated with tamoxifen or corn oil. Nuclei (DAPI, blue). Scale bars: 50 μm. **b)** Representative of ChIP-seq reads of ETV1, H3K4me1 and H3K4me3 at KIT transcription start site (H3K4me3) and enhancer regions (H3K4me1 and ETV1) in human GIST48 cells. **c)** ETV1 is knockdown with siRNA in GIST882 cells and ChIP for KIT enhancers were performed with anti-ETV1 antibody.

Figure 3: Dual inhibition of KIT and ETV1 synergistically induce apoptosis in GIST

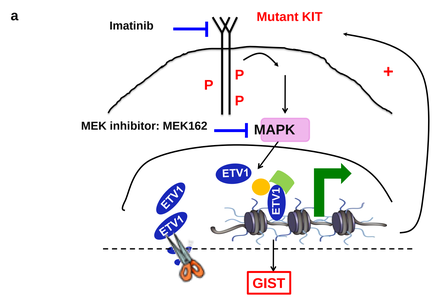
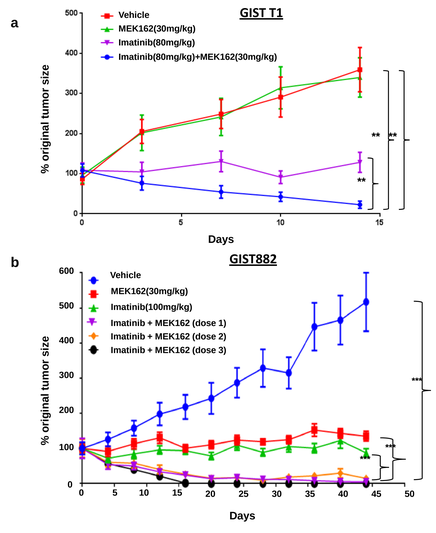


Figure3 a) Schematic model for GIST pathogenesis. **b)** Western blot of GIST882 cells treated with Imatinib and MEK162 for 60hrs.

Figure 4: Dual inhibition of KIT and ETV1 results in GIST xenograft tumor regression *in vivo*



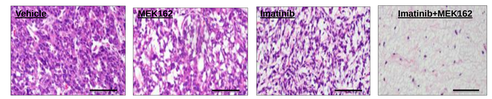
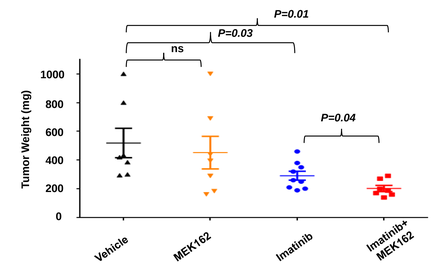


Figure4 a) Tumor Size measurement of GIST T1 xenograft tumors after treatment. Mice were treated vehichle, Imatinib(80mg/kg, BID), MEK162(30mg/kg, BID), Imatinib(80mg/kg, BID)+MEK162(30mg/kg, BID). The result was presented as percentage tumor growth by normalizing to the first mean tumor volume measured for each group ± SEM (n = 10 mice/group). **P<0.005. **b)** Tumor Size measurement of GIST882 xenograft tumors after treatment. Mice were treated with vehichle, Imatinib(100mg/kg, BID), MEK162(30mg/kg, BID), dose 1(Imatinib, 100mg/kg, BID+MEK162, 10mg/kg, BID), dose 2 (Imatinib, 50mg/kg, BID+MEK162, 30mg/kg, BID) and dose 3 (Imatinib, 100mg/kg, BID+MEK162, 30mg/kg, BID) and presented as in **a**. *** p<0.001 **c)** Representative histology of GIST882 xenograft tumor after 1 month treatment. Scale Bars: 100uM.

Figure 5: Dual inhibition of KIT and ETV1 results in GIST GEMM tumor regression *in vivo*



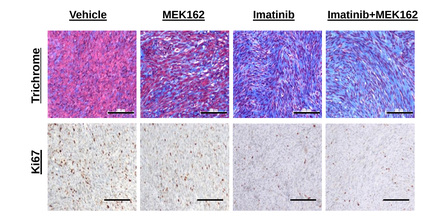


Figure5 a) Cecal tumor weight meausrement of *Etv1*$^{f/f}$*;Kit*$^{V558\Delta/+}$ mice treated with vehicle, MEK162(30mg/kg, BID), Imatinib(50mg/kg, BID), Imatinib(50mg/kg, BID)+MEK162(30mg/kg, BID) for 5 days. **b)** Representative trichrome staining and Ki67 immunohistochemistry staining of tumor masses 5days after treatment. Scale bars: 100uM.

CONCLUSION

- ***Etv1* is required for GIST tumor initation and maintanence *in vivo*.**
- ***Etv1* positively regulates *KIT* expression through binding to its enhancer.**
- **Targeting ETV1-KIT feed forward circuits by combined therapy of MEK162 and imatinib leads to more enhanced inhibition of GIST growth than either single agent *in vitro* and *in vivo*.**

REFERENCE

1. Chi, P. *et al. Nature* **467,** 849–853 (2010).
2. Verweij, J. *et al.Lancet* **364,** 1127–34 (2004).
3. Balachandran, V. P. & Dematteo, R. P. *Surg. Oncol. Clin. N. Am.* **22,** 805–21 (2013).