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Original Article

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GAS2–Calpain2 axis contributes to the growth of leukemic cells

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Abstract

Growth arrest specific 2 (GAS2) modulates cell cycle, apoptosis, and Calpain activity. GAS2–Calpain2 axis is required for the growth of BCR-ABL$^+$ hematopoietic cells and chronic myeloid leukemia cells. However, the expression of GAS2 in acute leukemia patients remains unclear and what role GAS2–Calpain2 axis plays in these leukemic cells is not known yet. In this study, GAS2 was found to have significantly higher expression in 16 various leukemic cell lines than in control cells. Using THP-1 cells (from acute myeloid leukemia patient, AML) and Jurkat cells (from acute lymphoid leukemia patient, ALL) as models, we found that GAS2 silence led to elevated Calpain activity, decreased cellular growth, and inhibition of colony-forming cell (CFC) production; and these effects could be rescued by GAS2 re-expression. Moreover, GAS2 silence prevented tumor formation of THP-1 cells in nude mice. In both THP-1 and Jurkat cells, GAS2 interacted with Calpain2 rather than Calpain1. The dominant negative form of GAS2 (GAS2DN, GAS2Δ171-313) had similar effects on leukemic cells through the activation of Calpain. Importantly, Calpain2 silence abolished the proliferation inhibition induced by GAS2 targeting. We also found that GAS2 was aberrantly expressed and Calpain activity was decreased in clinical isolates from acute leukemia patients. Taken together, our results demonstrated the deregulation of GAS2 in both AML and ALL and the requirement of GAS2–Calpain2 axis for the growth of leukemic cells, which will help to understand the molecular pathogenesis of hematological malignancies and possibly to develop novel approaches to treat these deadly diseases.

Key words: GAS2, Calpain2, RNAi, GAS2DN, immunoprecipitation

Introduction

A group of genes are up-regulated when NIH-3T3 cells are subject to serum deprivation, one of which is named as growth arrest specific 2 (*GAS2*) gene [1]. It is well established that GAS2 is a microfilament-associated protein with diverse functions to regulate cell cycle and apoptosis [1–7]. At the meantime, GAS2 has been identified as an endogenous inhibitor of m-Calpain (Calpain2), a calcium-activated enzyme. It is proposed that the N-terminus of GAS2 binds with m-Calpain and the C-terminus of GAS2 inhibits the protease activity of Calpain, thus the N-terminus of GAS2 acts as the dominant negative form of GAS2 (GAS2DN). In addition, endogenous GAS2 interacts with Calpain2 in NIH-3T3 cells [8].

Conflicting data have suggested diverse functions of GAS2 in cancer biology. Benetti *et al*. [8] have shown that GAS2 is able to stabilize p53, which sensitizes cancer cells to the treatments of etoposide and methyl methanesulfonate. Costa-Mattioli *et al*. [9] have demonstrated

that 4E-BPs (eIF4E-binding proteins) promote cellular senescence and transformation resistance, in which p53 stability conferred by GAS2 is critical. Kondo *et al*. [10] have shown that GAS2 expression is impaired in breast cancer due to the accumulation of histone H3 lysine 27 trimethylation and the re-expression of GAS2 in tumor cells facilitates the apoptosis induced by etoposide. On the contrary, GAS2–Calpain2 axis controls a GSK3β-independent pathway to degrade β-catenin, and targeting GAS2 with both RNAi and GAS2DN results in the growth inhibition of colon cancer cells (HCT116) and BCR-ABL$^+$ hematopoietic cells [11,12]. Our previous study has demonstrated that GAS2 is up-regulated in chronic myeloid leukemia (CML) cells including stem/progenitor enriched CD34$^+$ cells. Targeting GAS2 leads to growth inhibition and sensitizes leukemic cells to the treatment of imatinib mesylate (IM). Interestingly, the inhibitory effect of GAS2DN on leukemic progenitor cells is significantly stronger than that against normal hematopoietic progenitor cells, suggesting the therapeutic potential of this approach [13]. We have also shown that Calpain2 silence is able to rescue GAS2DN-induced CML cells inhibition [13]. However, the interaction between GAS2 and Calpain2 in hematopoietic cells has not been reported yet. In addition, whether the deregulation of GAS2 is limited to CML and not in other types of leukemia especially acute leukemia is not known yet. Huang *et al*. [12] have revealed that GAS2 is regulated by interferon consensus sequence binding protein 8 (IRF8) and Ets variant 6 (ETV6). IRF8 deficiency may promote hematological transformation [14], and numerous mutations of ETV6 occur in various hematological malignancies [15]. Therefore, the hypothesis that GAS2 is deregulated in acute leukemia was proposed. If so, it will be interesting to explore the functional role of GAS2 in these cells. To date, IM treatment largely improves the management of CML [16]; however, the treatments of adult acute leukemia (AML and ALL) remain miserable [17]. Thus, it is critical to investigate the role of GAS2–Calpain2 axis in these malignant cells, which will shed new light on the molecular insights of these deadly diseases.

In the present study, the expression of GAS2 in a variety of leukemic cell lines was detected, and the function of GAS2 in leukemic cells *in vitro* and *in vivo* was studied. Immunoprecipitation was performed to analyze whether endogenous GAS2 interacts with Calpain2 in leukemic cells. The requirement of Calpain2 activity for the growth inhibition upon targeting GAS2 was also studied. Finally, the expression of GAS2 in primary leukemia samples was assessed and compared with that from the healthy donors. Our data demonstrated the aberrant expression of GAS2 in acute leukemia and revealed the importance of GAS2–Calpain2 axis in the growth of leukemic cells, which will deepen our understanding of the function of this molecular machinery in controlling the growth of leukemic cells, and possibly will help to develop alternative strategies to improve the management of these deadly diseases.

Materials and Methods

Cells and culture media

SHI-1 cell is a gift from Dr Suning Chen of the First Affiliated Hospital of Soochow University (Suzhou, China). All other leukemic cell lines used in this study were purchased from the Cell Bank of Chinese Academy (Shanghai Institutes for Biological Sciences, Shanghai, China). These cells were all maintained in RPMI-1640 medium plus 10% fetal bovine serum. The bone marrow samples of leukemia patients and healthy donors were collected in the Department of Hematology, the First Affiliated Hospital of Soochow University, with written informed consent approved by the Ethical Committee of Soochow University. The clinical characteristics of these patients are summarized in Supplementary Table S1. After gradient centrifugation with Lympholyte-H cell separation media (Cedarlane Laboratories, Burlington, USA), the yielded nucleated cells were used for confocal analysis, RNA extraction, and Calpain activity analysis.

RNA extraction and real-time reverse transcription–polymerase chain reaction

RNAprep Pure Micro kit (Tiangen, Beijing, China) was used to extract RNA. DNaseI (Life Technologies, Grand Island, USA) treatment was applied according to the manufacturer's protocol to minimize the contamination with genomic DNA. RNA was reversely transcribed with SuperScript III (Life Technologies) to generate the first strand of cDNA, and real-time reverse transcription–polymerase chain reaction (qRT–PCR) was performed using SYBR Green PCR MasterMix with 7500 real-time PCR system (Applied Biosystems, Foster City, USA). The primers for GAS2 were, forward, 5′-GCAACCCAGAGAAGTG TGTCT-3′; and reverse, 5′-CAGGAGGCTCCACACCAT-3′. The primers for Calpain2 were, forward, 5′-GCAGCCATTGCCTCCCTC AC-3′; and reverse, 5′-ACCTCCACCCACTCGCCGTA-3′. The primers for actin were, forward, 5′-CACCATTGGCAATGAGCGGTT CC-3′; and reverse, 5′-GTAGTTTCGTGGATGCCACAGG-3′.

Western blot analysis

Protein samples were prepared with the protein lyssis buffer (Beyotime, Shanghai, China) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF), and protein concentrations were determined using the BCA kit (Beyotime). Protein samples with same amount of protein (15 μg/lane) were separated by SDS–PAGE and transferred to the Immobilon™ polyvinylidene fluoride membrane (Millipore, Billerica, USA) using Bio-Rad gel system (Hercules, USA). The blot was performed following the instructions of the suppliers of various antibodies, including anti-GAS2 (ab109762; Abcam, Cambridge, USA), anti-Calpain2 (ab75994; Abcam), anti-Calpain1 (ab49652; Abcam), anti-Caspase-3 (9662S; Cell Signaling Technology, Danvers, USA), and anti-Tubulin (T6074; Sigma, St Louis, USA). After incubation with the corresponding HRP-conjugated secondary antibodies, the blot was developed with chemiluminescence substrate provide in the ECL kit (GE Healthcare Life Sciences, Piscataway, USA).

Immunoprecipitation assay

Cell pellets were prepared with protein lysis buffer (Beyotime) plus 1 mM PMSF. Protein concentrations were determined using the BCA kit (Beyotime). Protein lysate (500 μg) was incubated with anti-GAS2 antibody (ab109762; Abcam; 5 μg/ml) and isotype control antibody (I5006; Sigma; 5 μg/ml) at 4°C overnight. Then, the mixture was incubated with protein G Plus-Agarose (L-00209; GenScript, Piscataway, USA) for 4 h at 4°C. The yielded precipitates were analyzed with antibodies against GAS2 (ab109762; Abcam), Calpain1 (ab49652; Abcam), and Calpain2 (ab75994; Abcam), respectively.

Immunofluorescence assay

A total of 1×10^5 cells were transferred to coated slides (Thermo Fisher, Waltham, USA) with a cytospin centrifuge. After being air-dried for 20 min at room temperature, the cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 for 5 min. Then, cells were blocked in Tris-buffered saline with 5% bovine serum albumin for 30 min, and incubated with primary antibody

against GAS2 (ab109762; Abcam; 1/100) overnight at 4°C in a humidifying container. The cells were washed three times with phosphate-buffered saline (PBS) and incubated with FITC-conjugated goat anti-rabbit IgG secondary antibody (1:200; Multisciences, Hangzhou, China) for 1 h. After being washed three times with PBS, the cells were stained with Hoechst 33342 (Sigma) and covered with Prolong Gold Antifade reagent (Life Technologies). The expression of GAS2 was analyzed with a confocal microscope (FV1000MPE-share; Olympus, Tokyo, Japan). Hoechst and FITC were excited by 355 and 485 nm lasers and the emission signals were captured with 460 and 535 nm filters, respectively.

Calpain assays

Calpain activity assays were carried out using a commercially available kit (Biovision, Mountain View, USA) following the instruction of the supplier. In brief, the cells were washed three times with PBS before protein extraction, and then equal amount of protein lysate was mixed with fluorescence-labeled substrate for fluorometric detection with a microplate reader (SpectraMax M5; Molecular Devices, Sunnyvale, USA).

Lentiviral production and transduction

The lentiviral vector expressing full-length GAS2 and GAS2DN was generated as previously described [13]. The lentiviral vectors containing shRNAs against *GAS2* and *Calpain2* together with the negative control (shNC) were from GenePharma (Shanghai, China). The viral production was performed with a standard method as previously described [13]. A total of 1×10^5 THP-1 or Jurkat cells were mixed with 50–100 μl virus containing media overnight, and then the cells were washed with PBS for three times. These cells were cultured for another 3 days before the purification of fluorescent cells with a cell sorter (AriaIII; Becton Dickinson, Franklin Lakes, USA).

Apoptosis analysis

Cells were stained with Annexin V-PE/7-AAD according to the manufacturer's recommendations (Becton Dickinson), and then the cell apoptosis was analyzed by using a flow cytometer (Calibur; Becton Dickinson).

Cell proliferation and CFC assay

A total of 2×10^4 sorted cells were seeded into 24-well plates (2×10^4 cells/ml/well), and then the viable cells were counted 3 and 6 days later with Trypan Blue exclusion staining. The FACS-purified cells were cultured in methylcellulose media (MethoCult™ H4230; Stem Cell Technologies, Vancouver, Canada) and the colonies were counted after 10–14 days of incubation.

siRNA transfection

Double-stranded RNA against Calpain2 was purchased from GenePharma. The forward sequence was 5′-GGAACUACCCG AACACAUUCUU-3′ and the reverse sequence was 5′-AAGAAU GUGUUCGGGUAGUUCC-3′. The transfection procedure was performed with spermine-introduced pullulan as previously described [18], and the transfection efficiency for THP-1 cells was as high as 90%.

Xenograft experiment with nude mice

Nude mice (6–8 weeks old) were purchased from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (SLACCAS, Shanghai, China), and then raised in the animal facility of Soochow University. All animal experiments were approved by the Animal Experimental Committee of Soochow University and performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. A total of 1×10^7 virus transduced THP-1 cells were injected subcutaneously, and then the growth of the tumors was observed closely every 2 days. No mouse died within the period of observation. The tumors were analyzed with luminescence detector (IVIS Lumina II, Caliper Life Sciences, Hopkinton, USA), and then dissected and fixed for hematoxylin and eosin staining (H&E staining). The tumor-free survival was analyzed with Kaplan–Meier method.

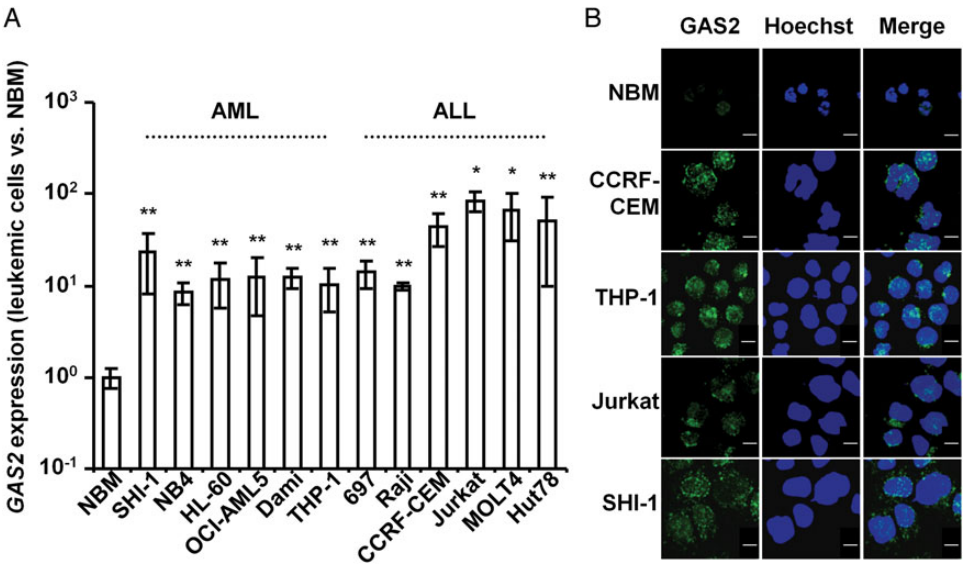

Figure 1. GAS2 is up-regulated in various acute leukemia cell lines (A) GAS2 expression of various leukemia cell lines was compared with cells from NBM. (B) GAS2 expression was analyzed with confocal microscopy. Scale bar = 7.5 μm. *$P < 0.05$ and **$P < 0.01$. NBM, normal bone marrow.

Statistical analysis

All values were presented as the mean ± SEM from at least three independent experiments, and the statistical analysis was performed with Student's *t*-test, in which a *P*-value <0.05 was considered statistically significant.

Results

GAS2 is deregulated in various acute leukemia cells

To assess the expression of GAS2, we compared GAS2 transcript in six AML cell lines (SHI-1, NB4, HL-60, OCI-AML5, Dami, and THP-1) and six ALL cell lines (697, Raji, CCRF-CEM, Jurkat, MOLT4, and Hut78) with that in normal bone marrow (NBM, $n = 7$) cells. The results showed that the expression of GAS2 was significantly higher in every cell line ranging from 8- to 85-fold in comparison with that in NBM cells (**Fig. 1A**). Confocal analysis demonstrated that GAS2 protein level was also elevated in leukemic cells when compared with that in NBM cells. Among the four cell lines analyzed with confocal microscopy, THP-1 and Jurkat cells had the most significant GAS2 expression (**Fig. 1B**).

GAS2 contributes to the growth of leukemic cells

Two independent sequences against GAS2 were delivered to both THP-1 and Jurkat cells. Results showed that GAS2 transcript expression was reduced by ~80% ($P < 0.05$) and GAS2 protein expression was decreased as well by these two shRNAs (**Fig. 2A,B**). The Calpain

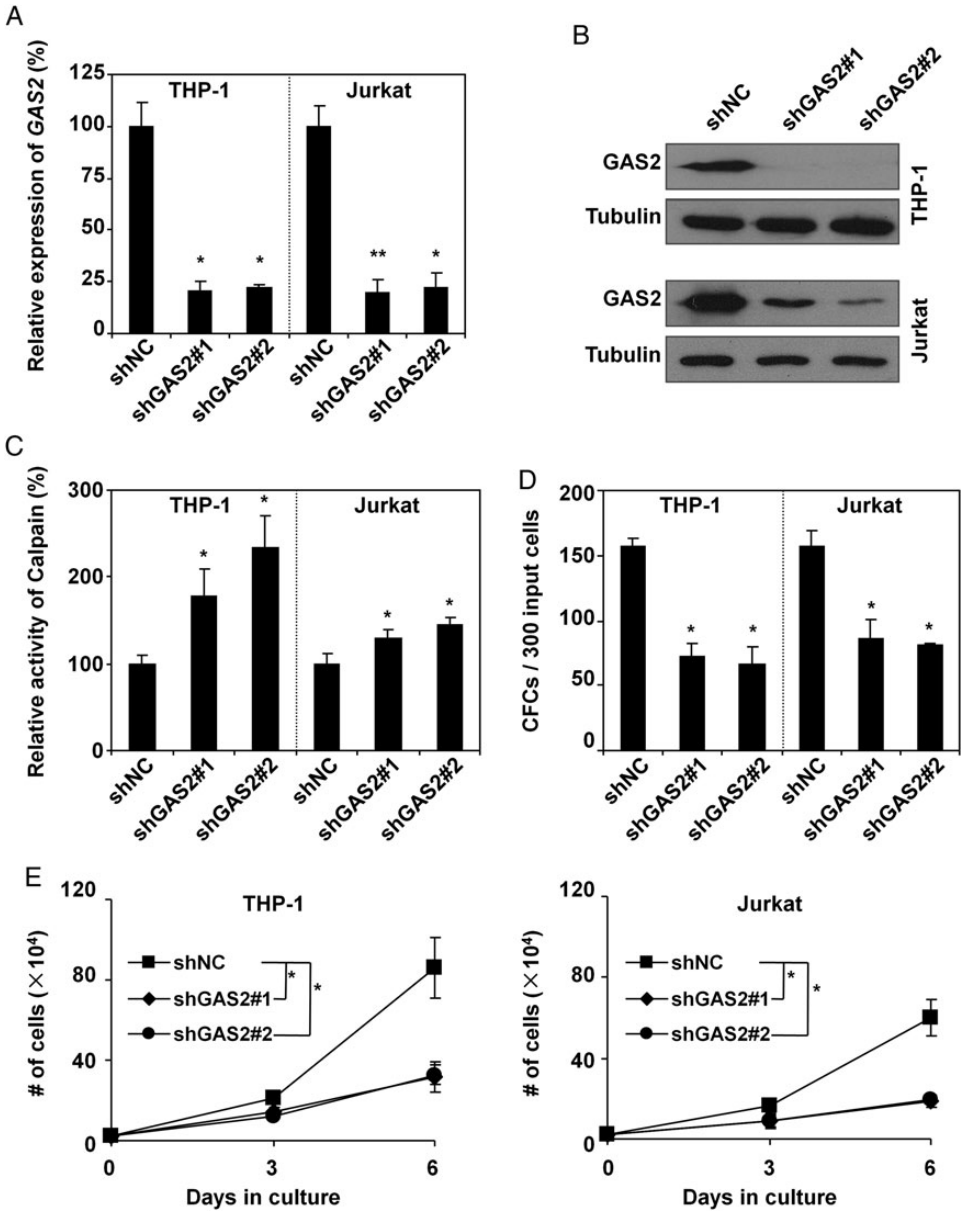

Figure 2. GAS2 silence enhances Calpain activity and inhibits leukemic cell growth Two independent shRNA sequences (shGAS2#1 and shGAS2#2) were delivered to both THP-1 and Jurkat cells with lentiviral vectors. (A, B) The GAS2 transcript and GAS2 protein in control (shNC) and shRNA delivered cells were analyzed with qRT–PCR and western blot analyses, respectively. (C–E) The Calpain activities, the CFC capacities, and the growth of these cells were assessed. *$P < 0.05$ and **$P < 0.01$.

activities in GAS2-silenced THP-1 and Jurkat cells were significantly enhanced ($P < 0.05$; **Fig. 2C**). The CFC production in both THP-1 and Jurkat cells was significantly inhibited ranging from 45% to 57% ($P < 0.05$; **Fig. 2D**). In addition, the growth of GAS2-silenced cells in liquid culture was significantly decreased in comparison with the control cells (**Fig. 2E**). Conversely, we constructed a lentiviral vector to deliver the full-length GAS2, which was capable to re-express GAS2 in GAS2-silenced THP-1 and Jurkat cells (**Fig. 3A**). The re-expression of GAS2 suppressed the enhanced Calpain activity (**Fig. 3B**, upper panel). It also partially rescued the inhibited CFC production (**Fig. 3B**, lower panel) and the growth in both THP-1 and Jurkat cells (**Fig. 3C**).

To address whether GAS2 silence was sufficient to suppress the *in vivo* growth of leukemic cells, control and GAS2-silenced THP-1 cells were injected subcutaneously into nude mice as we did in our previous study [19]. Within 1 month post-injection, tumors were obtained in seven of eight (~88%) mice for the control group; while only one tumor was observed in the shGAS2#1 group (one of eight, ~13%) and no tumor was found in the shGAS2#2 group. All the tumors were fluorescently analyzed with IVIS imaging system and H&E staining. The representative photos of tumors and the H&E staining were shown in **Fig. 4A,B**. GAS2-silenced tumor grew much slower than the control ones (**Fig. 4C**). Moreover, GAS2-silence significantly increased the tumor-free survival analyzed with Kaplan–Meier method (**Fig. 4D**).

Control and GAS2-silenced THP-1 cells were further analyzed by 7-AAD/Annexin V staining. Results showed that GAS2 silence induced cell apoptosis (**Supplementary Fig. S1A**). In addition, cleaved Caspase-3 was detected in GAS2-silenced THP-1 cells in comparison with the control cells (**Supplementary Fig. S1B**). Thus, these proteins, such as GAS2 and charged multivesicular body protein 5 [20], whose silence induces apoptosis of leukemic cells, are of great interest for further investigations, which will possibly provide new clues to improve leukemia treatment.

GAS2 interacts with Calpain2 at endogenous levels

In this study, Calpain2 but not Calpain1 was detected in the immunoprecipitate captured with GAS2 antibody in both THP-1 and Jurkat cells (**Fig. 5A**), though Calpain1 and Calpain2 are constitutively expressed and similar in terms of structure [21]. It has been shown that GAS2DN is able to activate Calpain and inhibits the growth of BCR-ABL$^+$ leukemic cells [12,13], because GAS2DN is capable to compete wild-type GAS2 to interact with Calpain2, but fails to suppress the activity of Calpain2. Herein, GAS2DN was delivered to both THP-1 and Jurkat cells. Similar to GAS2 silence, this action significantly increased the Calpain activity ($P < 0.01$; **Fig. 5B**), and decreased the CFC production and growth of both THP-1 and Jurkat cells ($P < 0.05$; **Fig. 5C,D**).

Calpain2 is required for GAS2 silence-induced growth inhibition

To address the requirement of Calpain2 in the growth inhibition upon GAS2 silence, Calpain2 expression was suppressed using RNAi. Our results showed that both transcript and protein expressions of Calpain2 were evidently suppressed in control and GAS2-silenced THP-1 cells (**Fig. 6A,B**). Calpain activity was significantly reduced in both control and GAS2-silenced THP-1 cells as well ($P < 0.05$; **Fig. 6C**). The inhibited CFC production and cell proliferation upon GAS2 silence were significantly rescued by additional Calpain2 knockdown (**Fig. 6D,E**). Similarly, it was found that Calpain2 silence

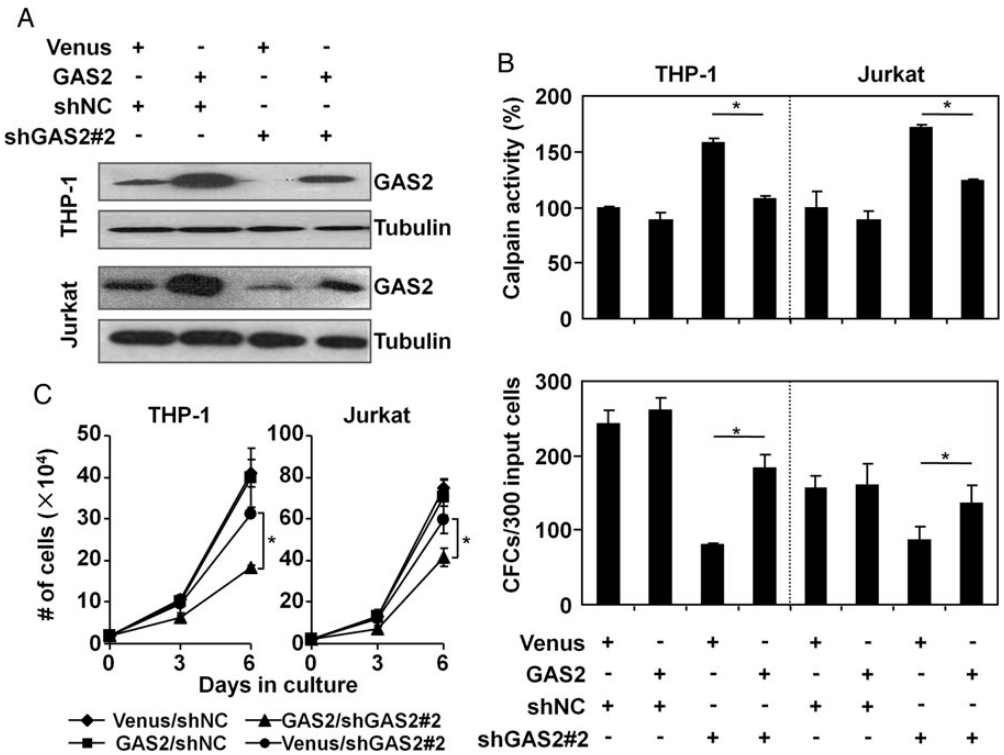

Figure 3. GAS2 re-expression rescues GAS2 silence-induced growth inhibition (A) GAS2 expression in various lentiviral vector transduced cells was analyzed by western blot analysis. (B, C) The Calpain activities, the CFC capacities, and the growth of these variously treated cells were assessed. *$P < 0.05$.

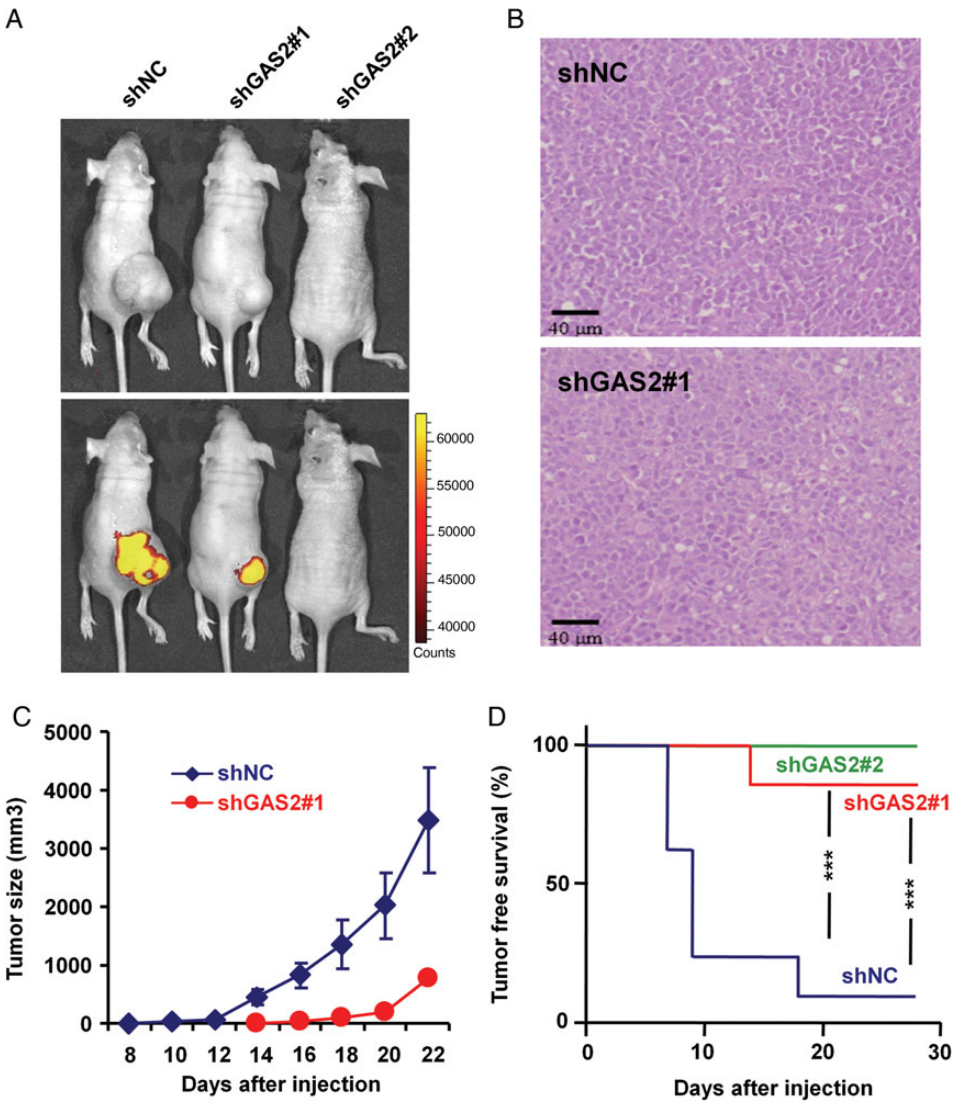

Figure 4. GAS2 silence inhibits the tumor formation ability of THP-1 cells in nude mice (A) Equal amount control and GAS2-silenced THP-1 cells (1×10^7) were injected subcutaneously into nude mice. A representative photograph of tumors in nude mice was shown, and the fluorescence was monitored with IVIS II imaging system. (B) The tumors were also analyzed with H&E staining. (C) The growth of tumor was monitored. (D) The tumor-free survival of the mice within 30 days post-injection was analyzed with Kaplan–Meier method. ***$P < 0.001$.

was able to rescue the growth inhibition induced by the expression of GAS2DN in Jurkat cells (**Supplementary Fig. S2**).

GAS2 is deregulated in primary acute leukemia patients

The expression of GAS2 transcript in a number of acute leukemia patients was assessed. Fifty-seven AML patients, 20 ALL patients, and 7 healthy donors were recruited. GAS2 expression was significantly elevated in both AML and ALL patients compared with healthy donors (**Fig. 7A**). Further statistical analyses confirmed that the differential expressions of GAS2 between patients and healthy donors were independent of age or gender (data not shown). Moreover, the GAS2 expression in subtypes of AML and ALL patients was analyzed. M1 ($n = 10$), M4 ($n = 10$), and M5 ($n = 10$) AML patients had significant higher GAS2 expression than healthy donors (Supplementary Fig. S3A); both T-cell ($n = 11$) and B-cell ($n = 9$) ALL patients had higher GAS2 expression compared with healthy donors as well (Supplementary Fig. S3B). Then, protein samples from 22 AML patients, 19 ALL patients, and 7 healthy donors were collected and the Calpain activity of each group was assessed. The results showed that Calpain activity was significantly lower in leukemia patients than that in healthy donors ($P < 0.05$; **Fig. 7B**).

Discussion

The deregulation of GAS2 in blood disorders has been reported, including the stem/progenitor cell enriched $CD34^+$ subset of myeloproliferative disease (MPD) and CML [13,22–24]. However, the expression of GAS2 in other types of blood disorders has not been elucidated yet. In this study, results showed that GAS2 was upregulated in a variety of leukemic cell lines, and confirmed that the aberrant expression of GAS2 occurred in acute leukemia patients, suggesting an important role of GAS2 in acute leukemia as well. Huang *et al.* [12] have reported that the expression of GAS2 is negatively regulated by IRF8, which provides a clue to understand the

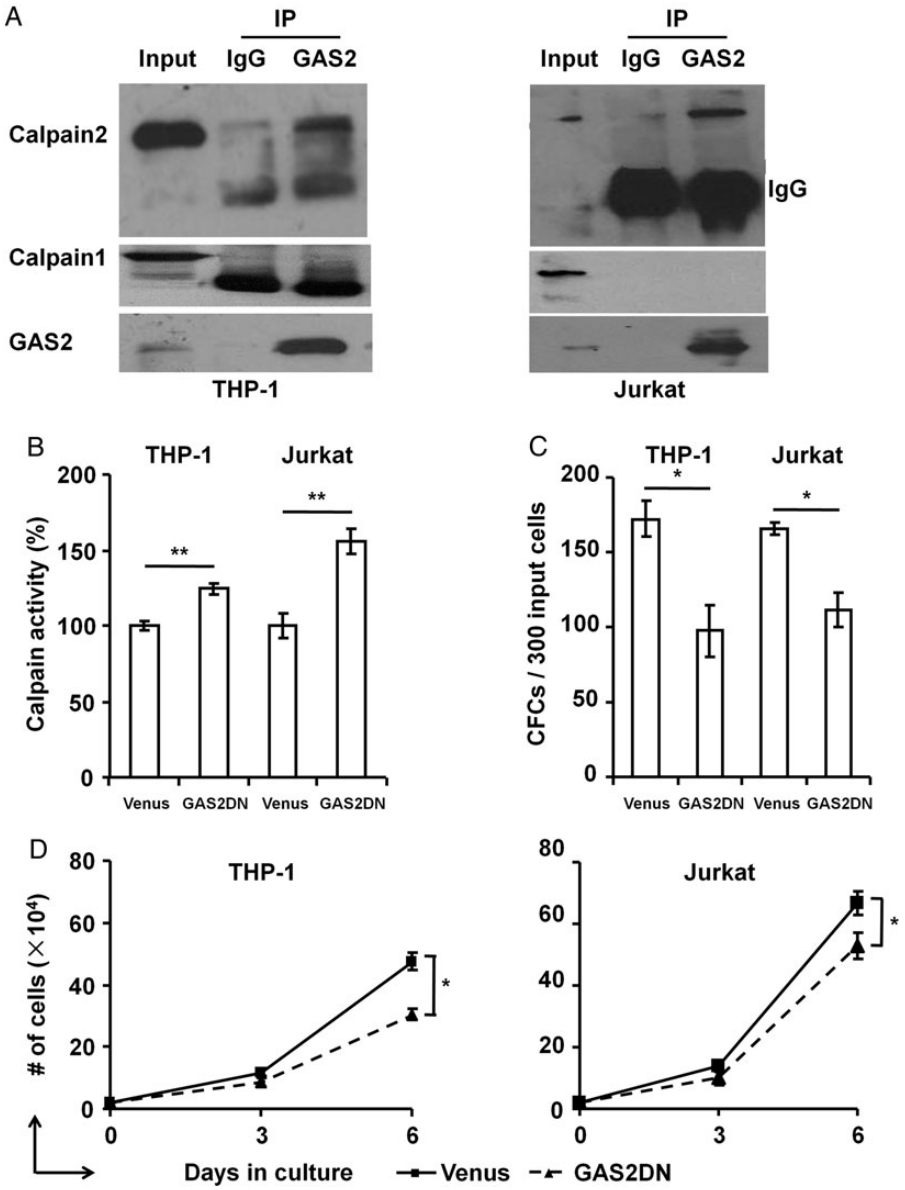

Figure 5. Detection of GAS2–Calpain2 interaction in both THP-1 and Jurkat cells (A) THP-1 and Jurkat cells were used for immunoprecipitation analysis. (B–D) The dominant negative form of GAS2 (GAS2DN) was delivered to both THP-1 and Jurkat cells with lentiviral vector, and then the Calpain activities, the CFC capacities, and the growth of control and GAS2DN-expressing cells were compared. $*P < 0.05$ and $**P < 0.01$.

aberrant expression of GAS2 in myeloid leukemia, as IRF8 is a critical regulator of normal hematopoiesis and acts as a tumor-suppressor in human myeloid leukemia [17]. Nevertheless, the factors contributing to the deregulation of GAS2 in hematological malignancies are largely unknown.

Although the aberrant expression of GAS2 has been reported in both MPD and CML, the functional role of GAS2 has been studied only in CML cells, which revealed that the GAS2–Calpain2 axis is required for the growth of these cells [13]. In this study, our results demonstrated that GAS2 silence could lead to growth inhibition of both THP-1 and Jurkat cells. Moreover, our results clearly showed that GAS2 interacts with Calpain2 in these two cells. As far as we know, it is the first evidence that these two proteins bind together in hematopoietic cells. The interaction was further supported by the facts that GAS2DN was able to inhibit the growth of leukemic cells and that Calpain2 was required for targeting GAS2-induced growth inhibition. We have demonstrated that Calpain2 activation is a critical event in GAS2 silence-induced growth inhibition. Similarly, Ishihara *et al.* [25] reported that Calpain2 loss was detected in adult T-cell leukemia (ATL) cells and, Δ_{19}Calpain2 (with constitutive activity) was able to inhibit the growth of various ATL cells including SO-4, KOB, and KK1 cells that are associated with reduction of α-II spectrin. Taken together, the present study indicates that GAS2–Calpain2 axis plays a novel and ubiquitous role in the growth of leukemic cells, which possibly will help to develop novel therapeutic strategies to improve the management of blood disorders.

Accumulating evidence has proved the important role of Calpain in cancer biology. First, Calpains expression/activities are associated with tumor histopathological features and clinical parameters.

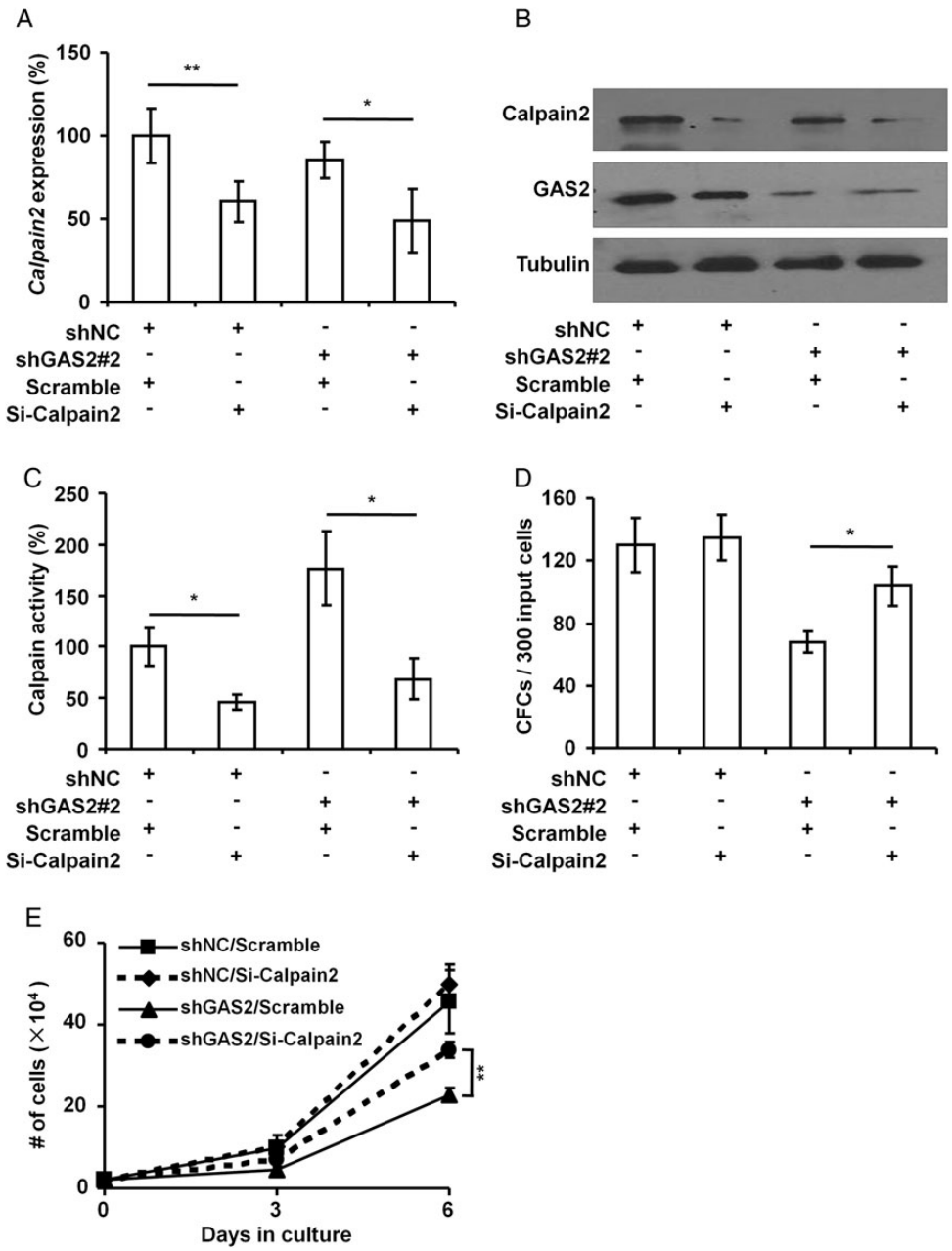

Figure 6. Calpain2 is required for GAS2 silence-induced growth inhibition shRNA against GAS2 was delivered with lentiviral vector and siRNA against Calpain2 was delivered with a modified pullulan carrier to THP-1 cells, and then (A) the Calpain2 transcript, (B) the protein expressions of Calpain2 and GAS2, (C) the Calpain activity, (D) the CFC capacities, and (E) the growth of variously treated cells were assessed. *$P < 0.05$ and **$P < 0.01$.

For instance, aberrant expression/activities of Calpain have been reported in gastric cancers, colorectal cancers, and melanomas [26–28]. Secondly, many consolidated or novel compounds with proven anti-cancer efficacy are able to modulate Calpain activities [20]. Thus, the roles and underlying mechanisms of Calpains in tumor cells are critical to be investigated.

The key to understand how Calpains exert their function on tumors is to identify the targets of aberrantly expressed Calpains. To date, numerous targets of Calpains in this context have been reported, such as c-Myc and β-catenin. Two independent groups have claimed that β-catenin is an important target of Calpain2 in BCR-ABL$^+$ cells [12,29]. Our previous report failed to show that K562 and MEG-01 cells have basal transcriptional activity of β-catenin, though targeting GAS2 is able to activate Calpain2 and inhibits the growth of leukemic cells. Therefore, we propose that the GAS2–Calpain2 axis has alternative pathway, rather than the β-catenin degradation, to inhibit the growth of CML cells [13]. Intensive studies are required to understand the complicated mechanisms of GAS2–Calpain2 axis involved in the growth maintenance.

Our study provides an example that GAS2–Calpain2 axis plays a critical role in hematological malignancies, and the intensive investigations on this axis will deepen the understanding of Calpain in blood disorders and provide novel clues to improve the treatment of these deadly diseases.

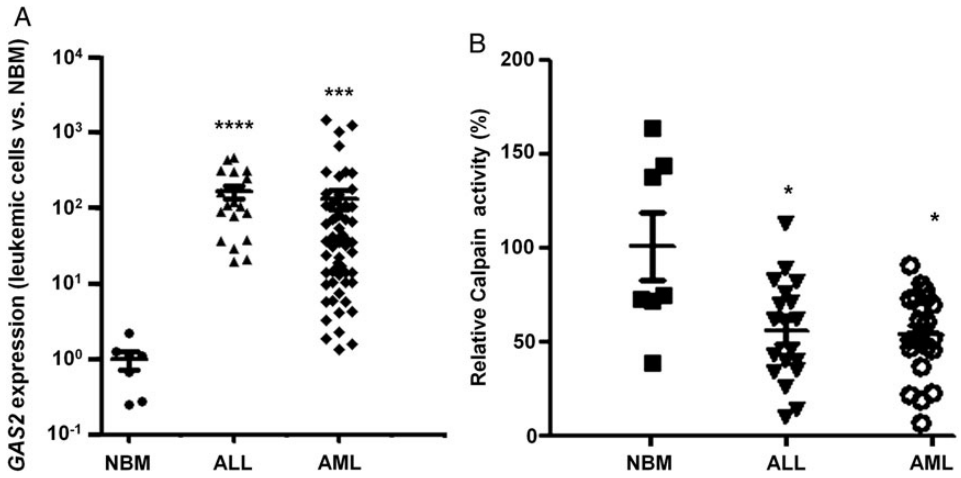

Figure 7. The aberrant expression of GAS2 in acute leukemia patients and the deregulation of Calpain activity (A) The transcript expression of GAS2 was quantified with qRT–PCR. Totally 84 samples from healthy donor and leukemia patients were recruited, including NBM ($n=7$), AML ($n=57$), and ALL ($n=20$). (B) Protein samples from healthy donors ($n=7$), ALL ($n=19$) and AML patients ($n=22$) were collected, whose Calpain activities were assessed and compared with those of THP-1 cells. The relative Calpain activities of healthy donors, ALL and AML patients were then compared. *$P<0.05$; ***$P<0.001$; ****$P<0.0001$. NBM, normal bone marrow.

Supplementary Data

Supplementary data is available at *ABBS* online.

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