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ORIGINAL RESEARCH

FLI1 Regulates Histamine Decarboxylase Expression to Control Inflammation Signaling and Leukemia Progression

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Aim: Histamine decarboxylase (HDC) catalyzes decarboxylation of histidine to generate histamine. This enzyme affects several biological processes including inflammation, allergy, asthma, and cancer, although the underlying mechanism is not fully understood. The present study provides a novel insight into the relationship between the transcription factor FLI1 and its downstream target HDC, and their effects on inflammation and leukemia progression.

Methods: Promoter analysis combined with chromatin immunoprecipitation (ChIp) was used to demonstrate binding of FLI1 to the promoter of *HDC* in leukemic cells. Western blotting and RT-qPCR were used to determine expression of HDC and allergy response genes, and lentivirus shRNA was used to knock-down target genes. Proliferation, cell cycle, apoptosis assays and molecular docking were used to determine the effect of HDC inhibitors in culture. An animal model of leukemia was employed to test the effect of HDC inhibitory compounds in vivo.

Results: Results presented herein demonstrate that FLI1 transcriptionally regulates *HDC* by direct binding to its promoter. Using genetic and pharmacological inhibition of HDC, or the addition of histamine, the enzymatic product of HDC, we show neither have a discernable effect on leukemic cell proliferation in culture. However, HDC controls several inflammatory genes including IL1B and CXCR2 that may influence leukemia progression in vivo through the tumor microenvironment. Indeed, diacerein, an IL1B inhibitor, strongly blocked Fli-1-induced leukemia in mice. In addition to allergy, FLI1 is shown to regulate genes associated with asthma such as IL1B, CPA3 and CXCR2. Toward treatment of these inflammatory conditions, epigallocatechin (EGC), a tea polyphenolic compound, is found strongly inhibit HDC independently of FLI1 and its downstream effector GATA2. Moreover, the HDC inhibitor, tetrandrine, suppressed HDC transcription by directly binding to and inhibiting the FLI1 DNA binding domain, and like other FLI1 inhibitors, tetrandrine strongly suppressed cell proliferation in culture and leukemia progression in vivo.

Conclusion: These results suggest a role for the transcription factor FLI1 in inflammation signaling and leukemia progression through HDC and point to the HDC pathway as potential therapeutics for FLI1-driven leukemia.

Keywords: HDC, histamine, FLI1, inflammation, allergy, asthma, leukemia progression

Introduction

Histamine decarboxylase (HDC) is the sole enzyme required for the synthesize of histamine from histidine in eukaryotes and mammals.[1,2] Histamine is known for its multiple roles in immune response and allergic reaction.[1,3] In addition to these biological activities, HDC expression and histamine synthesis have also been found in various cancer cell lines from different malignancies.[4,5] Whether HDC expression also contributes to malignant transformation is not fully understood.

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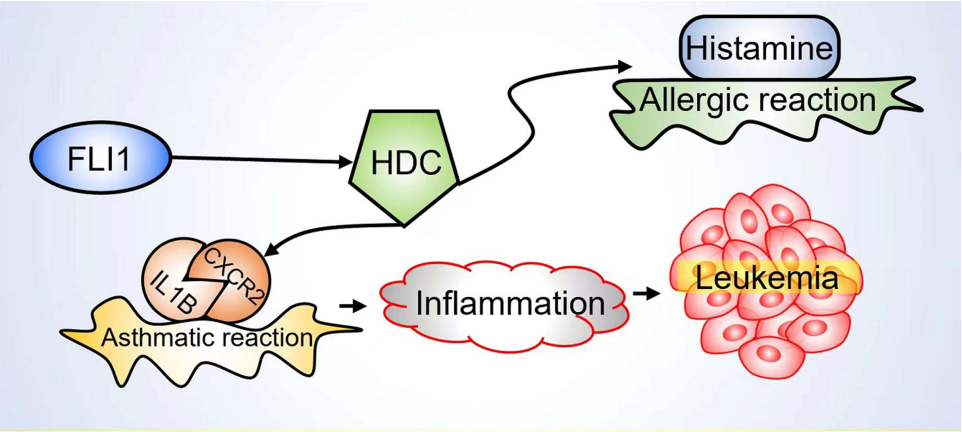
Graphical Abstract



HDC catalyzes a single step decarboxylation of histidine to form histamine, which is required for various functions including neurotransmission, immune system and gastric acid production.[1,2] In 2010, a rare nonsense mutation in the *HDC* gene was found in a patient with Tourette syndrome (TS) and related conditions.[6] Subsequent studies clearly found a strong association between HDC signaling and TS.[7] Histamine is released via paracrine and/or autocrine mechanisms.[1] The highest concentration of this enzyme is found in the stomach, lymph nodes, and thymus.[3] HDC expression is mainly found in mast cells, skin cells, platelets, basophils, immature myeloid cells (IMC's) and gastric enterochromaffin-like cells.[4] HDC knockout mice exhibit major defects in mast cell number and function.[8] As histamine mediates allergy through interaction with four receptors, H1, H2, H3, H4, each involved in a unique biological response.[9]

The role of Histamine in cancer is controversial as it can promote or suppress proliferation in different tumor cells. High HDC expression is detected in lung, breast, colon and stomach cancers.[10] In these tumors high histamine levels are required for growth as inhibition of HDC in these cells leads to antitumor reaction.[5,10] In pancreatic cancer cell lines, a positive or negative proliferative response to HDC depends on the dose of histamine administration,[11,12] likely reflecting different affinity to its receptors H1-H4HR.[9]

In Leukemia, histamine was proposed to suppress leukemogenesis by enhancing immunity against cancer cells.[13,14] Herein, we show that erythroleukemia cells expressing high levels of FLI1 also produce high levels of HDC. FLI1, a member of the ETS gene family of oncogenic transcription factor, was originally identified as the site of proviral integration in erythroleukemia induced by Friend Murine Leukemia Virus (F-MuLV).[15,16] FLI1 was later shown to be involved in the development of leukemia, lymphomas, myeloma and other types of cancer.[17–20] In this study, FLI1 for the first time is shown to directly bind the promoter of *HDC*, leading to higher expression of this enzyme, which controls histamine production. By using HDC knockdown and inhibitors of this enzyme, we show that HDC induction by FLI1 does not influence leukemia cell proliferation in culture. Instead, HDC appears to regulate many inflammatory genes that may affect leukemia progression through changes in tumor microenvironment. Accordingly, inhibitors of HDC pathway are shown to delay progression of leukemia in a mouse model in which FLI1 activation is essential for cancer initiation.[16,17] This study demonstrates a critical role of FLI1 in the regulation of HDC and implicates this enzyme in leukemia progression.

Materials and Methods

Cells Culture

The erythroleukemia cell line HEL (human origin) was originally obtained from ATCC (HEL 92.1.7), and maintained mycoplasma free used in all experiments.[19,20] The generation of inducible K562-fli1 cells is described elsewhere.[20] These

cells were maintained in Dulbecco's Modified Eagle Medium supplemented with HyClone 5% fetal bovine serum (GE Healthcare).

The HEL cells were treated with indicated drugs, used for cell counting/MTT assay (to determine the proliferation index) and for protein/mRNA extraction (for Western blotting and RT-qPCR). Famotidine was purchased from (APExBIO), Tetrandrine from (Solarbio, CN), EGC from (Selleckchem, CN) and Diacerein from (APExBIO).

Apoptosis and Cell Cycle

For apoptosis assay, cells were incubated with compounds or vehicle for 24 h, as previously described.[20] Treated cells were then washed by cold PBS, stained by Annexin V and PI apoptosis detection kit (BD Biosciences), following the kit guidelines and analyzed by flow cytometer. For cell cycle analysis, HEL cells were fixed by cold ethanol (75%) overnight at −20°C. Cells were then washed by cold PBS, stained in PI for 40 min at 37°C and analyzed by flow cytometer.

shRNA Expression

The construction of shRNA lentiviruses was previously described.[19] In brief, shHDC and scrambled control vectors constructed by cloning human HDC shRNA and scrambled DNAs into the BcuI restriction enzyme sites within the PLent-GFP expression vector that was purchased from Vigene Bioscience (Rockville, MD, USA). To produce life lentivirus particles, three shHDC DNAs (10 μg) co-transfected each with two packaging plasmids psPAX2 (5 μg) and pMD2.G (10 μg) (Addgene plasmid #12259 and #12260) into HEK293T cells, using Lipofectamine 2000 (Thermo Fisher Scientific, US).[19] Two days after transduction, the supernatant was collected and used to transduce HEL (1×10^6) cells in a 9 cm culture plates. One day after transduction the positive cells were selected after incubation with puromycin (5 μg/mL) [Solarbio]. The sequence of the shHDCs is shown in Table 1.

RNA Preparation and RT-qPCR

Total RNA was extracted from cells using TRIzol reagent (Life Technologies; Thermo Fisher Scientific, USA). CDNA was synthesized from mRNA using the reverse transcription reaction by the PrimeScript RT Reagent kit (Takara Bio, Beijing, China). RT-qPCR was carried out using the FastStart Universal SYBR-Green Master Mix (Roche, Shanghai, China) and the Step One Plus Real-time PCR system (Applied Biosystems/Thermo Fisher Scientific, US). The expression of the tested genes was calculated as relative values to the expression of GAPDH. Three biological replicates were used for all the RT-qPCRs, each in triplicate (n=3). The primer sequences are listed in Table 2.

Western Blotting

Western blotting was done using protocol, as previously described.[19,21] The antibodies used are as follows: Polyclonal rabbit antibodies for FLI1 (Cat. no. ab133485) and HDC (Cat. no. ab137571) were obtained from Abcam, the monoclonal GAPDH (Cat. no. G9545) antibody was obtained from Sigma Aldrich; goat anti mouse and goat anti rabbit HRP conjugated antibodies were obtained from Cell Signaling Technology (Cat. nos. 5470s and 5151s, respectively). Antibody dilution was conducted according to the manufacturer's instructions. The Odyssey system (LI COR Biosciences) and Bio was used to image proteins in Western blot analysis.

Table 1 The Sequence of the shHDCs

Gene	Sequence
shHDC1	5'GGCAGCAAGGAAGAACAAATTCAAGAGATTTGTTCTTCCTTGCTGCCTTTTTT3'
shHDC2	5'GCAGACCTTCAGTGTGAATTTCAAGAGAATTCACACTGAAGGTCTGCTTTTTT3'
shHDC3	5'GCCGACTCCTTCACCTTTAATTTCAAGAGAATTAAAGGTGAAGGAGTCGGCTTTTTT3'

Table 2 The Primer Sequences are Listed

Genes	Forward	Reverse
FLI1	CAGCCCCACAAGATCAACCC	CACCGGAGACTCCCTGGAT
HDC	ATGCACGCCTACTACCCAG	CAGTCCATGACGTTCATCTCC
IL1B	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
BHLHE40	GACGGGGAATAAAGCGGAGC	CCGGTCACGTCTCTTTTTCTC
AHR	ACATCACCTACGCCAGTCG	CGCTTGGAAGGATTTGACTTGA
AHRR	TTGACCTCATGGACCTCGTAG	GGCATCTTCGGAAGTCAGAGT
CXCR2	CCTGTCTTACTTTTCCGAAGGAC	TTGCTGTATTGTTGCCCATGT
GATA2	GCAACCCCTACTATGCCAACC	CAGTGGCGTCTTGGAGAAG
MITF	CAGTCCGAATCGGGGATCG	TGCTCTTCAGCGGTTGACTTT
CPA3	GGGTTTGATTGCTACCACTCTT	GCCAAGTCCTTTATGATGTCTGC
HBA1	CCGGTCAACTTCAAGCTCCT	GCCGCCCACTCAGACTTTAT
HBA2	TCTCCTGCCGACAAGACCAA	GCAGTGGCTTAGCTTGAAGTTG
miR145	TCCCTAAGGACCCTTTTGACC	AGTCTCAGGGTCCGAGGTATTC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

Leukemia Induction in Mice and Drug Therapy

Friend Murine Leukemia virus (F-MuLV) was previously demonstrated to induced erythroleukemia in newborn mice associated with the activation of FLI1.[21] To generate erythroleukemia, a group of neonate BALB/c mice (N=6) were infected intraperitoneally (i.p.) with F-MuLV, as described.[21] Five weeks post viral infection, leukemic mice were treated every other day for a total of six injections with 3 mg/kg bodyweight of the indicated drugs or DMSO as control. Mice were then observed for the development of severe leukemia. Mice displaying typical signs of late-stage disease were sacrificed by experienced technician and the percent survival was measured, as previously described.[21]

Computer Docking and STRING Analysis

The three-dimensional structure of tetrandrine was analyzed and drawn in PubChem. The protein crystallographic structures of receptors FLI1 (PDB ID: 6VG2) retrieved from www.rcsb.org. Auto Dock tools 1.5.7 used to compute the molecular docking simulations following the standard protocol, as described in the software documentation. Furthermore, the interacting sites were analyzed using PyMol analysis.

The interaction of HDC with other proteins was derived from STRING database (https://cn.string-db.org). The setting interaction was graphed using the default medium confident setting (a minimum required interaction of 0.4).

Chromatin Immunoprecipitation (ChIp) Analysis

The ChIp analysis was performed, as previously published.[19] In brief, erythroleukemia HEL cells were crosslinked in formaldehyde for 15 min, centrifugated and the pellet was resuspended in Magna ChIP A/G kit lysis buffer (Sigma-Aldrich, US). The fixed pellet was sonicated using a Sonics Vibra VCX150 (Ningbo Scientz Biotechnology, CN). At this stage, a small aliquot of the chromatin was removed to be used as input control. Protein G Sepharose beads (Cell Signaling Technology, US) were added to the chromatin and incubated for one hour at room temperature. The immunoprecipitations were performed overnight at 4°C with 1 µg of ChIp specific anti-FLI1 antibody (ab15289, Abcam, UK) and the negative control mouse immunoglobulin G (IgG) antibody (Cell Signaling Technology, US). After centrifugations, the chromatin precipitates were washed and reverse crosslinked according to the manufacturer's instructions. The precipitated chromatins were then incubated with proteinase K at 56°C for two hours, DNA purified with one phenol chloroform extraction and resuspended in TE buffer. RT-qPCR was performed using this DNA to determine the amount of FLI1 binding within the *HDC* promoter region. The percentage of input was calculated as previously described.[19] Amplified DNAs were also resolved on a 2% agarose gel and illustrated in Figure 1F. The ChIp experiment was performed at least in three independent experiments. The primer sequences for the ChIp PCRs are as follows:

HDC: Forward CAGCCAGAGATGTAGGAGGA

Reverse CCTTGGGGTTTGATTTCTGAGT

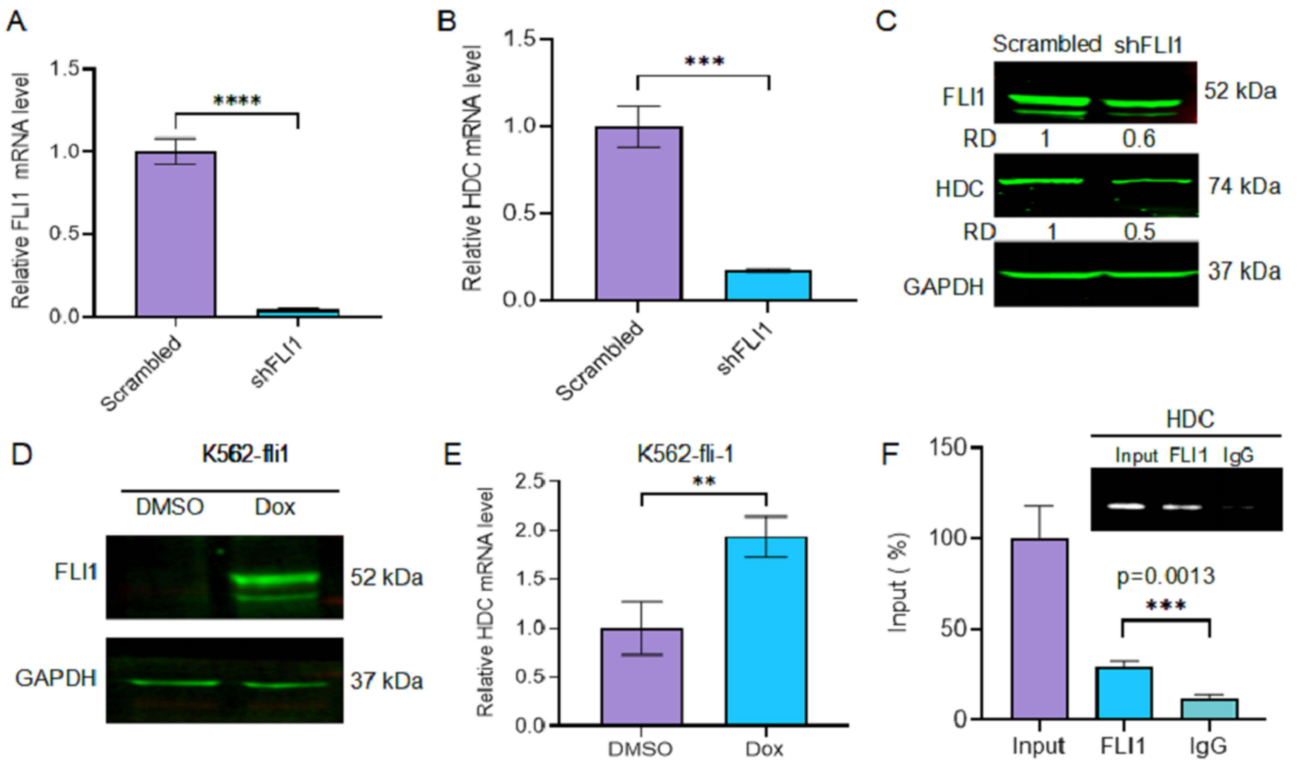


Figure 1 The HDC expression is regulated by FLI1. (**A** and **B**) ShFLI1 and control cells were subjected to RT-qPCR analysis for the expression of FLI1 (**A**) and HDC (**B**) genes. (**C**) ShFLI1 and scrambled control cells were examined for expression of FLI1 and HDC using Western blotting. Rd: Relative density determined using densitometer. (**D**) Relative expression of FLI1 protein in inducible K562-fli-1 cells after addition of Doxycycline. (**E**) Relative expression of HDC by RT-qPCR in K562-fli-1 cells after addition of doxycycline. (**F**) Chromatin immunoprecipitation (ChIp) analysis of the HDC promoter in HEL cells for binding to FLI1, by RT-qPCR (Lower panel). Upper panel represents the gel image of the immunoprecipitated PCR-amplified band relative to input. P=<0.01 (**), P=<0.001 (***) and P=<0.0001 (****) by two-tailed Student's t-test.

Statistical Analysis

A statistical analysis was measured using a two tailed Student *t*-test or a one-way ANOVA with Tukey's post hoc test, using Origin 3.5 software (Microcal Software). The P values were indicated within the figures using a standard scheme, P=<0.05 (*), P=<0.01 (**), P=<0.001 (***) and P=<0.0001 (****). Where appropriate, the data were displayed using the mean ± the SEM from at least 3 independent experiments.

Results

Direct Correlation Between the FLI1 and HDC Expression in Erythroleukemia Cells

We previously knocked-down FLI1 via a lentivirus shRNA (shFLI1) in human HEL cells, which normally overexpress this transcription factor,[19] and performed RNA-seq analysis.Re-analysis of these RNA-seq data revealed a 95% down-regulation of HDC in shFLI1-HEL cells compared to scrambled control cells. This correlation was confirmed by Q-RT-PCR (Figure 1A and B) and Western blot analysis (Figure 1C), demonstrating that FLI1 knockdown strongly down-regulates HDC expression. To further corroborate these results, we transfected a doxycycline inducible FLI1 expression vector into erythroleukemia cell line K562, which does not express this transcription factor,[21] yielding K562-fli1 cells. Induction of FLI1 in these erythroleukemia cells (Figure 1D) enhanced HDC expression (Figure 1E). We subsequently identified a FLI1 binding site (FBS) [ACTGGAAAC, position −485 to −493] within the *HDC* promoter {Supplemental Figure 1}. To ask whether FLI1 is directly recruited to the *FLI1* promoter, we employed Chromatin immunoprecipitation (ChIp) analysis. We found that anti-FLI1 antibodies effectively immunoprecipitated a DNA fragment encompassing the FBS site compared to control IgG (Figure 1F). These results demonstrate that HDC, involved in biosynthesis of amino-acid histamine, is a direct target of FLI1.

HDC Induction by FLI1 Does Not Affect Cell Proliferation

As FLI1 plays critical roles in cell proliferation, survival and differentiation,[17,19,21] we examined whether some of these biological functions are mediated through HDC regulation. To this end, HDC was knocked-downed by lentivirus shRNAs in HEL cells. Three shRNAs (shRNA1-3) were transduced into HEL cells and relative expression was determined by RT-qPCR (Figure 2A) and Western blot (Figure 2B) analyses. ShHDC-2 and shHDC-3 cells exhibited the strongest level of HDC depletion; shHDC2 was used in subsequent studies (Figure 2B). HDC knocked-down marginally increased proliferation in culture compared to scrambled-control cells (Figure 2C). Famotidine, a H2-receptor blocker[22] is commonly used to inhibit histamine production, only had a marginal effect on proliferation of HEL cells in culture even at high dose of 40 μM (Figure 2D).

Previously, supplementing histamine to cell culture was reported to accelerate cancer cell proliferation.[5,10] However, addition of histamine (10 μM) to HEL cell culture did not affect their proliferation rate (Figure 2E). However, we observed a slight reduction in cell proliferation with 20 μM of histamine at day 4 of incubation. Similar results were also seen in shHDC2 versus control cells treated with 10 μM of histamine (Figure 2F).

As FLI1 expression blocks erythroid differentiation and its inhibition induces this maturation process,[20] we asked whether this effects are regulated by HDC. We found that the expression of globin HBA1 and HBA2 genes was not

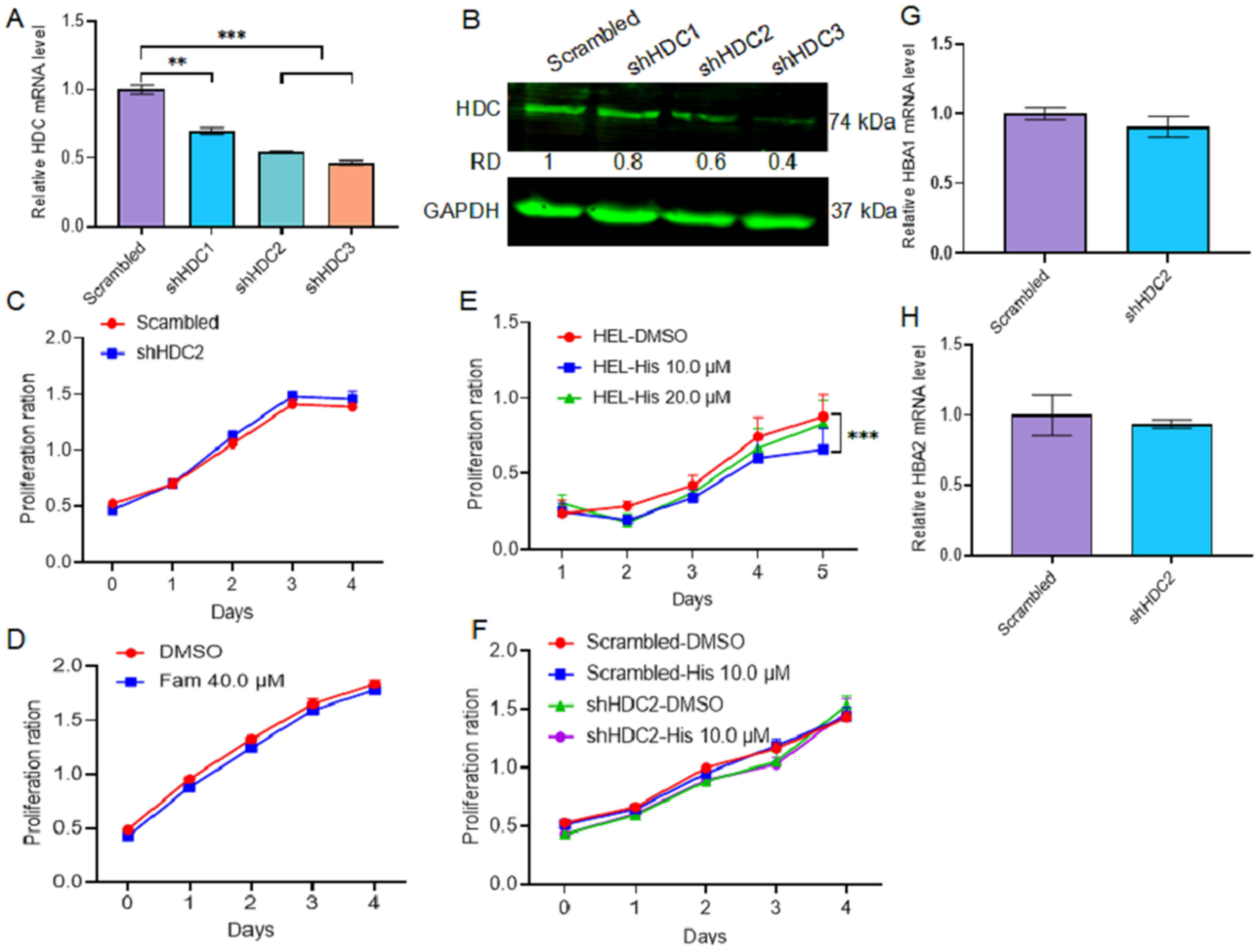


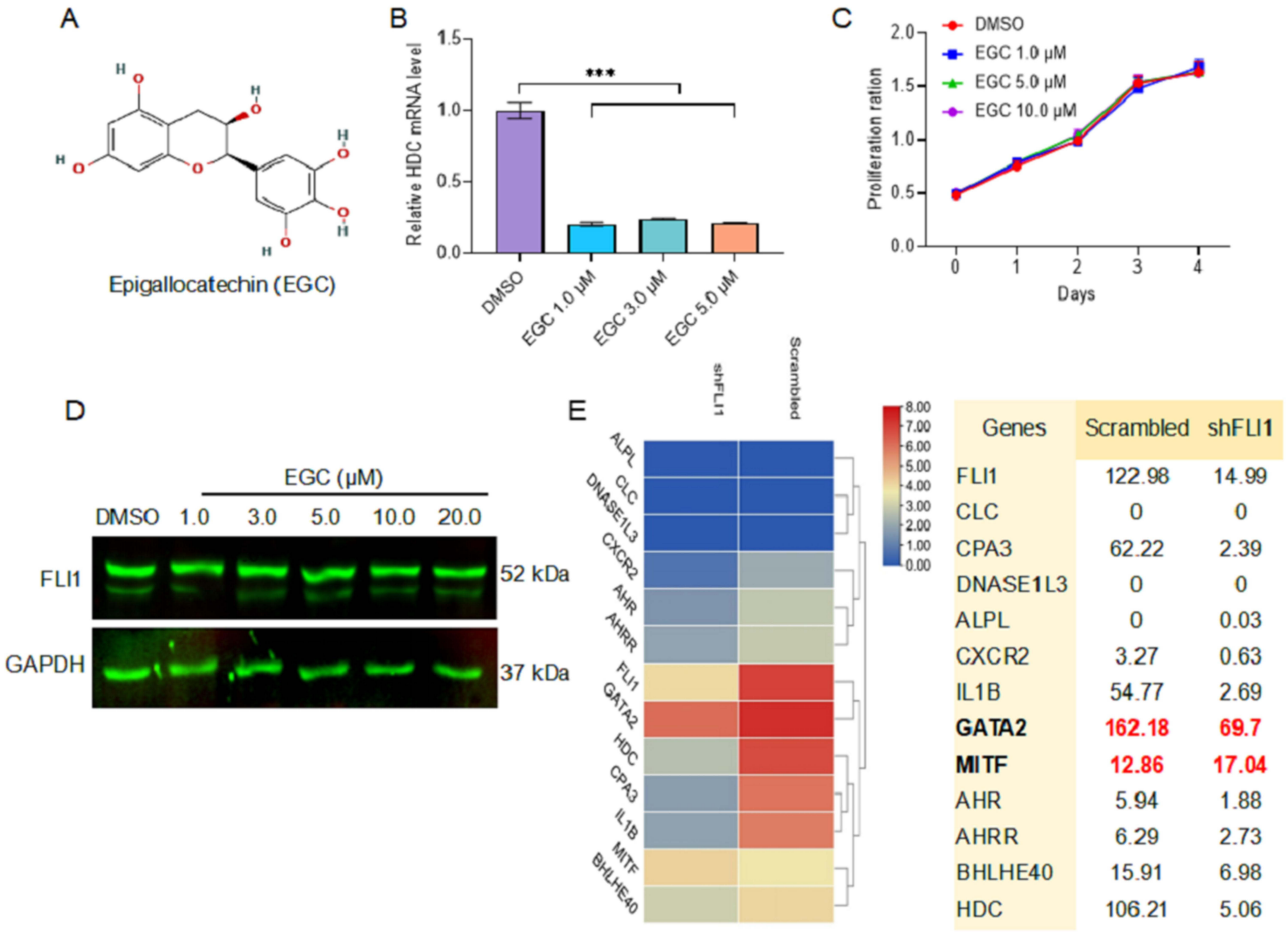
Figure 2 The HDC knockdown does not affect cell proliferation in culture. (**A** and **B**) Relative expression of HDC in shHDC1-3 cells by RT-qPCR (**A**) and Western blot (**B**). (**C**) Cell proliferation index in scrambled and shHDC2 cells. (**D** and **E**) Cell proliferation of HEL cells in culture following addition of the indicated concentrations of famotidine (**D**) and histamine (**E**). (**F**) Cell proliferation in shHDC2 and control cells with or without addition of histamine. (**G** and **H**) Relative expression of globin genes HBA1 (**G**) and HBA2 (**H**) in shHDC2 and control cells, by RT-qPCR. P=<0.01 (**) and P=<0.001 (***) by two-tailed Student's t-test.

affected in shHDC2 relative to control cells (Figure 2G and H), respectively. These results suggest that leukemia cell proliferation in culture is not regulated by HDC or its product histamine.

Epigallocatechin (EGC) Inhibits HDC to Lower Growth Rate

As HDC is essential for induction of allergy, targeting this enzyme has important therapeutic implications. Epigallocatechin gallate (EGCG), a tea polyphenolic compound, was reported to suppress histamine release from the mast cell line RBL-2H3 by inhibiting tyrosine phosphorylation of proteins such as Focal Adhesion Kinase (pp125FAK).[23] Interestingly, a derivative of EGCG (Epigallocatechin or EGC; Figure 3A), was found to strongly inhibit transcription of HDC in HEL cells, as shown in Figure 3B. Inhibition of HDC by EGC, like in HDC knockdown cells (Figure 2C), resulted in no significant growth inhibition (Figure 3C). The effect of EGC on FLI1 protein expression is negligible (Figure 3D), indicating a FLI1-independent inhibition of HDC by this compound.

The mechanism by which EGC suppresses HDC is unknown. Interestingly, a previous study demonstrated the involvement of transcription factors GATA2 and MITF in regulating HDC during IgE/mast cell-induced anaphylaxis.[24] Indeed, our RNAseq analysis of FLI1 knockdown cells (shFLI1) revealed that while GATA2 is strongly downregulated, MITF transcript level increased in HEL cells (Figure 3E).[20] In mast cells, while GATA2 controls the transcription of Mitf, Ahr, Ahrr, and Bhlhe40 genes, the expression of only Mitf, but not ahr, ahrr, or bhlhe40, was implicated in anaphylaxis reaction.[24] Interestingly, our RNAseq data clearly showed that similar to GATA2, expression of human AHR, AHRR and BHLHE40 is downregulated in shFLI1 versus control cells (Figure 3E), raising the possibility that



Genes	Scrambled	shFLI1
FLI1	122.98	14.99
CLC	0	0
CPA3	62.22	2.39
DNASE1L3	0	0
ALPL	0	0.03
CXCR2	3.27	0.63
IL1B	54.77	2.69
GATA2	162.18	69.7
MITF	12.86	17.04
AHR	5.94	1.88
AHRR	6.29	2.73
BHLHE40	15.91	6.98
HDC	106.21	5.06

Figure 3 Epigallocatechin (EGC) suppresses HDC transcriptional expression in HEL cells independent of FLI1. (**A**) Chemical structure of EGC. (**B**) Expression of HDC in HEL cells treated with the indicated concentration of EGC, as determined by RT-qPCR. (**C**) EGC marginally affect cell proliferation of HEL cells in culture. (**D**) HDC expression does not affect the expression of FLI1, as determine by Western blot. (**E**) Heatmap of expression of the indicated genes in shFLI1 versus control cells (Left panel). The relative expression level was determined by RNAseq analysis, as shown in the right panel. P=<0.001 (***) by two-tailed Student's t-test.

these genes are regulated by GATA2. In contrast, expression of MITF is upregulated in shFLI1 cells (Figure 3E). Treatment of HEL cells with EGC slightly increased GATA2, but strongly upregulated MITF expression (Supplemental Figure 2A and B). However, while EGC sharply blocked HDC transcription (Figure 3B), it had no effect on expression AHR and AHRR (Supplemental Figure 2C and D), but induced BHLHE40 (Supplemental Figure 2E). These results suggest that EGC controls HDC transcription independently of FLI1 and GATA2, but likely through MITF, as previously reported.[24] EGC-mediated induction of BHLHE40 is likely through an independent mechanism.

FLI1 Regulates Transcription of Inflammatory-Response Genes Associated with Asthma Through HDC

During asthmatic reaction, expression of 6 biomarkers (6GS), including Charcot-Leyden crystal galectin [CLC]; carboxypeptidase 3 [CPA3]; deoxyribonuclease 1-like 3 [DNASE1L3]; alkaline phosphatase, liver/bone/kidney [ALPL]; C-X-C motif chemokine receptor 2 [CXCR2]; and interleukin 1B [IL1B] predicts inflammatory response in patients' saliva.[25] By RNAseq analysis, while CLC, DNASE1L3 and ALPL expression is negligible in leukemic HEL cells, transcription of the inflammatory genes CPA3, IL1B and CXCR2 is strongly suppressed in FLI1 knockdown cells (Figure 3E). We previously showed that IL1B is regulated by FLI1 in leukemic cells.[26] We confirmed the expression pattern of CPA3, IL1B and CXCR2 using RT-qPCR in knockdown HEL (shFLI1) versus scrambled control cells (Figure 4A–C). Interestingly, while expression of CPA3 was slightly increased in shHDC-2 and shHDC-3 cells, IL1B and CXCR2 transcription was downregulated when compared to scrambled control (Figure 4D–F). Since FLI1

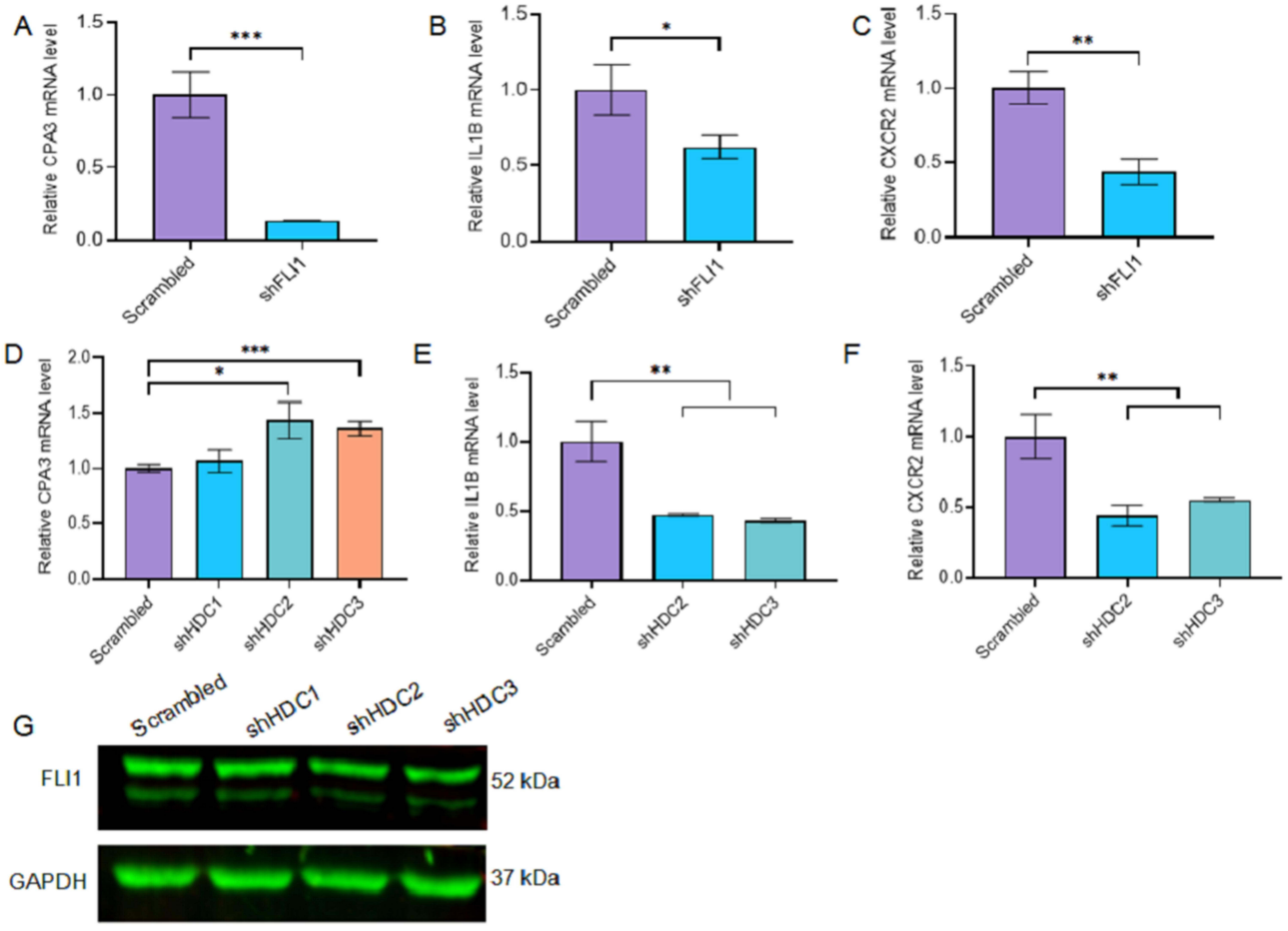


Figure 4 FLI1 regulates genes associated with the asthmatic reaction. (**A**–**C**) Relative expression of the CPA3 (**A**), IL1B (**B**) and CXCR2 (**C**) genes in shFLI1 versus control cells, by RT-qPCR. (**D**–**F**). Relative expression of the CPA3 (**D**), IL1B (**E**) and CXCR2 (**F**) genes in shHDC2-3 cells, by RT-qPCR. (**G**) Relative expression of FLI1 in shHDC1-3 cells, by Western blot. P=<0.05 (*), P=<0.01 (**) and P=<0.001 (***) by two-tailed Student's t-test.

expression is not changed in shHDC1-shHDC3 cells (Figure 4G), HDC controls these inflammatory response genes independently of FLI1.

Protein-protein interaction database (STRING) predicts that HDC interacts with CPA3 (Supplemental Figure 3) and functions as a biomarker for allergy and asthmatic reaction.[27,28] The above results therefore suggest that FLI1 controls a subset of inflammation response genes in asthma through HDC.

Tetrandrine Inhibits HDC Through Downregulation of FLI1

Similar to EGCG, the natural compound tetrandrine (Figure 5A) was previously reported to reduce HDC activity, although the mechanism is unknown.[29] Treatment of HEL cells with various concentration of tetrandrine resulted in a dose-dependent downregulation of FLI1 protein (Figure 5B) and mRNA (Figure 5C). While HDC protein and mRNA level is downregulated by tetrandrine (Figure 5B and D), the inhibition level at 3 and 5 μM drug concentrations is very similar, suggesting a strong but restricted role for FLI1 in transcriptional regulation of HDC. This is indeed consistent with the results (Figure 3B) in which other transcriptional factors control HDC independently of FLI1. Interestingly, the extent of FLI1 protein inhibition by tetrandrine is much higher than transcriptional inhibition, raising the possibility that the drug may bind to FLI1 and inhibit its function. Indeed, in molecular docking analysis, tetrandrine binds strongly to the ETS DNA binding domain of FLI1, with an affinity of −7.9 (Supplemental Figure 4). We have previously shown that FLI1 negatively regulates miR145, that binds to untranslated region in FLI1 mRNA and downregulates protein

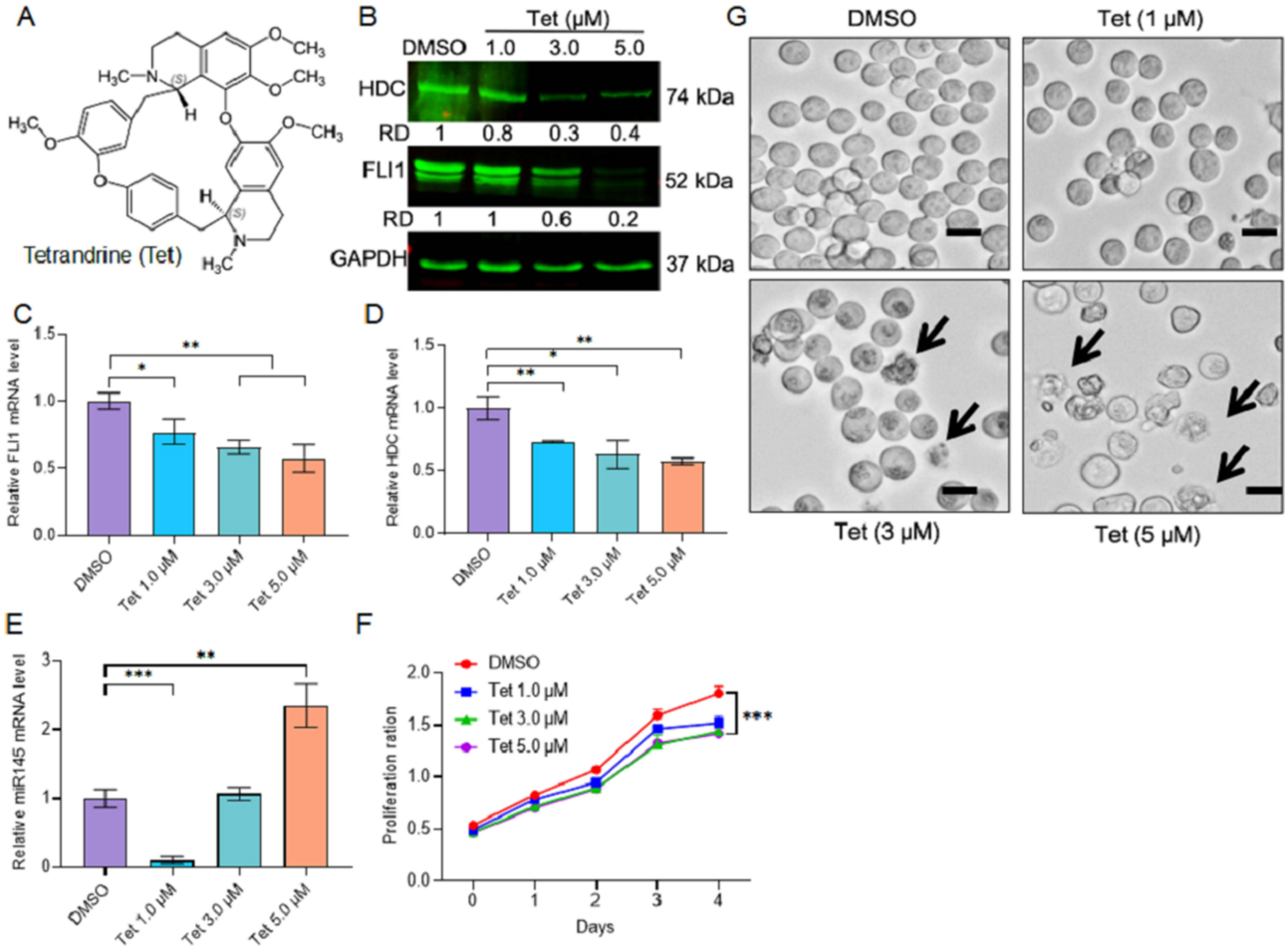


Figure 5 Tetrandrine inhibits HDC through downregulation of FLI1. (**A**) Chemical structure of tetrandrine. (**B** and **C**) Tetrandrine inhibits FLI1 protein (**B**) and its transcript (**C**) in HEL cells in a dose dependent manner. (**D**) Tetrandrine inhibit HDC in a dose dependent manner, as determined by RT-qPCR. (**E**) Tetrandrine activated the expression of miR145 in a dose dependent manner. (**F**) Tetrandrine inhibits HEL cell proliferation in culture. (**G**) Tetrandrine induces cell death of HEL cells in a dose dependent manner. (**G**) Cells were photographed 24 h post-drug treatment (−50μm; magnification 20X). Black arrows shows death cells. P=<0.05 (*), P=<0.01 (**) and P=<0.001 (***) by two-tailed Student's t-test.

expression.[19] In accordance, tetrandrine strongly upregulated miR145 in a dose dependent manner (Figure 5E). Higher miR145 expression induced by tetrandrine correlated with lower expression of FLI1 in Western blots (Figure 5B).

At 5 μM concentration, tetrandrine strongly inhibited cell proliferation (Figure 5F) and promoted cell death (Figure 5G) of HEL cells in culture. Indeed, tetrandrine induces apoptosis (Supplemental Figure 5A) and S-phase cell cycle arrest (Supplemental Figure 5B) of HEL cells in a dose-dependent manner. This result shows, for the first time, that tetrandrine blocks HDC expression through direct binding to FLI1 and inhibition of its transcriptional activity.

HDC Accelerates Leukemogenesis Independently of the Histamine Synthesis Pathway

To determine the role of HDC and its downstream pathway in leukemia progression, we utilized a mouse model of erythroleukemia induced by Friend Murine Leukemia Virus (F-MuLV), in which FLI1 activation is a critical step.[15,16] Mice infected at birth with F-MuLV develop leukemia 2–4 months post-viral infection.[21] Leukemia-bearing mice at 5 weeks post-viral infection were treated with either histamine or the H2 blocker Famotidine (5 mg/Kg) for two weeks every other day (3 times/week, Figure 6A and B). Leukemia survival rate was scored when mice succumbed to the disease.[20,21] Relative to DMSO-control treated mice, histamine and famotidine treatment resulted in similar survival rates, indicating that histamine biosynthesis is not involved in leukemia progression. While HDC inhibition had no effect on cell proliferation in culture (Figure 2C), this enzyme may alter tumor microenvironment through regulation of inflammatory gene(s), leading to leukemia progression. Indeed, Diacerein (3 mg/Kg), a potent inhibitor of the inflammatory genes IL6,[30] TNF[31] and IL1B[31,32] strongly suppressed leukemia progression (Figure 6C).

As tetrandrine (3 mg/Kg) blocks FLI1, leading to cell cycle arrest and inhibition of proliferation, this compound also strongly inhibited leukemia progression (Figure 6D), confirming previous reports in which FLI1 inhibition was shown to attenuate leukemia progression.[19] Overall, our results suggest a model in which FLI1 controls HDC expression, leading to activation of its biological functions including activation of inflammatory genes associated with allergy and asthma

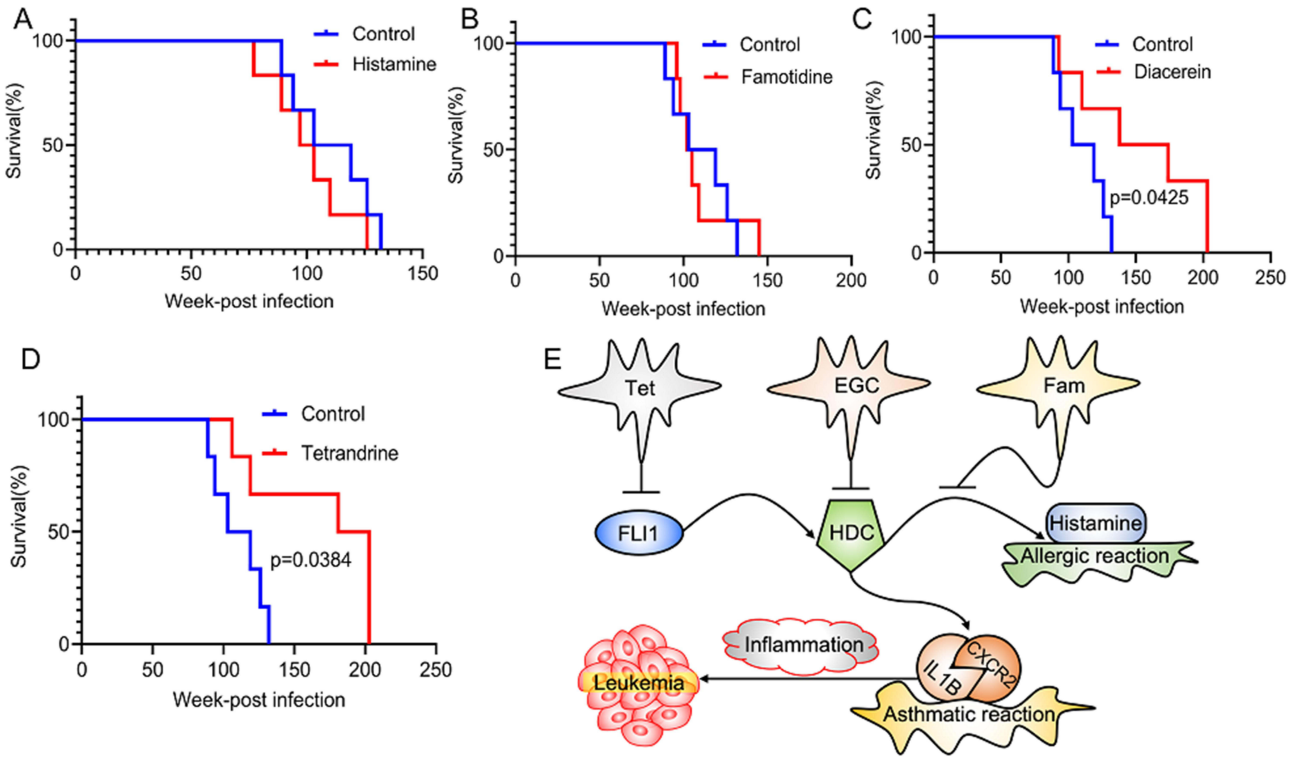


Figure 6 Diacerein and tetrandrine inhibit leukemia progression in vivo. (**A**–**D**) Leukemic mice induced by F-MuLV were treated with 5 mg/kg histamine (**A**), 3 mg/kg famotidine (**B**), 3 mg/Kg diacerein (**C**) and 3 mg/Kg tetrandrine (**D**) for two weeks. Mice were observed for the development of leukemia and scored at the onset of death. The tumor growth was calculated by Student's t-test and death was used to plot a Kaplan–Meier survival curve. (**E**) In this model, FLI1 controls HDC transcription, which itself regulates histamine production involved in allergenic reaction. HDC is also controls CXCR2 and IL1B associated with asthmatic reaction. These inflammatory factors likely effects leukemia progression through inflammation. While tetrandrine inhibits HDC through FLI1, EGC controls transcription of HDC through another mechanism.

(Figure 6E). Some of the inflammatory genes associated with asthmatic reaction, including IL1B and CXCR2,[25,26] may control leukemia progression through the tumor microenvironment.[13,14] Thus, HDC pathway inhibitors in addition to allergy and asthma, may be repurposed for the treatment of leukemia.

Discussion

HDC expression is required for various biological function and cancer, although the mechanism by which this enzyme is regulated are poorly understood. The oncogenic transcription factor FLI1, plays a major role in various biological functions, including hematopoiesis and immune function.[17] FLI1 regulates multiple genes in different context.[17] In this study, we showed that FLI1 directly regulates the *HDC* gene in leukemic cell lines (HEL and K562). Moreover, we show that FLI1 upregulates genes associated with asthmatic response and inflammation through HDC dependent and independent mechanisms. Unlike FLI1 which controls leukemia induction, proliferation and tumor progression, we demonstrated that HDC inhibition does not affect leukemic cell proliferation in culture. However, inhibition of inflammatory genes some of which are induced by HDC blocks leukemia progression emphasizing the importance of HDC and inflammation in leukemia progression.[26] This study suggests that FLI1 regulation of HDC is required for its normal biological functions and likely in leukemia progression.

Murine Fli-1 was historically identified as an oncogene in leukemias and is involved in oncogenic translocation that drives human Ewing's sarcoma.[17] We have previously identified several inflammatory genes including *IL1B* and *TNF*, regulated by FLI1 that may accelerate leukemia progression via the tumor microenvironment.[26] However, in contrast to other reports,[5,10] exogenous administration of histamine or histamine biosynthesis inhibitors did not modulate leukemia cell proliferation in culture. Similarly, knockdown of HDC had no effect in cell proliferation. However, in FLI1 and HDC knockdown cells, the expression of the inflammatory genes IL1B and CXCR2 is downregulated in leukemic cells. As FLI1 is known to directly bind to the IL1B promoter and regulate its expression, HDC is also shown here to induce IL1B independently of FLI1, suggesting multiple mechanisms of regulation of this inflammatory factor. Interestingly, diacerein inhibits several inflammatory factors including IL1B,[30–32] and exhibits a strong inhibitory effect on cancer progression in a mouse model of leukemia driven by high Fli-1. These results raised the possibility that HDC may affect leukemia progression through regulation of the inflammatory genes within tumor microenvironment. These results are supported by previously published reports indicating a role for histamine in regulation of cytokine expression in tumor models.[11,33] How critical is the contribution of HDC in leukemia is an interesting area of research that may require further confirmation in future studies.

During asthmatic reaction, expression of 6 biomarkers (6GS) predicts inflammatory response in patient's saliva.[25] Interestingly, three of these genes, CPA3, IL1B and CXCR2, are regulated by FLI1. In protein-protein interaction database (STRING), based on co-expressed genes and data mining, predicts CPA3 interacts with HDC protein. CPA3 could then bind to HDC and alter its biological function. Indeed, co-expression of both CPA3 and HDC was previously reported in mast cells.[34,35] CPA3 is also known for its association with inflammation, allergy and asthmatic reaction.[27,28] Regulation of both HDC and CPA3 by FLI1 may therefore indicate a role for this TF in allergy and asthma. Whether CPA3 interaction with HDC can alter allergy and asthmatic reactions is an interesting question that needs further investigation.

Since HDC controls various diseases including allergic and asthmatic reactions, significant effort has been invested to uncover its regulation. FLI1 is shown to play a critical role in regulating several genes including HDC, GATA2, AHR, AHRR, BHLHE40 and MITF that are involved in asthmatic reaction. A strong correlation was also seen between FLI1, GATA2 and its downstream targets AHR, AHRR and BHLHE40. However, FLI1 appears to negatively regulate MITF whose expression correlated with its downstream target HDC.[24] These results suggest that AHR, BHLHE40 and AHRR are targets of FLI1-induced GATA2, but HDC likely regulates MITF, as previously described.[24] Further studies are necessary to investigate this relationship at the molecular level.

As HDC plays a critical role in development of allergy and asthma, extensive research has been conducted to develop inhibitors of HDC to combat diseases induced by this enzyme. Epigallocatechin gallate (EGCG) was previously identified as a strong inhibitor of histamine release.[23] Herein, for the first time we showed that its derivative EGC blocks transcription of HDC. We showed that suppression of HDC by EGC is independent of FLI1 and its downstream

effector GATA2, but is likely regulated by MITF. Interestingly, EGC appears to activate BHLHE40 independently of GATA2, through a mechanism that remained to be elucidated in future studies.

Tetrandrine is also identified as potent compound that suppresses HDC transcription,[29] but the underlying mechanism is unknown. Here we showed that this compound strongly inhibits FLI1 protein expression, but at lower level its transcription. Molecular docking analysis revealed high affinity interaction of tetrandrine to the FLI1 DNA-binding domain. We previously reported that FLI1 negatively regulates miR145, which controls FLI1 protein expression.[19] Higher expression of miR145 due to FLI1 inhibition triggers downregulation of FLI1, that further restricts its transcriptional activation. As expected, FLI1 inhibition by tetrandrine also attenuates cell proliferation in culture and leukemia progression in a mouse model of erythroleukemia in which FLI1 activation is the major driver of leukemogenesis.[16] These results implicate tetrandrine as a novel and strong inhibitor of FLI1.

While the results demonstrate a role for HDC downstream of FLI1, the exact effect of histamine on leukemia progression and microenvironment remains to be fully elucidated. Whether FLI1 affects solid cancer inflammatory response has not been explored in this study. How FLI1 orchestrates an inflammation response and its role in asthma and allergy as well as whether FLI1 inhibitory drugs can attenuate these diseases needs further investigation. Finally, whether FLI1 controls other enzymes that like HDC controls Allergy and asthma, is a critical question that remains to be addressed in future studies.

Conclusion

FLI1 is shown herein to regulate HDC, which alters inflammatory signal associated with allergy, asthmatic reactions and likely cancer. In cell lines, while HDC inhibition does not affect cell proliferation, inflammatory genes induced by this enzyme in response to FLI1 activation may contribute to leukemia progression through the tumor microenvironment. Among inhibitors targeting HDC, we showed that EGC blocked transcription of this enzyme independently of FLI1 and GATA2. Another inhibitor, Tetrandrine was found to inhibit HDC through direct binding to FLI1, leading to suppression of its transcriptional activation ability. These results demonstrate the critical role by which HDC regulation by FLI1 plays in controlling inflammatory signals and leukemia progression.

Abbreviation

HDC, Histamine decarboxylase; EGC, epigallocatechin; TS, Tourette syndrome; IMC's, immature myeloid cells; F-MuLV, Friend Murine Leukemia virus; FBS, FLI1 binding site; EGCG, Epigallocatechin gallate; CLC, Charcot-Leyden crystal galectin; CPA3, carboxypeptidase 3; DNASE1L3, deoxyribonuclease 1-like 3; liver/bone/kidnalkaline phosphatase, ALPLey; CXCR2, C-X-C motif chemokine receptor 2; IL1B, interleukin 1B; Tet, Tetrandrine; Fam, Famotidine; His, Histamine.

Data Sharing Statement

All data generated or analyzed during this study are included in this article.

Ethical Approval

The animal study was carried out in accordance with the ARRIVE guidline.[36] Animal care and experimental procedures were conducted with the accredited animal ethics committee of the Guizhou Medical University and Council of Animal Care (Approval ID #1900373). The animal welfare guidelines were then carried out according to Institutional Animal Care and Use Committee guidelines of Guizhou Medical University.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest.

References

1. Huang H, Li Y, Liang J, Finkelman FD. Molecular regulation of histamine synthesis. *Front Immunol*. 2018;9:1392. doi:10.3389/fimmu.2018.01392
2. Kennedy L, Hodges K, Meng F, Alpini G, Francis H. Histamine and histamine receptor regulation of gastrointestinal cancers. *Transl Gastrointest Cancer*. 2012;1(3):215–227.
3. Thangam EB, Jemima EA, Singh H, et al. The role of histamine and histamine receptors in mast cell-mediated allergy and inflammation: the hunt for new therapeutic targets. *Front Immunol*. 2018;9:1873. doi:10.3389/fimmu.2018.01873
4. Höcker M, Zhang Z, Koh TJ, Wang TC. The regulation of histidine decarboxylase gene expression. *Yale J Biol Med*. 1996;69(1):21–33.
5. Medina VA, Rivera ES. Histamine receptors and cancer pharmacology. *Br J Pharmacol*. 2010;161(4):755–767. doi:10.1111/j.1476-5381.2010.00961.x
6. Ercan-Sencicek AG, Stillman AA, Ghosh AK, et al. L-histidine decarboxylase and Tourette's syndrome. *N Engl J Med*. 2010;362(20):1901–1908.
7. Pittenger C. Histidine decarboxylase knockout mice as a model of the pathophysiology of Tourette syndrome and related conditions. *Handb Exp Pharmacol*. 2017;241:189–215. doi:10.1007/164-2016-127
8. Ohtsu H, Tanaka S, Terui T, et al. Mice lacking histidine decarboxylase exhibit abnormal mast cells. *FEBS Lett*. 2001;502(1–2):53–56. doi:10.1016/s0014-5793(01)02663-1
9. Blaya B, Nicolau-Galmés F, Jangi SM, et al. Histamine and histamine receptor antagonists in cancer biology. *Inflamm Allergy Drug Targets*. 2010;9(3):146–157. doi:10.2174/187152810792231869
10. Medina VA, Brenzoni PG, Lamas DJ, et al. Role of histamine H4 receptor in breast cancer cell proliferation. *Front Biosci*. 2011;3(3):1042–1060. doi:10.2741/e310
11. Cricco G, Martin G, Labombarda F, Cocca C, Bergoc R, Rivera E. Human pancreatic carcinoma cell line Panc-I and the role of histamine in growth regulation. *Inflamm Res*. 2000;49(Suppl 1):S68–S69. doi:10.1007/PL00000188
12. Cricco GP, Mohamad NA, Sambuco LA, et al. Histamine regulates pancreatic carcinoma cell growth through H3 and H4 receptors. *Inflamm Res*. 2008;57(Suppl 1):S23–S24. doi:10.1007/s00011-007-0611-5
13. Grauers Wiktorin H, Nilsson MS, Kiffin R, et al. Histamine targets myeloid-derived suppressor cells and improves the anti-tumor efficacy of PD-1/PD-L1 checkpoint blockade. *Cancer Immunol Immunother*. 2019;68(2):163–174. doi:10.1007/s00262-018-2253-6
14. Stadtmauer EA. Histamine dihydrochloride and interleukin-2 in the treatment of acute myeloid leukemia. *Semin Oncol*. 2002;29(3 Suppl 7):47–51. doi:10.1053/sonc.2002.33084
15. Ben-David Y, Giddens EB, Bernstein A. Identification and mapping of a common proviral integration site Fli-1 in erythroleukemia cells induced by Friend murine leukemia virus. *Proc Natl Acad Sci USA*. 1990;87(4):1332–1336. doi:10.1073/pnas.87.4.1332
16. Ben-David Y, Giddens EB, Letwin K, Bernstein A. Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the ets gene family, Fli-1, closely linked to c-ets-1. *Genes Dev*. 1991;5(6):908–918. doi:10.1101/gad.5.6.908
17. Ben-David Y, Gajendran B, Sample KM, Zacksenhaus E. Current insights into the role of Fli-1 in hematopoiesis and malignant transformation. *Cell Mol Life Sci*. 2022;79(3):163. doi:10.1007/s00018-022-04160-1
18. Cui JW, Vecchiarelli-Federico LM, Li YJ, Wang GJ, Ben-David Y. Continuous Fli-1 expression plays an essential role in the proliferation and survival of F-MuLV-induced erythroleukemia and human erythroleukemia. *Leukemia*. 2009;23(7):1311–1319. doi:10.1038/leu.2009.2
19. Wang C, Sample KM, Gajendran B, et al. FLI1 induces megakaryopoiesis gene expression through WAS/WIP-dependent and independent mechanisms; implications for Wiskott-Aldrich syndrome. *Front Immunol*. 2021;12:607836. doi:10.3389/fimmu.2021.607836
20. Liu T, Xia L, Yao Y, et al. Identification of diterpenoid compounds that interfere with Fli-1 DNA binding to suppress leukemogenesis. *Cell Death Dis*. 2019;10(2):117. doi:10.1038/s41419-019-1363-1
21. Liu T, Yao Y, Zhang G, et al. A screen for Fli-1 transcriptional modulators identifies PKC agonists that induce erythroid to megakaryocytic differentiation and suppress leukemogenesis. *Oncotarget*. 2017;8(10):16728–16743. doi:10.18632/oncotarget.14377
22. Langtry HD, Grant SM, Goa KL. Famotidine. An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in peptic ulcer disease and other allied diseases. *Drugs*. 1989;38(4):551–590. doi:10.2165/00003495-198938040-00005
23. Yamashita K, Suzuki Y, Matsui T, et al. Epigallocatechin gallate inhibits histamine release from rat basophilic leukemia (RBL-2H3) cells: role of tyrosine phosphorylation pathway. *Biochem Biophys Res Commun*. 2000;274(3):603–608. doi:10.1006/bbrc.2000.3200
24. Li Y, Liu B, Harmacek L, et al. The transcription factors GATA2 and microphthalmia-associated transcription factor regulate Hdc gene expression in mast cells and are required for IgE/mast cell-mediated anaphylaxis. *J Allergy Clin Immunol*. 2018;142(4):1173–1184. doi:10.1016/j.jaci.2017.10.043

25. Fricker M, Gibson PG, Powell H, et al. A sputum 6-gene signature predicts future exacerbations of poorly controlled asthma. *J Allergy Clin Immunol*. 2019;144(1):51–60. doi:10.1016/j.jaci.2018.12.1020
26. Chen B, Sheng D, Wang C, et al. FLI1 regulates inflammation-associated genes to accelerate leukemogenesis. *Cell Signal*. 2022;92:110269. doi:10.1016/j.cellsig.2022.110269
27. Dougherty RH, Sidhu SS, Raman K, et al. Accumulation of intraepithelial mast cells with a unique protease phenotype in T(H)2-high asthma. *J Allergy Clin Immunol*. 2010;125(5):1046–1053. doi:10.1016/j.jaci.2010.03.003
28. Takabayashi T, Kato A, Peters AT, et al. Glandular mast cells with distinct phenotype are highly elevated in chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*. 2012;130(2):410–420. doi:10.1016/j.jaci.2012.02.046
29. Teh BS, Seow WK, Chalmers AH, Playford S, Ioannoni B, Thong YH. Inhibition of histamine release from rat mast cells by the plant alkaloid tetrandrine. *Int Arch Allergy Appl Immunol*. 1988;86(2):220–224. doi:10.1159/000234575
30. Bharti R, Dey G, Ojha PK, et al. Diacerein-mediated inhibition of IL-6/IL-6R signaling induces apoptotic effects on breast cancer. *Oncogene*. 2016;35(30):3965–3975. doi:10.1038/onc.2015.466
31. Pasin JS, Ferreira AP, Saraiva AL, et al. Diacerein decreases TNF-alpha and IL-1beta levels in peritoneal fluid and prevents Baker's yeast-induced fever in young rats. *Inflamm Res*. 2010;59(3):189–196. doi:10.1007/s00011-009-0085-8
32. Mohan GC, Zhang H, Bao L, Many B, Chan LS. Diacerein inhibits the pro-atherogenic & pro-inflammatory effects of IL-1 on human keratinocytes & endothelial cells. *PLoS One*. 2017;12(3):e0173981. doi:10.1371/journal.pone.0173981
33. Takahashi K, Tanaka S, Furuta K, Ichikawa A. Histamine H(2) receptor-mediated modulation of local cytokine expression in a mouse experimental tumor model. *Biochem Biophys Res Commun*. 2002;297(5):1205–1210. doi:10.1016/s0006-291x(02)02360-4
34. Yan Z, Liu L, Jiao L, Wen X, Liu J, Wang N. Bioinformatics analysis and identification of underlying biomarkers potentially linking allergic rhinitis and asthma. *Med Sci Monit*. 2020;26:e924934. doi:10.12659/MSM.924934
35. Zhao X, Younis S, Shi H, et al. RNA-seq characterization of histamine-releasing mast cells as potential therapeutic target of osteoarthritis. *Clin Immunol*. 2022;244:109117. doi:10.1016/j.clim.2022.109117
36. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *BMC Vet Res*. 2020;16(1):242. doi:10.1186/s12917-020-02451-y