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Original article

Dandelion root extract suppressed gastric cancer cells proliferation and migration through targeting lncRNA-CCAT1

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ABSTRACT

Gastric cancer (GC) is one of the most common tumors worldwide. Standard treatment after early detection involves surgical excision (recurrence is possible), and metastatic gastric cancer is refractory to immuno-, radio-, and most harmful chemotherapies. Various natural compounds have shown efficacy in killing different cancers, albeit not always specifically. In this study, we show that dandelion root extract (DRE) specifically and effectively suppresses proliferation and migration in human gastric cells without inducing toxicity in noncancerous cells. Long noncoding RNAs (lncRNAs) are known to promote tumorigenesis in many cancer types. Here, we showed that the lncRNA colon cancer-associated transcript-1 (CCAT1) was down-regulated in dandelion-treated GC cells. Furthermore, downregulation of CCAT1 inhibited proliferation and migration of gastric cells. We also found that DRE exerted its function in GC cells partially through targeting CCAT1. This data will provide a basis on which further research in cancer treatment through DRE can be executed.

1. Introduction

Gastric cancer is the fourth most common malignancy and the second leading cause of cancer-related deaths worldwide, with approximately 1 million new cases and 0.7 million deaths per year [1]. Despite recent improvements in multimodal therapy including surgery, chemotherapy, radiotherapy, and targeted therapy, the prognosis of patients diagnosed with advanced GC remains unsatisfactory [2]. Therefore, it is urgent to elucidate the regulatory network underlying GC and improve the quality of life of patients already diagnosed with the disease.

Natural health products (NHPs) and natural products (NPs) have been identified as essential components in the development of many drugs. Besides, most of the currently available chemotherapies including paclitaxel are derived from natural sources [3]. Recent studies on the use of the NHPs for specific diseases yield some related scientific validation for their application [4]. Dandelions (Taraxacum spp), as one of the most common and recognizable weeds, can be found in almost every part of the world [5]. This plant has been used widely in Traditional Chinese Medicine and traditional Native American Medicine for its medicinal activity. Dandelions extracts could treat diseases ranging from diarrhea and digestive diseases to hepatitis. However, there are limited scientific studies investigating the anti-cancer activity of dandelion extracts and very little is known about the mechanism of action. Ovadje and his colleagues reported a strong anti-cancer activity of DRE in human leukemia, pancreatic cancer cells, colorectal cancer cells and drug-resistant human melanoma cells [6–10]. Nevertheless the efficacy of DRE in GC has not been reported.

Long non-coding RNAs (lncRNAs), consisting of more than 200 nucleotides with no or limited coding protein capacity, have been reported in many biological processes [11–15]. Colon-cancer-associated transcript-1 (CCAT1), a 2628-bp lncRNA located on chromosome 8q24.21, was first identified in colon cancer [16]. CCAT1 is upregulated in hepatocellular carcinoma, gallbladder cancer, and colon carcinoma tissues compared with adjacent

Abbreviations: GC, gastric cancer; NHPs, natural health products; NPs, natural products; DRE, dandelion root extract; CCAT1, colon-cancer-associated transcript-1; GES-1, gastric epithelium cell line.

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normal tissues [17]. However, the expression of CCAT1 in gastric cancer still remains unclear.

In the current study, we have investigated the anti-cancer activity of the DRE in gastric cancer cell lines. Our results showed that DRE could suppress the proliferation and decrease the metastatic capacity of GC cells by targeting CCAT1. We have also observed that CCAT1 was down-regulated in dandelion-treated GC cells. Furthermore, downregulation of CCAT1 also inhibited proliferation and migration of gastric cancer cells. Furthermore, DRE exerted its function in GC cells partially by targeting CCAT1. Though it is unclear which components of DRE are active in successfully killing human gastric cancer cells, our work with DRE presents a novel, natural chemotherapeutic agent that may be extended to cancer lines.

2. Material and method

2.1. Cell culture

Two gastric cancer cell lines (SGC7901, BGC823), and a normal gastric epithelium cell line (GES-1) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI 1640 or DMEM (GIBCO-BRL) medium supplemented with 10% fetal bovine serum (10% FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin in humidified air at 37 °C with 5% CO_2.

2.2. Dandelion root extraction & preparation

The dandelion roots used for this study were obtained from Premier Herbal Inc. (Lot No. 318121). The root extract was prepared according to the previously reported [18]. In detail, dried dandelion root was immersed in liquid nitrogen for about 5 to 10 min, until thoroughly frozen. The frozen pieces were ground up in an impingement grinder to an average particle size of ≤45 μm. Following grinding, dandelion root powder was extracted in boiling water on low heat for 3 h. The total extracted material was filtered through a NITEX nylon mesh filter (LAB PAK; Sefar BDH Inc. Chicoutini, Quebec CA) and the filtrate was spun down at 800 × g for 5 min at room temperature. The supernatant was filtered through a 0.45 μm filter, followed by lyophilization. The dried extracted material was reconstituted in water to give a final stock solution of 100 mg/ml and then passed through a 0.22 μm filter, in a biological safety cabinet and stored at 4 °C or −20 °C for long term storage. This material was used for all the experiments described in this study.

2.3. Real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA from cells was isolated with Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. Reverse transcription was performed with PrimeScript RT reagent Kit (Takara, Japan) according to the manufacturer's instructions. qRT-PCR was performed with SYBR Prime Script RT-PCR Kits (Takara, Japan) based on the manufacturer's instructions. Results were calculated with the $2^{-\Delta\Delta Ct}$ methods and normalized to the expression of GAPDH. All assays were performed in triplicate. The expression levels were relative to the fold change of the corresponding controls which were defined as 1.0.

2.4. Cell viability

Cells were seeded into 96-well plates (3×10^3 cells/well) directly or 24 h after transfection. After treatment with the indicated DRE for 48 h, cell viability was assessed via 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-trtrazolium bromide (MTT) assayAll samples were assayed in triplicate.

2.5. Colony formation assay

Cells (500 cells/well) were plated in 6-well plates and incubated in RPMI 1640 with 10% FBS at 37 °C. Two weeks later, the cells were fixed and stained with 0.1% crystal violet. The number of visible colonies was counted manually.

2.6. Cell transfection

The plasmids pcDNA3.1/CCAT1 (pcDNA3.1/empty vector as NC) and shCCAT1 (shRNA as control) were all synthesized by GenePharma (Shanghai, China). Transfections were performed

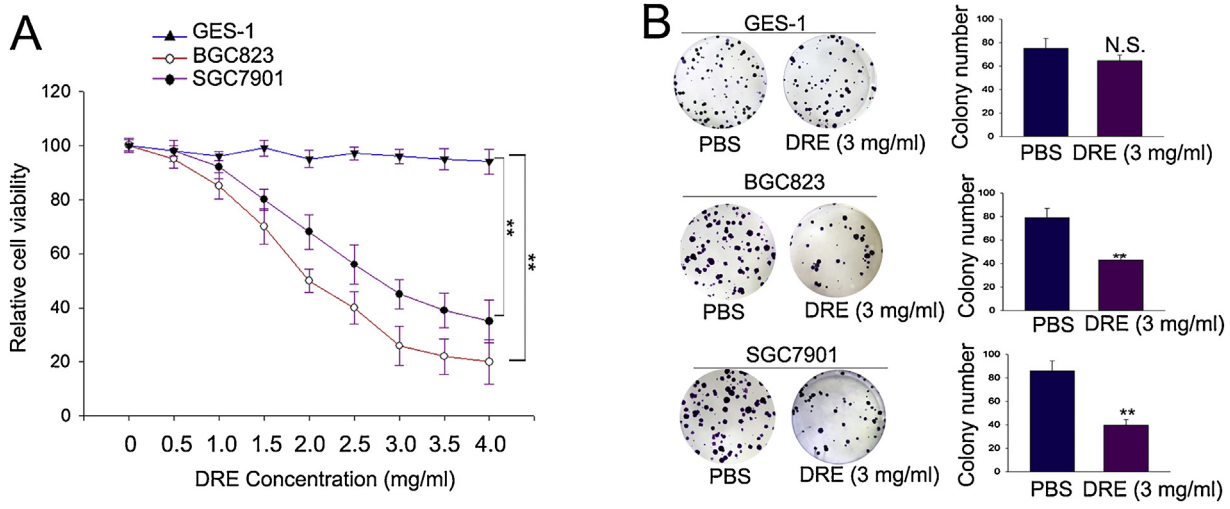

Fig. 1. DRE suppressed the proliferation of GC cells. A. MTT was employed to determine the effect of DRE on the viability of SGC7901, BGC823 and GES-1 cells treated with DRE. B. Colony formation was performed to measure the proliferation ability of SGC7901, BGC823 and GES-1 cells treated with or without DRE. Error bars represent the mean ± SD of at least three independent experiments. N.S.: no significant $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group.

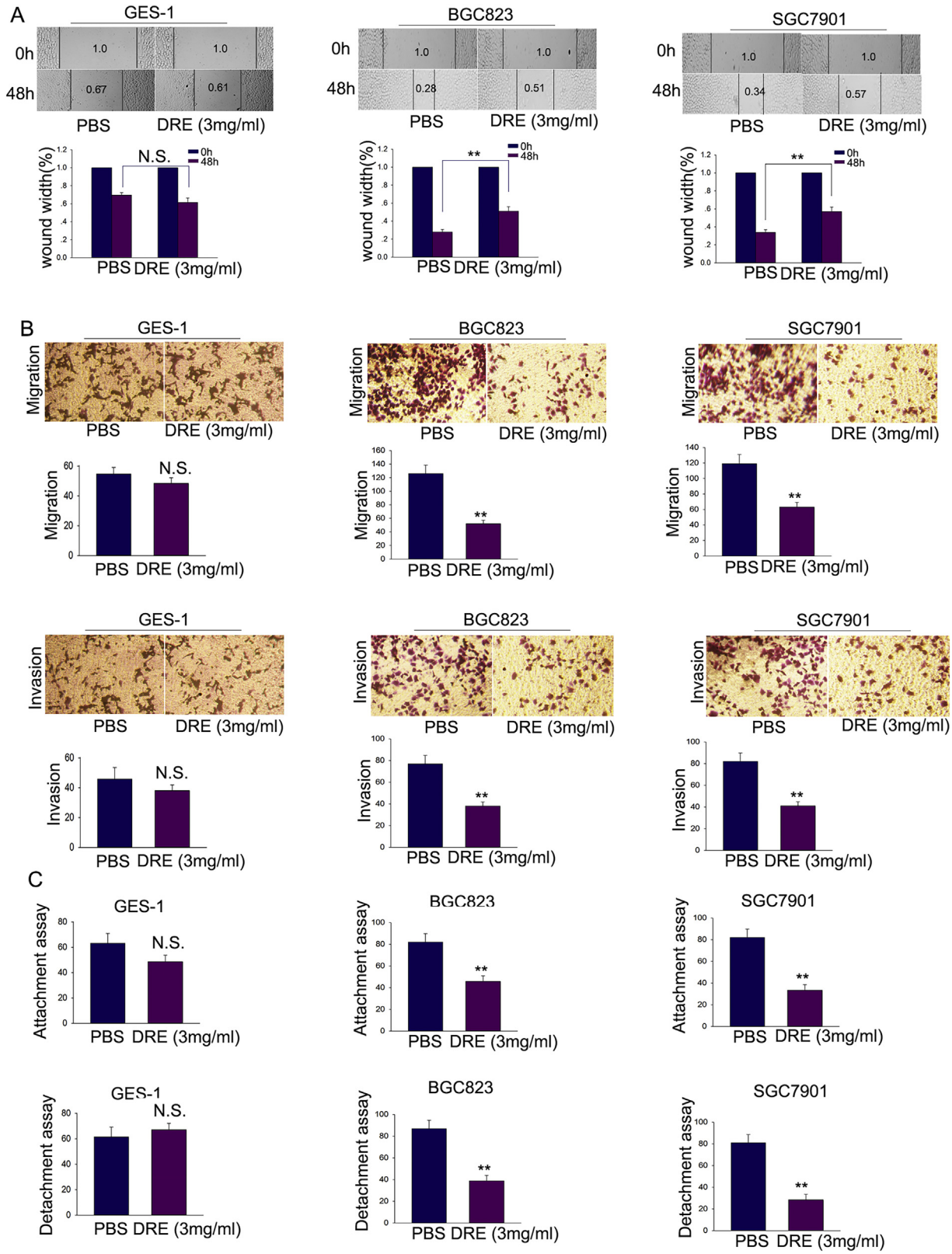

Fig. 2. Dandelion decreased the metastasis capacity of GC cells. A–C. Wound healing assay, transwell assay and attachment/detachment assay were performed to detect the effect of DRE on the migration of SGC7901, BGC823 and GES-1 cells. Error bars represent the mean ± SD of at least three independent experiments. N.S.: no significant $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group.

using Lipofectamine 2000 (Invitrogen, USA), according to the manufacturer's protocol.

2.7. Wound healing assay

Cell migration capacity was calculated by wound healing assay. 2×10^5 cells with or without transfection were plated into 12-well plates and incubated in DMEM with 10% FBS at 37 °C. After reaching 100% confluence, cells were wounded by scraping with a 200 μl tip, following washed 3 times in serum-free medium and incubated in regular medium. Wounds were observed at 0 and 48 h. The cell migration distance was calculated by subtracting the wound width at each time point from the wound width at the 0 h time point. Three independent assays were assayed.

2.8. Migration and invasion assays

Cell migration and invasion were measured by transwell chamber (8um pore size, Corning) and Matrigel invasion (Bection Dickinson), respectively. 48 h after transfection, cells in serum-free media were placed into the upper chamber coated with or without 10ug/ml Matrigel. Media containing 10% FBS were added into the lower chamber. Following 48 h incubation, cells remained in upper membrane were wiped, while cells migrated or invaded were fixed in methanol, stained with 0.1% crystal violet and counted under a microscope. Three independent experiments were carried out.

2.9. Attachment and detachment assays

For attachment assay, cells were seeded in 24-well plates at 5×10^4 cells per well. Unattached cells were removed after 1 h incubation, and the attached cells were counted after trypsinization. The data were presented as a percentage of the attached cells compared to total cells. For cell detachment assay, after 24 h incubation, the cells were incubated with 0.05% trypsin for 3 min to detach the cells. Then, the culture medium was added to inactivate the trypsin and the detached cells were collected. The remaining cells were incubated with 0.25% trypsin to detach and counted. The data were presented as a percentage of the detached cells to total cells.

2.10. Statistical analysis

All experiments were performed at least three times, and presented as mean ± SD. The SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Two group comparisons were performed with a Student *t*-test. Multiple group comparisons were analyzed with one-way ANOVA. All tests performed were two-sided. $P < 0.05$ or less was considered significant.

3. Results

3.1. The effect of DRE on the proliferation ability of GC cells

To detect the effect of dandelion on GC cells proliferation, two GC cells (SGC7901 and BGC823) were employed. For comparison, normal gastric epithelium cells (GES-1) were also used to assess the selectivity of DRE to GC cells. As present in Fig. 1A, results from MTT showed that the viability of SGC7901 and BGC823 cells exposed to the treatment of DRE was significantly suppressed, and this effect was dose dependent. The selectivity of DRE to cancer cells was confirmed, as normal GES-1 cells did not lose cell viability when exposed to the same doses. Meanwhile, the results from colony formation assays showed that proliferation ability was obviously suppressed when SGC7901 and BGC823 cells treated with DRE (3 mg/ml), while GES-1 cells, again, remained unaffected (Fig. 1B). These results confirmed the anti-cancer potential of DRE in GC cells.

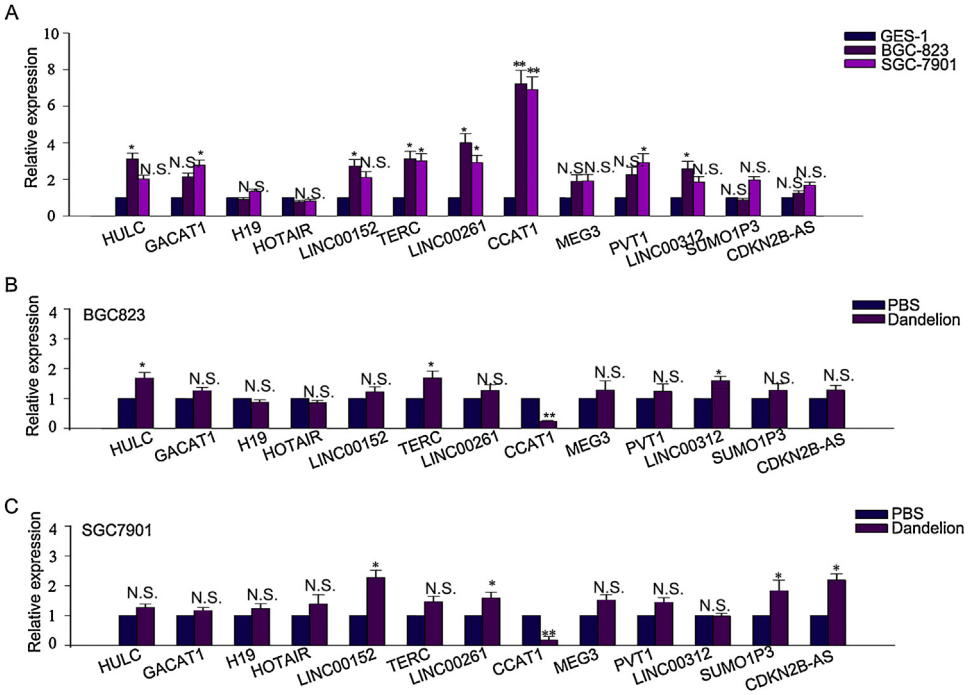

Fig. 3. CCAT1 was down-regulated in DRE-treated GC cells.
A. Expression profiles of 13 gastric cancer-associated lncRNAs in BGC-823, SGC-7901 and normal gastric mucosal cell GES-1. B–C. The level of 13 lncRNAs in BGC-823 and SGC-7901 cell treated with or without DRE. Error bars represent the mean ± SD of at least three independent experiments. N.S.: no significant *p < 0.05, **p < 0.01 vs. control group.

3.2. The effect of DRE on the metastasis capacity of GC cells

To determine if DRE can prevent invasive and metastatic behaviors in GC cells, the scratch wound healing assay, transwell assay and attachment/detachment assay were employed. As present in Fig. 2A–C, results from wound healing assay, transwell assay and attachment/detachment assay showed that treatment with DRE inhibited the ability of GC cells, SGC7901 and BGC823, to migration, while GES-1 cells, again, remained unaffected. Collectively, these findings indicate that DRE can inhibit the ability of GC cells to migrate and invade, and therefore metastasize to secondary locations.

3.3. CCAT1 was down-regulated in DRE-treated GC cells

LncRNAs are known to promote tumorigenesis in many cancer types. To explore whether lncRNAs mechanism were involved in the DRE-mediated anti-tumorigenesis of GC, RT^2 lncRNA PCR Arrays system (https://www.qiagen.com/cn/shop/pcr/primer-sets/rt2-lncrna-pcr-arrays/?catno=LAHS-002Z#geneglobe) was applied. We analyzed the expression profiles of 13 gastric cancer-associated lncRNAs in BGC-823, SGC-7901 and normal gastric mucosal cell GES-1 (Fig. 3A). We found that only CCAT1 was significantly increased in both BGC-823 and SGC-7901 cells. To further confirmed, we measured the expression level of 13 lncRNAs

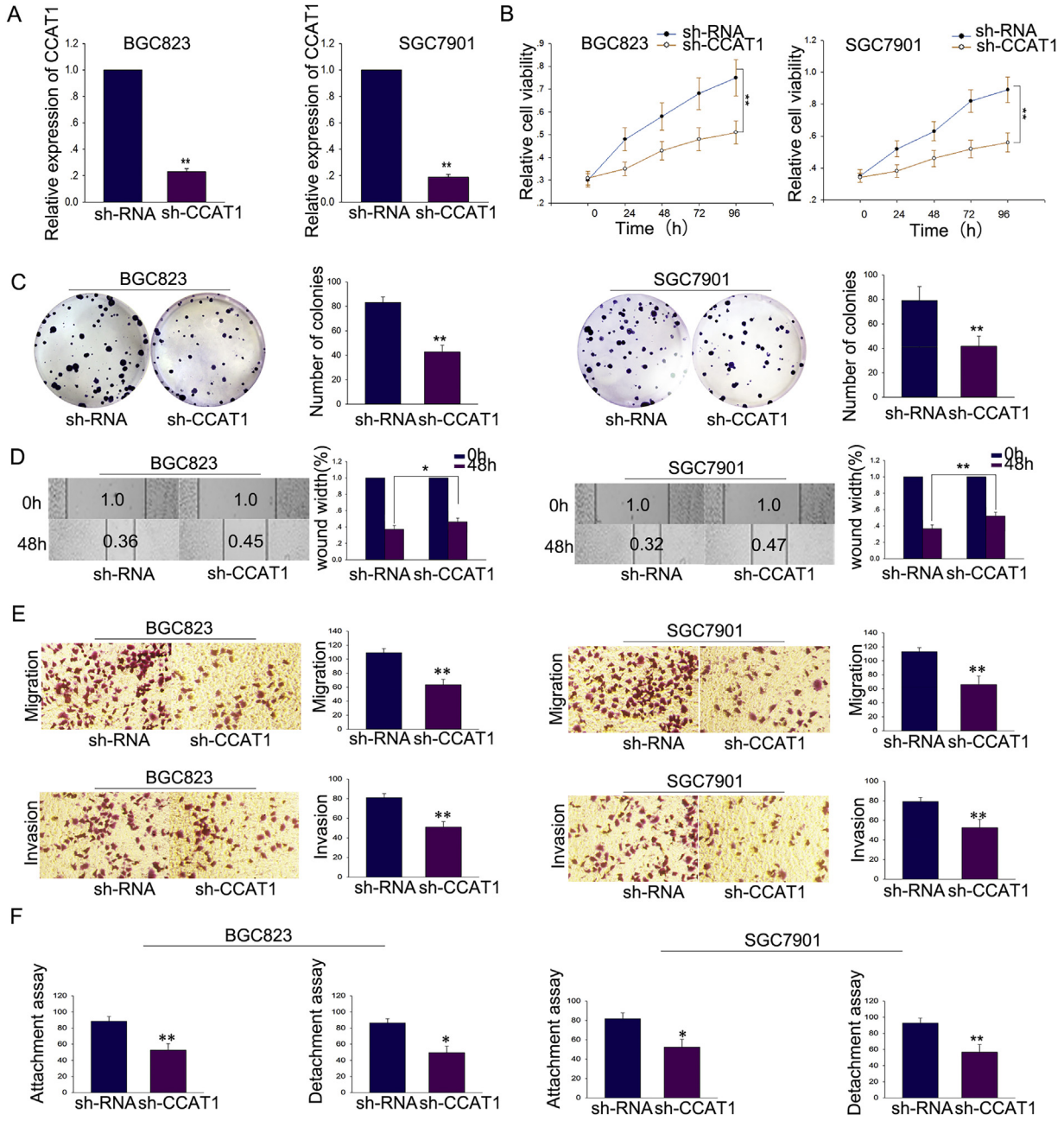

Fig. 4. Down-regulated CCAT1 suppressed the proliferation and migration of GC cells. A. Satisfactory transfection efficiency was obtained at 48 h post-transfection. B–C. MTT and colony formation were employed to measure the function of CCAT1 on GC cells proliferation. D–F. Wound healing assay, transwell assay and attachment/detachment assays were employed to measure the function of CCAT1 on GC cells migration. Error bars represent the mean ± SD of at least three independent experiments. N.S.: no significant $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group.

in BGC-823 and SGC-7901 cell with or without treatment of DRE. As shown in Fig. 3B–C, the level of CCAT1 could be significantly suppressed by DRE. Combined the three results (Fig. 3A–C), we focused on CCAT1, the greatest reduction one in response to DRE. Therefore, we selected CCAT1 as the possible target of DRE for the following experiments.

3.4. Down-regulated CCAT1 suppressed the proliferation and migration of GC cells

To confirm whether the effect of DRE on GC cells might be mediated by CCAT1, we firstly investigated the function of CCAT1 in GC cells. We transfected sh-CCAT1 into SGC7901 and BGC823 cells, and satisfactory transfection efficiency was obtained at 48 h post-transfection (Fig. 4A). Then, MTT and colony formation assay showed that silencing CCAT1 decreased the cells proliferation ability (Fig. 4B–C). Additionally, results from wound healing assay, transwell assay and attachment/detachment assays showed that cells down-regulated CCAT1 exerted an weakened migration capacity in cells transfected with sh-CCAT1 (Fig. 4D–F). These data revealed that the effect caused by down-regulated CCAT1 was consistent with the function of DRE, suggesting that dandelion might exert its function by targeting CCAT1.

3.5. DRE exerted its function in GC cells was at least partially through targeting CCAT1

To further confirm that DRE exerted its function through targeting CCAT1, rescue assays were performed. As present in Fig. 5A–B, the function of DRE on GC cells proliferation could be reversed when co-transfected with CCAT1. Additionally, results from wound healing assay, transwell assay and attachment/detachment assay showed that DRE on GC cells migration could be reversed when co-transfected with CCAT1 (Fig. 5C–E). These

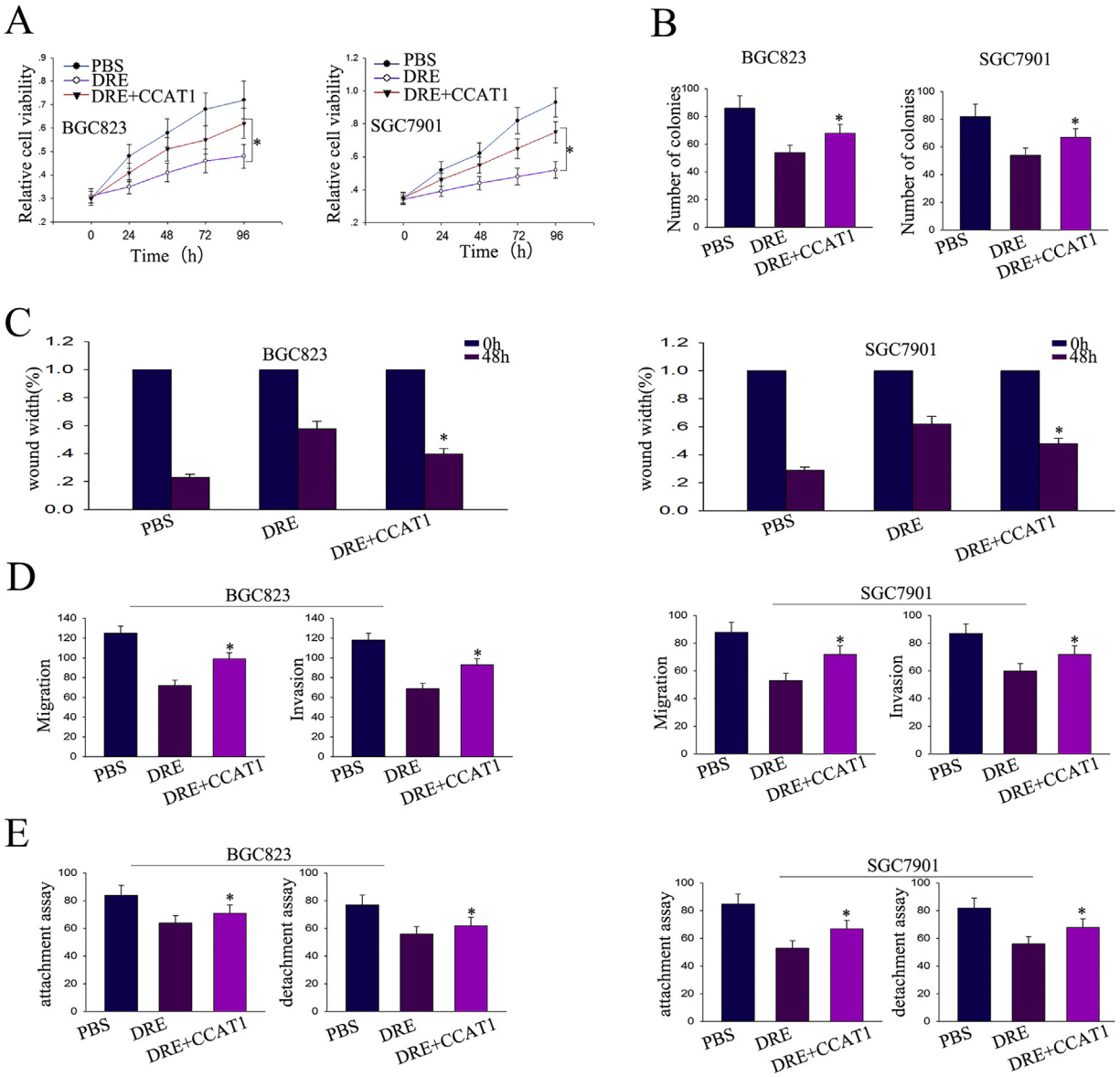

Fig. 5. DRE exerted its function in GC cells was at least partially through targeting CCAT1. A–B. Function of DRE on GC cells proliferation could be reversed when co-transfected with CCAT1. C–E. The function of DRE on GC cells migration could be reversed when co-transfected with CCAT1. Error bars represent the mean ± SD of at least three independent experiments. N.S.: no significant $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control group.

findings reflected that DRE could selectively inhibit cancer cell growth and such function was at least partially mediated through targeting CCAT1.

4. Discussion

DRE have been studied extensively in recent years for its anti-depressant and anti-inflammatory activity [19–24]. Besides, DRE has been used in traditional medicine as a detoxifying agent for digestive disorders [25]. However, there has been little scientific advancement made in this field about the effect of DRE on cancers including GC. In this study, we show that DRE can specifically target human gastric cancer cell without inducing toxicity in noncancerous cells, making it a valuable chemotherapeutic. We have investigated the inhibition of proliferation and migration in cancer cells and anti-cancer potential of DRE in GC cells.

Long non-coding RNAs (LncRNAs), consisting of more than 200 nucleotides with no or limited coding protein capacity, have been reported in many biological processes [26–33]. To explore the underlying lncRNAs mechanism in the dandelion-mediated anti-tumorigenesis of GC, RT^2 lncRNA PCR Arrays system was applied. Finally, we chose the greatest reduction one, CCAT1, in response to dandelion as the study object. CCAT1, located in the vicinity of c-MYC, was first found abnormally expressed in colon cancer, and has been elucidated to be dysregulated in many other cancers [34–41]. However, the mechanism by which CCAT1 exhibits its oncogenic functions in the dandelion-mediated anti-tumorigenesis of GC remains to be investigated.

Firstly, we employed loss-of-function to investigate the effect of CCAT1 in GC cells. We found that deletion of CCAT1 significantly decreased the cells proliferation and migration. What's more, rescue assays revealed that the function of dandelion root extracts could be partially reversed by introduction with CCAT1. All these together suggested that dandelion root extracts exerted in GC cells was through targeting CCAT1.

5. Conclusion

In general, we confirmed the function of DRE and first revealed that DRE could suppress gastric cancer cells proliferation and migration by targeting lncRNA-CCAT1. Our results scientifically validate the use of DRE as potential anti-cancer agents, which might represent a novel non-toxic alternative to conventional cancer therapy available today.

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Disclosure

None.

Competing interests

The authors declare no competing or financial interests.

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