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SERPINB13 is a novel RUNX1 target gene

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Abstract

RUNX1 is a critical transcription factor during embryogenesis and neoplastic disease. To identify novel transcriptional targets of RUNX1 in the context of chromatin, we performed genome wide location analysis (ChIP-on-chip). Here we report that SERPINB13, a gene downregulated in head and neck cancers, is a novel RUNX1transcriptional target. RUNX1 binds the SERPINB13 promoter in chromatin to repress its transcription. Mutation of either RUNX1 binding site in the SERPINB13 promoter increased the activity of the promoter. Finally, overexpression of RUNX1 and concomitant decrease in SERPINB13 expression led to increased activity of cathepsin K, an enzyme inhibited by SERPINB13. These data demonstrate that RUNX1 is an important regulator of SERPINB13 and cathepsin K activity.

Keywords

RUNX; transcription; SERPINB13; chromatin immunoprecipitation

Introduction

RUNX1 is a transcription factor that binds to TGt/cGGT consensus site in gene regulatory elements [1; 2]. The last three nucleotides are critical for binding to RUNX1 [3] and 5 of the 6 nucleotides are important for RUNX proteins to bind the TCR enhancer [4]. RUNX1 is essential during development [5; 6] and conditional knockout of RUNX1 revealed that it is critical for the development of platelets and T-cells [7; 8].

RUNX1 is also involved in human malignancies. Sporadic and germline mutations and translocations in the RUNX1 gene are linked to leukemogenesis [9]. These alterations affect the DNA binding and transcriptional activities of RUNX1, indicating that RUNX1 transcriptional targets regulate proliferation and differentiation. Overexpression of RUNX1

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in endometrial carcinoma has been reported [10] and in a subset of B-ALL patients and AML patients with trisomy 21 [reviewed by Blyth et al [11].

Consensus RUNX1 binding sites were confirmed by EMSA in several genes [12]. Reporter studies demonstrate that RUNX1 regulates transcription through these binding sites. In hematopoietic cells, it upregulates expression of M-CSFR [13], GM-CSF [14; 15], CD4 silencer [16], IL-3 [17], and BLK [18]. Little is known, however, about RUNX1 regulation of these genes the natural context of chromatin.

To identify novel transcriptional targets of RUNX1 in chromatin, we performed genome wide location or ChIP-on-chip. We identified novel RUNX1 genes including SERPINB13, an inhibitor of cathepsins K and L, which are associated with inflammation, survival, cancer development [19], and bone remodeling [20]. RUNX1 repressed SERPINB13 resulting in increased cathepsin K enzymatic activity. We propose that RUNX1 repression of SERPINB13 alters cathepsin activity in normal cells and perhaps in tumor cells.

Materials and Methods

Cell culture

HaCaT and K562 were maintained in RPMI 1640 medium (Invitrogen) and 293T cells were maintained in DMEM supplemented with 10% FBS (Omega), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen).

Plasmids

pCAPP-HA-RUNX1b contains the HA-RUNX1b cDNA via SmaI. The pCAPP vector was created by digesting pCAGGS vector with HindIII and blunt-ending with T4 polymerase. The phosphoglycerate kinase promoter (PGK) regulates the puromycin resistance gene and was derived from the MSCVpac vector (Clontech).

MSCV-HA-RUNX1b-puro contains the HA-RUNX1b cDNA in the HpaI site of MSCV-puro (Clontech).

For control shRNA, primers used were: 5’-GATCCCCCCTCGAAGACATCGGCAGATTCAAGAGATCTGCCGATGTCTTCGAGGTTTTTA-3’ (top) and 5’-AGCTTAAAAACCTCGAAGACATCGGCAGATCTCTTGAATCTGCCGATGTCTTCGAGGGGG-3’ (bottom).

For RUNX1 shRNA, the primers used: 5’-GATCCCCACTTTCCAGTCGACTCTCATTCAAGAGATGAGAGTCGACTGGAAAGTTTTTTA-3’ (top) and 5’-AGCTTAAAAAACTTTCCAGTCGACTCTCATCTCTTGAATGAGAGTCGACTGGAAAGTGGG-3’ (bottom)

Oligos were inserted into pSuper.retro.puro (Oligoengine) via BglII-HindIII sites.

Wild-type SERPINB13 p romoter plasmids were provided by Dr. Clayman (MDACC) [21]. Mutagenesis of the wild-type promoter was performed with the primers (Quikchange XL, Stratagene):

For TGTGGT consensus site 1 mutation: 5'-GCTCTGTCCATCTGTTAGAAGTAAAGTCTGCAGTTAATGTATGTGTTCATCAGGC-3', 5’-

GCCTGATGAACACATACATTAACTGCAGACTTTACTTCTAACAGATGGACAGAG C-3'

For the consensus TGCGGT site 2: 5' - TCCCAGCGTTGGCGGTACCTTGTAATCACCTGCTAGGATAAAAATTCTGATGCCT GG- 3', 5' - CCAGGCATCAGAATTTTTATCCTAGCAGGTGATTACAAGGTACCGCCAACGCTG GGA- 3'

The pRL-null (Promega) was co-transfected with reporter plasmids to normalize transfections with *Renilla* luciferase.

Generation of HA-RUNX1 K562 cells

Approximately 1×10^7 K562 cells were electroporated in 400 µl serum free media at 250V/ 950 µF with 50 µg of linearized pCAPP-HA-RUNX1b. Two days after electroporation, cells were selected in 4 µg/ml puromycin. Resistant cells were pooled and HA-RUNX1 expression was confirmed by immunoblotting with anti-HA antibody (Covance). Cell cycle analysis was performed as described [22].

Generation of retroviruses and HaCaT stable lines

Approximately 5×10^6 293T cells were co-transfected with 15 µg MSCV-puro vector (Clontech) or MSCV-HA-RUNX1b-puro and 10 µg of amphotrophic packaging pCL10A1 using calcium phosphate precipitation. Retrovirus was collected 48 hours after transfection and added to 5×10^5 HaCaT cells/well with 16 µg/ml polybrene (Sigma). Cells were centrifuged at 1400× g for 2.5 hours at 32°C followed by selection in 4 mg/ml puromycin.

For RUNX1 shRNA HaCaT pools, retroviruses were prepared from control vector pSuper (Oligoengine), pSuper-RUNX1 control shRNA, or pSuper-RUNX1shRNA. Reduction of endogenous RUNX1 was detected by immunoblotting with a RUNX1 specific antibody [23].

Chromatin Immunoprecipitation

Approximately 4×10^8 HA-RUNX1 K562 cells were crosslinked in PBS containing 1.1% formaldehyde for 10 minutes on ice followed by 0.125 M glycine treatment. Nuclei isolation was performed as described [24] and DNA was sonicated to an average length of 0.5 – 1 kb. Soluble chromatin was precleared with 100 µl sepharose beads (GE Healthcare) followed by dilution in 0.1% Triton X-100 and 0.1% sodium deoxycholate to 1 ml. Immunoprecipitations with 5 µg of anti-HA (Covance) or mouse IgG control (Sigma I5381) were performed at 4°C overnight. Immune complexes were obtained with 30 µl protein G sepharose (GE healthcare) including 1 µg/µl BSA and 1 µg/µl herring sperm DNA for 1 hour at 4°C. Immunoprecipitates were washed 7 times in 1 ml of RIPA buffer with 5 minute rotations between washes. A final wash with 1 ml of TE was performed. Crosslinks were reversed by incubation at 65°C overnight. DNA was purified by RNase and proteinase K, followed by phenol:chloroform extraction [24] and Qiaquick column (Qiagen).

Preparation of DNA for microarray

DNA from ChIP was used in a positive control PCR reaction for enrichment of human PU.1 URE [28]. PU.1 URE primer sequences were: forward 5'-GTTTCTCTGGGCCGCTGT-3', reverse 5'-AGCTGCCCCTGTTTCCACATC-3'. DNA was prepared for microarray to the promoter microarray as described [25]. Statistical analysis was performed after scanning fluorescent microarray slides using GenePix Pro software (Molecular Devices). Putative target promoters were identified as enriched relative to total input (genomic DNA) with a P

value of 0.001. Each promoter microarray was performed two independent times using independent ChIP.

SERPINB13 promoter PCR

Primers used for SERPINB13 promoter regions: Region 1 F: 5'-AAGCATTATGGCCGACTCAG-3' Region 1R: 5'-AGGGCATATGGGTCATGTG-3', Region 2F: 5'-GTGTGTGCGTGTGTGTGTG -3' and Region 2R: 5'-CACCGCAGGTGATTACAATG-3'.

Approximately 0.1% of DNA from ChIP was used in amplification of each promoter region using quantitative real-time PCR reactions with (Platinum SYBR green QPCR, Invitrogen) and 0.5 μM of each primer. Duplicate reactions were analyzed by iCycler iQ (Bio-Rad Laboratories) with melt curve analysis. Products were analyzed by gel electrophoresis. Relative expression was calculated by correcting for Ct value differences between IgG control and HA immunoprecipitates. Promoter binding in IgG control was set to 1 and the relative binding of HA-RUNX1 is graphed.

Luciferase Reporter Assays

2×10^6 cells were electroporated with 12μg of DNA in 180 μl serum free RPMI at 250V/950 μF and assayed 24 hours later.

Ten μg of reporter plasmid were co-transfected with 1 μg of vector control or transcription factor indicated, and 0.2 μg of pRL-null. Electroporated cells were cultured for one day following transfection and then lysed for luciferase assays using the Dual Reporter Assay (Promega). Relative light units were measured using a luminometer (BD Monolight, BD Biosciences). Data from three independent experiments were averaged.

Quantitative RT-PCR

RNA was prepared using RNAzolB Reagent (TEL-TEST). cDNA was prepared (SuperScript II RT, Invitrogen) for quantitative real-time PCR reactions with Platinum SYBR green QPCR Supermix (Invitrogen) and 0.5μM of each primer. Duplicate reactions were analyzed using the iCycler iQ (Bio-Rad Laboratories) with melt curve analysis. Products were analyzed by agarose gel electrophoresis. For each gene analyzed, cDNA from at least three independent vector control and RUNX1 expressing pools were analyzed. Relative expression was calculated by correcting for Ct value differences for human GAPDH loading. Vector control was set to 1 and the relative fold change is graphed for SERPINB13 mRNA.

Primers used were hGAPDHF: 5'- GAGCTGAACGGGAAGCTCACTGG-3', hGAPDHR: 5'- CAACTGTGAGGAGGGGAGATTCAG-3', SERPINB13F: 5'-AGCCGATGAAAGTCGAAAGA-3', SERPINB13R: 5'-CTGCAAGTCCTCCAGGAAAG- 3'.

Cathepsin activity assay

1×10^5 cells were plated on 22 mm square coverslips. 16–20 hours after plating, cathepsin substrates were added at 1× concentration (Cell Technology, CDK200-1, CDK300-1) to media for 45 minutes at 37°C, followed by Hoechst 33342 staining for 10 minutes at 37°C. Coverslips were washed twice in 1× PBS and mounted on glass slides. Fluorescence was detected using a BP 360–400 filter for Hoechst 33342 and BP 515–560 filter for the cathepsins, and images were acquired using a Spot Digital camera (Diagnostic Instruments). Multiple images were acquired from 3 independent vector control pools or HaCaT-HA-RUNX1 expressing pools.

Results

RUNX1 Chromatin IP and promoter microarray

To identify novel RUNX1 target genes, we generated K562 cells expressing HA-tagged RUNX1 for chromatin immunoprecipitation assays (ChIP) (Fig. 1A). Expression of HA-RUNX1 in K562 cells did not inhibit cell proliferation compared to the vector control as reflected in the cell cycle distributions (Fig. 1B). The K562 HA-RUNX1 cells were expanded to perform ChIP with HA antibody or control mouse IgG. To validate the specificity of RUNX1 chromatin immunoprecipitation we analyzed binding to a known target gene, human PU.1 upstream regulatory region (URE) [25]. The PU.1 URE was enriched in HA-RUNX1 chromatin immunoprecipitates but not in the IgG control (Fig. 1C). This confirmed that the HA-RUNX1 ChIP was successful and this chromatin was used for hybridization to a promoter microarray.

DNA that co-precipitated with either IgG or HA antibody was purified, amplified by ligation mediated PCR (LM-PCR) as described [24] and hybridized individually to human promoter microarrays containing approximately 8000 upstream regulatory regions. Following microarray analysis combined with statistics, genes enriched in HA-RUNX1 immunoprecipitates compared to genomic input with varying P values between 0 and .0088 were considered as putative RUNX1 target genes. Promoters enriched in control IgG micorarrays were considered false positives. Among the 27 novel RUNX1 target genes identified (Table 1), four genes were identified in two independent experiments, including ETF1, SERPINB13, HSD3B2, and cyclin A1.

Eight putative RUNX1 targets from Table 1 were selected for confirmation. As shown in Table 2, ChIP assays with promoter specific primers revealed that the RUNX1 bound the promoters of SERPINB13, RCC1, RNF19, HSP 105B, and ZNF225. In contrast, we were unable to detect association with several targets including RB1, TNFRSF21, and ETF1, which may be due to transient association of RUNX1 with these promoters or insufficient sensitivity of our ChIP assay.

SERPINB13 is a novel RUNX1 target gene

The SERPINB13 gene was selected for further study since it was one of the most highly enriched in RUNX1 ChIP. The SERPINB13 gene was identified as a gene modulated in squamous cell carcinomas and regulated by UV irradiation [26] [27]. The human SERPINB13 promoter [21] contains consensus RUNX, C/EBP and Ets binding sites (Fig. 2A). We hypothesized that RUNX1 may directly regulate the SERPINB13 promoter through a consensus TGt/cGGT binding site or instead indirectly occupy the promoter through known interacting partners Ets-1 [28; 29] or C/EBP [30].

To confirm promoter microarray results, chromatin immunoprecipitates of HA-RUNX1 or control IgG were used to amplify two different regions of the SERPINB13 promoter. The locations of promoter regions amplified are indicated as R1 and R2 in figure 2A. Region 1 does not contain consensus RUNX1 binding sites and showed an average of 5-fold enrichment in HA-RUNX1 ChIP compared to IgG control. Region 2 containing two consensus sites was enriched 28-fold in HA-RUNX1 immunoprecipitates compared to IgG (Fig. 2B). These data confirm that RUNX1 binds the human SERPINB13 promoter.

We next tested if RUNX1 regulates SERPINB13 transcription. RT-PCR analysis of SERPINB13 was performed in K562 vector control or HA-RUNX1 expressing cells that were used in genome wide location analysis. SERPINB13 mRNA levels in K562 cells were difficult to detect by RT-PCR (data not shown). We therefore examined RUNX1 regulation

of SERPINB13 transcription in HaCaT cells, a cell line reported to express high levels of functional SERPINB13 [26; 27] which we confirmed by QPCR (data not shown).

HaCaT cells express endogenous RUNX1 isoforms that were decreased by RUNX1 shRNA but not by a negative control shRNA (Fig. 3A). We examined three independent control pools or RUNX1 shRNA pools for changes in SERPINB13 expression by real-time RT-PCR. SERPINB13 mRNA levels increased in cells expressing reduced levels of RUNX1 (Fig. 3B), suggesting that RUNX1 may repress SERPINB13 expression. Stable overexpression of RUNX1 in HaCaT cells was also performed (Fig. 3C). Three independent control pools or HA-RUNX1 expressing pools were examined for SERPINB13 expression. RUNX1 overexpressing cells had significantly less SERPINB13 mRNA levels (11-fold repression) compared to vector control cells (Fig. 3D). These data indicate that RUNX1 is a negative regulator of SERPINB13 transcription.

We next examined the mechanism of RUNX1 repression of SERPINB13 levels. As diagrammed in figure 2A, the SERPINB13 promoter contains two consensus RUNX1 sites, a TGTGGT site 1 and TGCGGT site 2 in Region 2. The consensus site 1 and the consensus site 2 were mutated to TGTTAG or TGCTAG, respectively, which eliminates binding of RUNX1 to target sites in EMSA analysis [3]. HaCaT cells were transiently transfected with either the wild-type SERPINB13 reporter (WT), the site 1, or the site 2 mutant reporters. Interestingly, mutation of the consensus site 1 or the site 2 consistently led to increased reporter activity in HaCaT cells (Fig. 4A) and in K562 cells (Fig. 4B). Additionally, transfection of HaCaT cells with the DNA biding domain of RUNX1 was sufficient to inhibit promoter activity (data not shown). These data suggest that RUNX1 represses SERPINB13 transcription by binding to consensus sites in the promoter.

RUNX1 upregulates cathepsin K activity

SERPINB13 protein inhibits lysosomal cathepsins K and L in vitro [31; 32]. Therefore, we tested whether repression of SERPINB13 by RUNX1 could increase cathepsin K or L enzymatic activity in cells. Control or RUNX1 expressing HaCaT cells from three independent pools were incubated with a cell permeable substrate specific for either cathepsin K or L. Following cleavage of the cathepsin substrate by active enzyme, fluorescence was visualized by microscopy. RUNX1 expressing cells contained higher cathepsin K activity compared to vector control cells (Fig. 4C), however no significant increase in cathepsin L activity was detected (Fig. 4C). These data further support that RUNX1 regulates SERPINB13 expression and enzymatic activity in cells.

Discussion

Cellular proliferation and differentiation involves the activity of transcription factors that activate or repress critical target genes. Knowledge of RUNX1 target gene regulation in the context of chromatin is lacking for many genes. With the combination of chromatin crosslinking of RUNX1 and the high throughput microarray technique, we have identified several new RUNX1 target genes and characterized one novel target gene, SERPINB13.

SERPINB13 is an inhibitor of lysosomal cathepsin enzymes K and L [31; 32] and is downregulated in head and neck cancers [21] suggesting that these tumors may have elevated activity of cathepsins K and L. Cathepsin K facilitates invasion of tumor cells due to degradation of the ECM and is expressed in breast [33] and prostate cancer [34], indicating that SERPINB13 may be repressed in some of these tumors. Future studies should explore if RUNX1 is overexpressed in these cancers, disrupting the ratio of SERPINB13 and cathepsin activity. The advantage of downregulating SERPINB13 in tumors may be linked to its anti-angiogenic activity [35].

We demonstrate for the first time that reduction of SERPINB13 levels alters cathepsin K activity in cells, supporting previous in vitro enzymatic studies [31; 32]. Cathepsin K is the major proteinase secreted by osteoclasts during bone remodeling. Bone remodeling by osteoclasts that line the bone marrow facilitates mobilization of hematopoietic progenitor cells [36]. RUNX1 regulates the number of hematopoietic stem cells in the bone marrow, and cathepsin K knockout mice have alterations in the number of hematopoietic cells in the bone marrow [37]. Therefore, RUNX1 may influence cathepsin K activity in the bone marrow by altering SERPINB13 levels.

Cyclin A1 was also identified in this study. Overexpression of cyclin A1 leads to abnormal myelopoiesis and leukemia development [38], similar to mutation of the RUNX1 gene in patients with FPD or sporadic gene mutations. Therefore, it is possible that RUNX1 may negatively regulate cyclin A1 expression in hematopoietic cells. Other microarray targets worthy of further investigation include IL-18, which is important for angiogenesis [39] and immune modulation [40], two processes regulated by RUNX1 in vivo. Thus, genome wide location of RUNX1 has provided a platform to identify novel functions associated with differentiation and transformation.

Highlights

Highlights of Boyapati et al BBRC submission 5–2011

- Genome wide location analysis identifies novel RUNX1 targets
- SERPINB13 is repressed by RUNX1
- RUNX1 increases cathepsin K activity

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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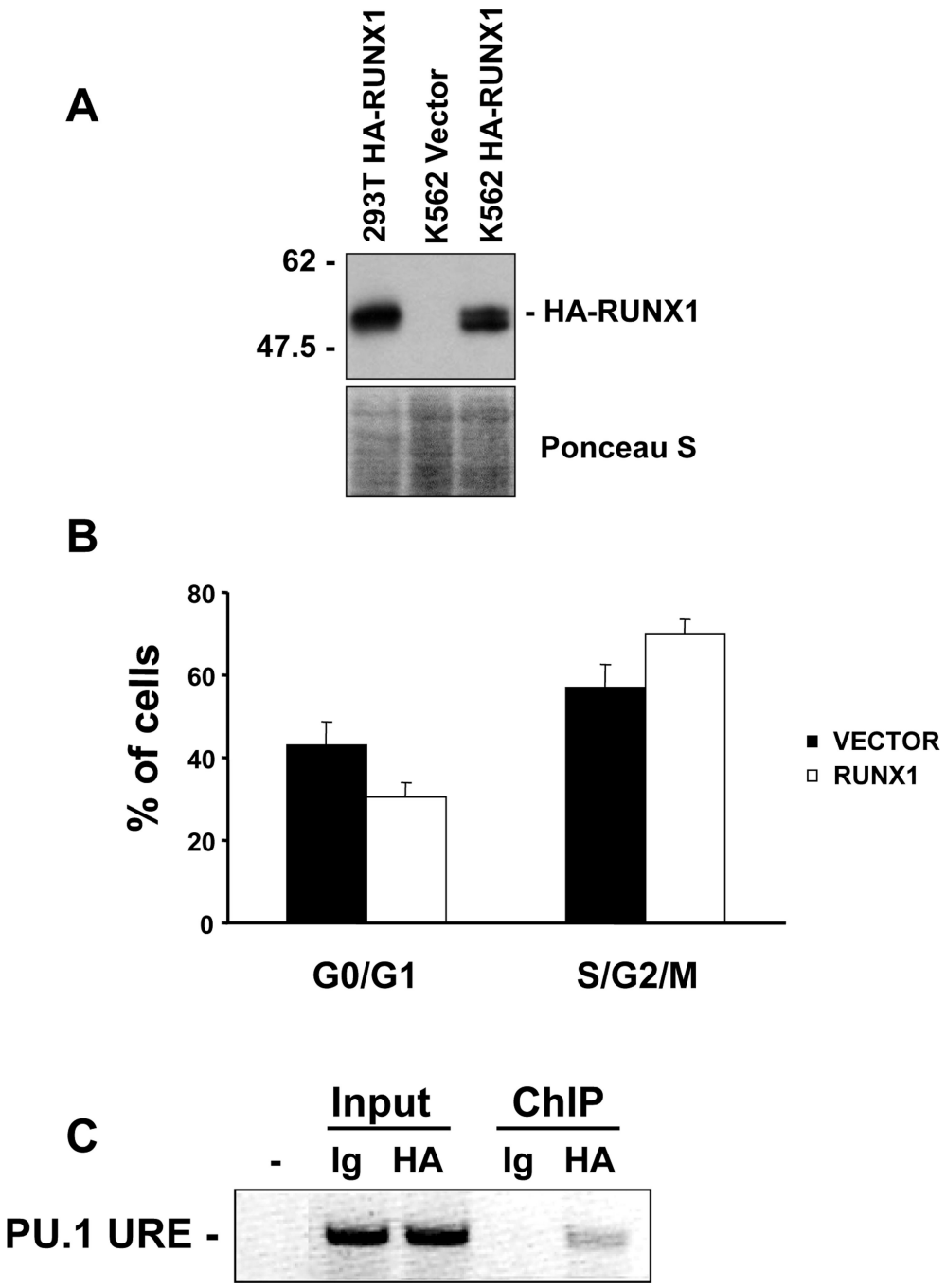

Fig. 1.

Analysis of K562-HA-RUNX1b cells. (A) Immunoblot from K562 control pool or HA-RUNX1b expressing pool. 293T cells transfected with the HA-RUNX1b expression plasmid were used as a positive control. Ponceau S stain reflects relative protein loading. (B) Cell cycle distributions of vector control pools and HA-RUNX1b expressing pools. Cells were plated at the same density and stained with propidium iodide. The bar graph shows the average percentages of cells in G0/G1 or in S/G2/M from ModFit analysis. (C) ChIP of K562-HA-RUNX1b cells. Soluble chromatin was used for immunoprecipitation with either IgG control antibody (Ig) or HA antibody (HA). An inverted ethidium bromide gel is shown after PCR for the human PU.1 URE. Input lanes reflect the relative chromatin input for each

immunoprecipitation and the ChIP lanes show the relative enrichment of the PU.1 URE in the HA-RUNX1 ChIP.

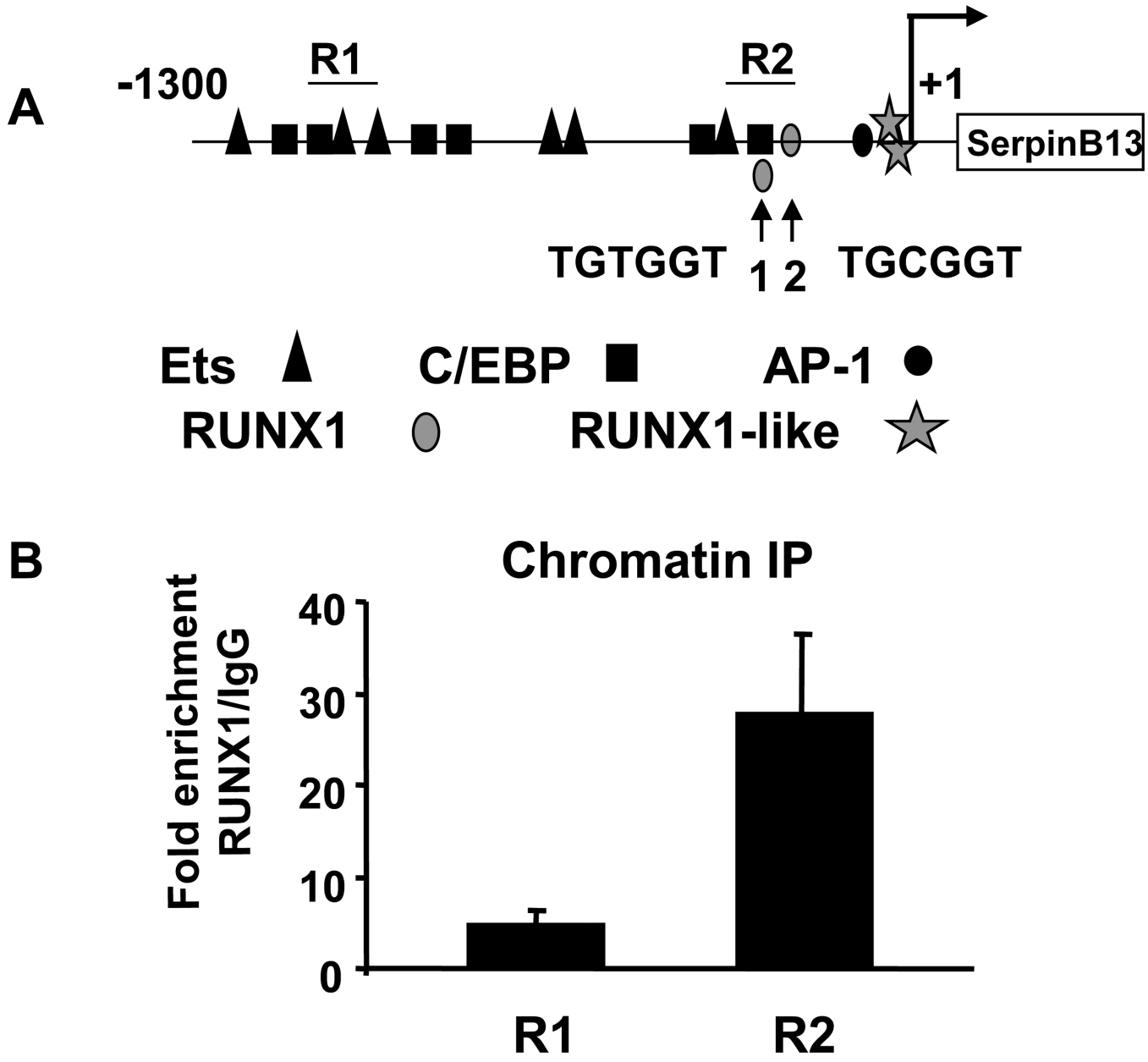

Fig. 2.

RUNX1 binds the SERPINB13 promoter. (A) Schematic of the human SERPINB13 promoter with putative binding sites for C/EBP, Ets, RUNX1, and AP-1. Two different regions amplified in quantitative ChIP are indicated as R1 and R2. Two consensus RUNX1 sites are indicated and are located at −265 and −178 relative to the start site identified by Nakashima et al [24]. (B) ChIP of IgG and HA-RUNX1 from K562-HA-RUNX1 cells. Precipitated DNA was used in real-time PCR of the SERPINB13 promoter using primers that encompass either R1 or 2. The fold enrichment of DNA in HA-RUNX1 chromatin IP compared to control IgG was calculated from Ct values after correcting for input DNA.

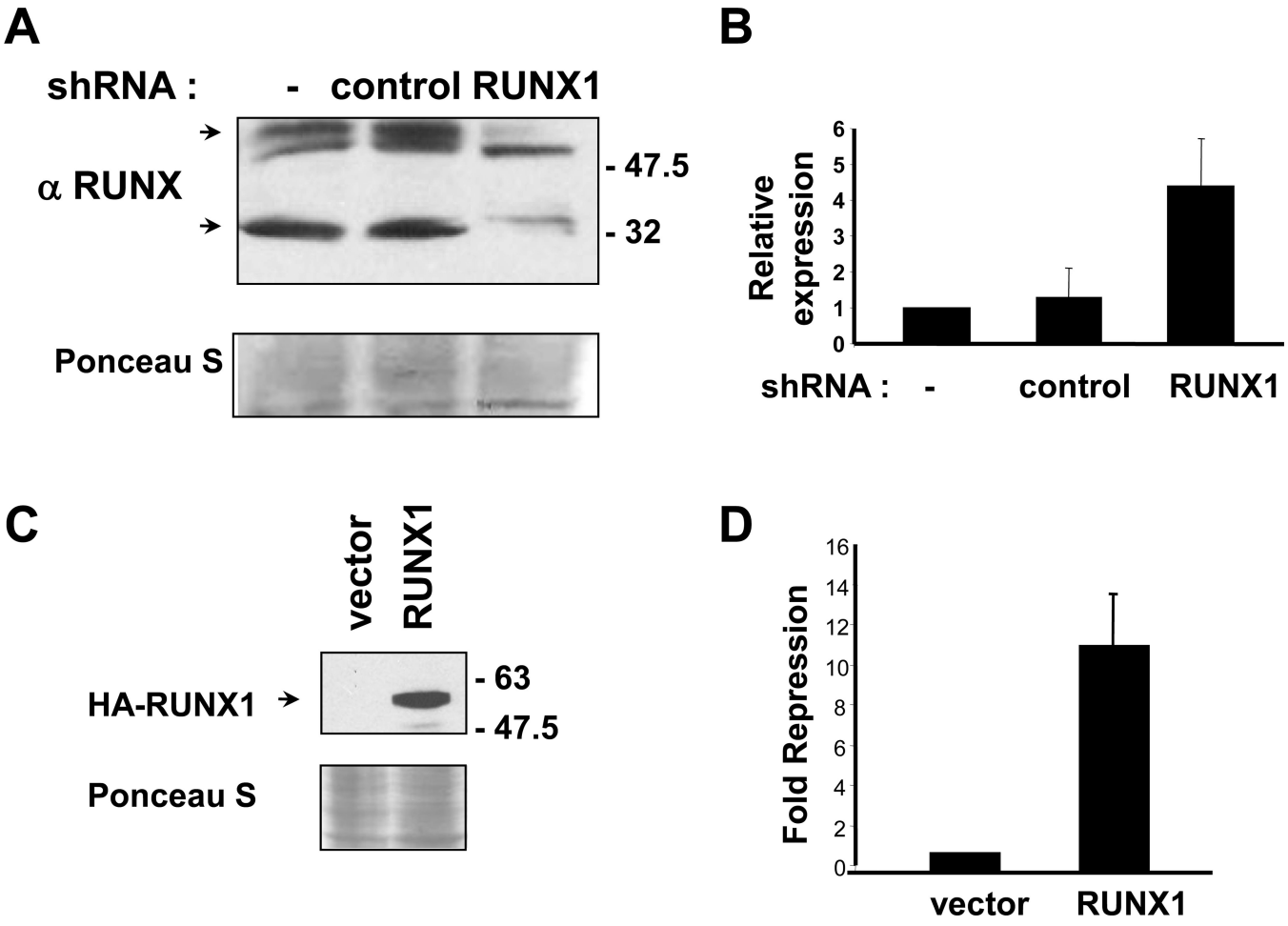

Fig. 3.

RUNX1 regulates SERPINB13 expression. (A) Immunoblotting for RUNX1 from HaCaT cells stably expressing control or RUNX1 shRNA or vector control (−). Ponceau S staining shows the relative protein loading. Arrows indi cate RUNX1 isoforms decreased by shRNA. (B) Quantitative RT-PCR analysis of SERPINB13 mRNA using RNA prepared from 3 independent vector control (−) pools, or 3 control or RUNX1 shRNA pools. The relative expression was calculated after correcting for cDNA input using human GAPDH primers. Error bars indicate the differences for data averaged from 3 pools. (C) HA immunoblot from HaCaT stably expressing HA-RUNX1 or vector control. (D) Quantitative RT-PCR analysis of SERPINB13 mRNA using RNA prepared from 3 independent vector control pools or 3 HA-RUNX1 expressing pools. The fold repression was calculated after correcting for cDNA input as described in B.

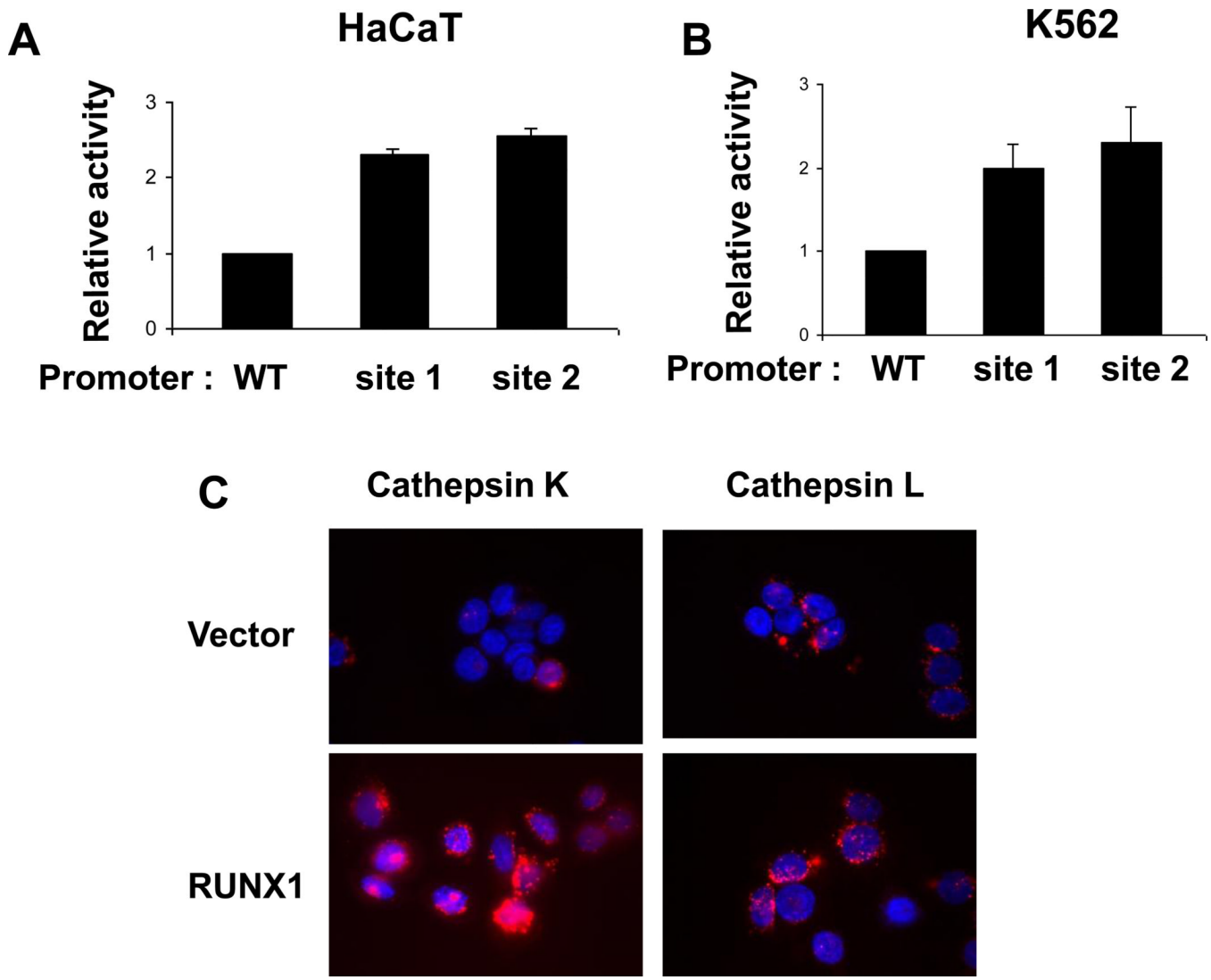

Fig. 4.

Regulation of SERPINB13 promoter activity. (A) Relative activities of the WT, RUNX1 site 1 mutant, or RUNX1 site 2 mutant SERPINB13 reporters in HaCaT cells. The activity of the WT promoter was set to 1. The average of three independent experiments is indicated and error bars show the standard deviations. (B) Same as A except the luciSferase activity was measured in K562 cells. (C) Micrographs of HaCaT vector control cells or HA-RUNX1 cells incubated with cathepsin K or cathepsin L substrates. Hoechst staining shows nuclei (blue) and red fluorescence in lysosomes reflects the cathepsin enzymatic activity.

Table 1
Putative RUNX1 targets from promoter microarray

The results from two independent genome wide location analyses are listed. The gene symbols and the P values and fold enrichment in HA ChIP relative to genomic input are indicated.

Gene	Accession	P value	Array
CCKAR	NM_000730	1.00E-06	2.61
SLC22A5	NM_003060	0	3.98
* ETF1	NM_004730	0	3.25
SF3B2	NM_006842	0	2.51
HSP105B	NM_006644	7.97E-04	2.06
CCL19	NM_006274	0	2.48
RCC1	NM_001048194	0	4.61
RB	NM_000321	0	2.60
TNFRSF21	NM_014453	3.30E-05	2.26
* SERPINB13	NM_012397	0	10.15
IL18	NM_001562	1.50E-05	2.03
* HSD3B2	NM_000198	3.38E-03	10.90
GALK2	NM_002044	4.31E-03	9.39
GHSR	NM_004122	6.00E-03	6.74
MUSK	NM_005592	7.55E-03	6.34
* CCNA1	NM_003914	6.94E-03	7.98
ZNF225	NM_013362	8.81E-03	5.89
UQCRB	NM_006294	9.36E-04	2.67
ADRB2	NM_000024	3.36E-04	3.12
RNF19	NM_183419	3.40E-05	9.46
HIST1H3I	NM_003533	9.34E-04	2.67
GPC6	NM_005708	6.28E-04	2.82
PFTK1	NM_012395	8.59E-04	2.72
RBM8A	NM_005105	7.68E-04	2.74
RPS6	NM_001010	4.73E-04	2.93
HIST1H2BC	NM_003526	9.59E-04	2.67
REG1A	NM_002909	5.80E-04	2.85

* Genes identified in two independent microarrays

Table 2

Genes confirmed by promoter ChIP

Genes listed with GenBank Accession numbers, known functions, enrichment in microarray and whether confirmed to interact with HA-RUNX1 in ChIP (YES) or not detected (ND).

Gene	Accession	Function	Microarray	ChIP
ETF1	NM_004730	Translation	3.3	ND
HSP105B	NM_006644	Chaperone	2.1	YES
RB	NM_000321	Tumor Suppressor	2.6	ND
RCC1	NM_001048194	Ran GNEF	4.6	YES
RNF19	NM_183419	E3 ligase	9.5	YES
SERPINB13	NM_012397	Cathepsin inhibitor	10	YES
TNFRSF21	NM_014453	Apoptosis	2.3	ND
ZNF225	NM_013362	Transcription Factor	5.9	YES