The Oncologist, 2022, **27**, e64–e75
https://doi.org/10.1093/oncolo/oyab015
Advance access publication 27 January 2022
Original Article

GBAS Regulates the Proliferation and Metastasis of Ovarian Cancer Cells by Combining with eEF1A1

Xin Ning[1], Guangyue Shi[2], Sujing Ren[1], Shuang Liu[1], Jing Ding[1], Ruichun Zhang[1], Lianwei Li[1], Qin Xie[1], Wei Xu[1], Fanling Meng[1,*], Rong Ma[1,*]

[1]Department of Gynecology, Harbin Medical University Cancer Hospital, Harbin, People's Republic of China
[2]Department of Oncology, Harbin Medical University Cancer Hospital, Harbin, People's Republic of China

*Corresponding authors: Fanling Meng, Department of Gynecology, Harbin Medical University Cancer Hospital, Harbin 150081, China. Tel: +86 451 85718069; Email: dr_mfl1979@126.com; Rong Ma, Department of Gynecology, Harbin Medical University Cancer Hospital, Harbin 150081, China. Tel: +86 451 85718058; Email: dr_marong2017@126.com

Abstract

Background: The glioblastoma-amplified sequence (GBAS) is a newly identified gene that is amplified in approximately 40% of glioblastomas. This article probes into the expression, prognostic significance, and possible pathways of GBAS in ovarian cancer (OC).

Method: Immunohistochemical methods were used to evaluate the expression level of GBAS in OC and its relationship with clinicopathological characteristics and prognosis. Glioblastoma-amplified sequence shRNA was designed to transfect into OC cell lines to silence GBAS expression, then detect the proliferation, apoptosis, and migration ability of the cell. Furthermore, an in vitro tumor formation experiment in mice was constructed to prove the effect of GBAS expression on the growth of OC in vivo. To further study the regulation mechanism of GBAS, we performed co-immunoprecipitation (Co-IP) and shotgun LC-MS mass spectrometry identification.

Results: Immunohistochemistry indicated that GBAS was markedly overexpressed in OC compared with normal ovarian tissue and was associated with lymph node metastasis. Inhibition of GBAS expression can significantly reduce OC cell proliferation, colony formation, promote cell apoptosis, and reduce the ability of cell migration and invasion. In vivo tumor formation experiments showed that the size and weight of tumors in mice after GBAS expression knockdown was significantly smaller. Glioblastoma-amplified sequence may be combined with elongation factor 1 alpha 1 (eEF1A1) to achieve its regulation in OC. Bioinformatics analysis data indicate that GBAS may be a key regulator of mitochondria-associated pathways, therefore controlling cancer progression. MicroRNA-27b, MicroRNA-23a, and MicroRNA-590 may directly targeting GBAS affects the biological behavior of OC cells.

Conclusion: The glioblastoma-amplified sequence may regulate the proliferation and metastasis of OC cells by combining with eEF1A1.

Key words: GBAS; ovarian cancer; eEF1A1; prognosis; proliferation; metastasis.

Implications for Practice

This article reports, for the first time, that glioblastoma-amplified sequence (GBAS) is highly expressed in ovarian cancer (OC) cells and is related to OC lymph node metastasis. Glioblastoma-amplified sequence regulates the proliferation and metastasis of OC cells at least to a certain extent by combining with elongation factor 1 alpha 1 and is related to the poor prognosis of OC. Knockdown of GBAS significantly inhibits the proliferation, invasion, and metastasis of OC cells and promotes apoptosis in the process of tumorigenesis of ovarian cancer.

Introduction

Ovarian cancer (OC) is one of the gynecological malignancies with the highest fatality rate.[1] The ovary is located in the deep part of the pelvic cavity, and the early lesions have no obvious symptoms and lack effective screening methods. Therefore, more than 70% of OC is diagnosed in the middle and late stages.[2] Surgery and chemotherapy are the main treatments for OC. Although the disease can be relieved after the initial treatment, most OCs will recur and metastasize within 5 years. Eventually, with chemotherapy resistance and difficulty in surgical resection, patients will die.[3] At present, there are some emerging strategies for the treatment of OC, such as the use of poly-ADP-ribose polymerase (PARP) inhibitors or bevacizumab for targeted therapy.[4,5] However, it is still necessary to further explore the pathogenesis of OC to find more valuable predictors and develop more targeted therapies.

Glioblastoma-amplified sequence (GBAS), a gene located on chromosome 7p12, was named for its amplification in approximately 40% of glioblastomas. It encodes proteins with recognizable signal peptides, transmembrane motifs, and two tyrosine phosphorylation sites.[6] Meanwhile, GBAS is also called 4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 2(NIPSNAP2), a member of the NIPSNAP family, contains the mitochondrial targeting sequence MTS at its N-terminus, which can be introduced into the mitochondria.[7] Research by Brittain et al showed that Nipsnap2 acts as a regulator of L-type Ca^{2+}, and its

Received: April 20, 2021. Editorial Acceptance: September 21, 2021.
© The Author(s) 2022. Published by Oxford University Press.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com.

overexpression can increase Ca^{2+} flux.[8] A report by Abudu et al pointed out that Nipsnap2 can be used as a signal marker for the degradation of damaged mitochondria—accumulating on the outer membrane of damaged mitochondria and continuously recruiting autophagy receptors during mitochondrial autophagy.[9] In addition, Yamamoto et al indicated that macrolide antibiotics can combine with NIPSNAP2 to inhibit the production of pro-inflammatory cytokines mediated by the NF-κB pathway.[10] At the same time, GBAS is also co-amplified with the epidermal growth factor receptor gene (EGFR). In recent years, GBAS has been studied more and more in cancer. Glioblastoma-amplified sequence expands in approximately 40% of glioblastomas. It has been reported that GBAS regulates the proliferation and apoptosis of oral squamous cell carcinoma through the p53 signaling pathway.[11] The level of GBAS affects the prognosis of early NSCLC patients undergoing surgical resection.[12]

Tissue elongation factor 1 alpha 1 (eEF1A1) is a protein ubiquitous in all eukaryotic cells, and it participates in the extension of peptide chains during protein translation.[13] There are two subtypes of eEF1A, eEF1A1 and eEF1A2. Among them, eEF1A1 is widely present in most cells, and eEF1A2 is mainly in the heart, brain and skeletal muscle.[14] They have the same function in protein translation. In addition to the conventional functions in translation, eEF1A1 also has unconventional functions such as signal transduction and heat shock process stimulation.[15] What's more, eEF1A1 can also exhibit chaperone-like activity, thereby participating in the regulation of cell proliferation and apoptosis.[16] eEF1A1 can also bind to actin and participate in the regulation of the cytoskeleton, thereby further affecting cell morphology.[17] The existence of eEF1A1 is essential for maintaining a complete cytoskeleton, because eEF1A1 can bind to actin with a unique bond, and is also related to the binding and breaking of microtubules and cell division.[18] The research of eEF1A1 in tumor has also made considerable progress. The truncated form of eEF1A1 has been reported to be carcinogenic and is translated from an mRNA variant of the prostate cancer-inducing gene PTI-1. The shortened eEF1A1 may affect the normal pathway of eEF1A1, interfere with its translation process, and may cause cell cycle out of control or even carcinogenesis.[19] Elongation factor 1 alpha 1 has been reported to participate in the vicious behavior of breast cancer, kidney cancer, colon cancer, and chronic lymphocytic leukemia.[14,16,20,21]

At present, the research on GBAS in OC is still blank. This article will analyze the overexpression of GBAS in OC and its relationship with prognosis, and simultaneously explore its role in the migration, invasion and other biological behaviors of OC.

Materials and Methods

Patient tissue samples

Paraffin-embedded (FFPE) tumor blocks were collected from 72 patients with OC who had not undergone any treatment, including radio-, chemical or immunotherapy, between January 2010 and June 2013. The cases were confirmed pathologically at the Harbin Medical University Cancer Hospital. The patients underwent ovarian surgery at the Harbin Medical University Cancer Hospital, and informed consent was obtained. Information regarding age, clinical stage (according to 2014 International Federation of Gynecology and Obstetrics (FIGO) stage of OC), pathological type, tissue differentiation, and lymph node metastasis was acquired from the medical record system of the hospital. The follow-up time was from the initial treatment time to March 2020 (median 112 months). The results of the follow-up were divided into two groups: death during the follow-up period was regarded as status 1; surviving or lost to follow-up was considered as status 0. The medical ethics committee of Harbin Medical University approved the study.

Immunohistochemistry

Immunohistochemical staining was performed on GBAS in paraffin-embedded tissue. The slides were incubated overnight in an oven at 80 °C, dewaxed with xylene and rehydrated with graded alcohol. Three percent of hydrogen peroxide was used to inactivate endogenous peroxidase, and sodium citrate for antigen retrieval. To reduce non-specific background staining, the sections were incubated with bovine serum albumin for 20 min at room temperature. Approximately 50 μL of diluted anti-GBAS antibody (1:100) was added dropwise to each section, and incubated overnight at 4 °C. The section with the same amount of PBS was used as a negative control. Drop the secondary antibody (ZLI-9018 DBA Kit) and incubate, diaminobenzidine tetrahydrochloride for immunostaining, hematoxylin counterstain to stain the nucleus, dehydrated with graded ethanol and xylene, and fixed in the medium On the resin. After drying, the slides were observed under a conventional optical microscope.

Scoring method

The scoring method is to add the color intensity score and the percentage of positive cells. According to the sum of the scores, the samples are divided into four levels, namely (−), (±), (+) and (++), where (+) and (++) mean positive. The percentage of positive cells is divided into five levels: 0 points mean no positive cells, 1 point ≤ 25%, 2 points 26%-50%, 3 points 51%-75%, and 4 points > 75%. The color intensity is 0 points for colorlessness, 1 point for light yellow, 2 points for brownish yellow, and 3 points for brown.

Cell Culture

Human OC cell lines HO-8910 and SK-OV-3 were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). The cells were cultured in DMEM (Corning, NY) cell culture medium supplemented with 10% fetal bovine serum (Ausbian, Australia) and Puromycin, and the incubator conditions were controlled at 37 °C and 5% CO_2.

Construction and Transfection of shRNA Lentivirus Vectors

To probe the influences of GBAS expression on the vicious behavior of OC, we designed specific shRNA knockdown GBAS in vitro. ShGBAS was designed according to GBAS target sequence AGATAAACACTACCCTTGT. The double-stranded DNA oligo was attached to the linearized vector, and the ligated product was introduced into *E. coli* cells. PCR identified the positive clones and sequenced them, and then the plasmid was extracted. Next, the plasmid and the packaged plasmid used to prepare the virus were introduced into 293T cells to prepare the lentiviral vector expressing the shGBAS plasmid. The empty GV115 lentivirus vector (shCtrl) serves as a control. Then, HO-8910 and SK-OV-3 cell lines were transfected with shGBAS

and shRNA, respectively. The shGBAS lentiviral vector and the interaction protein (EIF3F, ACTN4, eEF1A1, and ANXA2) overexpression vector were added to SK-OV-3 cells to prepare GBAS knockdown and interaction protein overexpression cells for functional recovery experiments. The efficiency of cell infection was observed by fluorescence microscopy after 72 h.

QRT-PCR Detection

Trizo reagent (Shanghai Pufei Biotechnology Co., Ltd., Shanghai, China) was used to extract mRNA from OC cells after transfection, and then use Promega M-MLV kit (Promega, Madison, WI) to synthesize cDNA, using LightCycler 480 II (Roche, Basel, Switzerland) real-time PCR was performed. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The GAPDH sequence is: 5′-TGA CTT CAA CAG CGA CAC CCA-3′ (forward), 5′-CAC CCT GTT GCT GTA GCC AAA-3′ (reverse).

Western Blot

After washing with PBS, the cells were lysed in RIPA buffer, and the supernatant was taken to determine the protein concentration using bicinchoninic acid (BCA) Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). Adjust the protein concentration of each sample to 2 μg/μL. Then use SDS-PAGE protein electrophoresis instrument (Shanghai Tianneng) for electrophoresis, and then transfer to polyvinylidene-fluoride (PVDF) membrane (Millipore, MA). Incubate the membrane in a TBST solution containing 5% skim milk for 1 h before adding the primary antibody (Anti-GBAS, 1:1000; Anti-EIF3F, 1:2000; Anti-ACTN4, 1:1000; Anti-eEF1A1, 1/40 000; Anti-ANXA2, 1:1000; Anti-CDK1, 1:1000), incubate overnight at 4 °C. The next day, wash the membrane and add a secondary antibody (Anti-Rabbit IgG; Anti-Mouse IgG) and incubate for 1 h. Among the antibodies, GBAS, EIF3F, ACTN4, eEF1A1, ANXA2 were from Abcam (Cambridge, UK), CDK1 and secondary antibodies were from CST (USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology, USA) was taken as an internal control. The experiment was repeated three times.

Cloning Detection

Monolayer cells at the logarithmic growth stage were digested with 0.25% trypsin and blown into single cells, then cell suspension was prepared with a new medium and counted, and cultured at 37 °C and 5% CO_2 for 72 h. Next, the cells were laid on a 6-well plate with a paving amount of 1×10^3/well. After 7 days, the cells were fixed with 4% paraformaldehyde, then 1000 μL of crystal violet dye was added to each well and the cells were counted.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Detection

To measure the proliferation ability of the cells, the transfected cell line was trypsinized and then made into a cell suspension and counted. Cells were inoculated into 96-well plates at a density of 2×10^3/well. For 5 consecutive days, 20 μL of 5 mg/mL MTT (Genview, USA) was added into the culture well and incubated for 4 h. After the supernatant was removed, 100 μL dimethyl sulfoxide (DMSO) (Corning, NY) was added to each well and shaken for 5 min at room temperature. At last, the OD value was detected at the wavelength of 490 nm by an enzyme marker.

Flow Cytometry

The transfected HO-8910 cell line was inoculated into a 6-well plate, cultured to 85% confluency, digested with trypsin, washed with PBS and centrifuged, and then added 200 μL of 1× binding buffer to configure the cell suspension. To detect cell apoptosis, the cell suspension was stained with 10 μL of FITC-annexin-V solution, incubated at room temperature and protected from light for 15 min, and then detected with BD C6 PLUS (BD, NJ) flow cytometer.

Transwell Migration Test, Invasion Test

Corning transfer kit and invasion kit (Corning, NY) were used for cell migration and invasion experiments. The invasion kit should be placed in a 37 °C incubator for 2 h to rehydrate the Matrigel matrix layer. The HO-8910 and SK-OV-3 cell lines were added to the kit at a density of 8×10^4/well and 5×10^4/well, respectively, 30% FBS medium was added to the lower chamber, and cultured for 24 h. Use a cotton swab to gently remove non-transferred cells in the chamber, then place the chamber in 4% paraformaldehyde fixative for half an hour and stain with 0.5% crystal violet. Count the cells under the microscope.

Wound Scratch

Inoculate the transfected cells into 96-well plates at a density of 5×10^4 and culture for 24 h to make the cell fusion rate reach 90%. Use a scratcher to gently push the center of the 96-well plate to form a scratch. Re-add low-concentration serum medium and culture at 37 °C and 5% CO_2. Take pictures at 0 and 24 h, and finally analyze the migration area with a Celigo cell counter (Nexcelom Bioscience, Lawrence, MA).

In Vivo Tumor Formation Experiment in Mice

The SK-OV-3 cell line transfected with shCtrl or shGBAS was cultured for 6 days and then inoculated into female BALB/c nude mice subcutaneously (4×10^4 cells/mouse). After 24 days, the tumor volume was measured and collected twice a week for a total of five times. On the 36th day, the mice were euthanized by an overdose of 2% sodium pentobarbital, and cervical spondylolysis was performed to confirm the death. Then, the tumor was taken out and the volume was measured and weighed. Tumor volume = $\pi/6 \times L \times W \times W$, that is, = $3.14/6 \times L \times W \times W$, where L represents the long diameter and W represents the short diameter. The experiment was authorized by the Ethics Committee of the Harbin Medical University Cancer Hospital.

CO-IP and Shotgun LC-MC Mass Spectrometry Analysis

Using PCR technology, the gene sequence encoding 3× FLAG tag was fused to the 5′-end of GBAS gene to generate 3× FLAG-GBAS fusion gene, and the fusion gene expression plasmid was prepared to infect SK-OV-3 cells. The control group used an empty Lentiviral plasmid. The cells were lysed with RIPA lysate and sonicated on ice. The total protein of the supernatant cells was collected by centrifugation, and the protein was quantified by the BCA method. Take the same amount of two groups of proteins and use FLAG-beads (Sigma, USA) for co-immunoprecipitation (Co-IP) to purify the protein complex. Co-immunoprecipitation samples were subjected to SDS-PAGE electrophoresis and

Coomassie brilliant blue staining. Subsequently, the obtained gel image was analyzed by Q Exactive Plus mass spectrometer (Thermo, MA), and the original spectrum file generated by Q Exactive was submitted to the MASCOT2.6 server for database using Proteome Discoverer 2.2 (Thermo Fisher Scientific) software Retrieval. Filter the data according to the standard of FDR < 0.01, and obtain highly reliable qualitative results. Finally, gene ontology (GO) analysis and KEGG analysis are performed on the protein list identified by shotgun mass spectrometry, and genes that may interact with the target gene are selected based on the biometric analysis for WB verification.

Statistical Analysis

GraphPad Prism 6.0 (GraphPad software, San Diego, CA) and SPSS 20.0 software (IBM SPSS, Chicago, IL) were used to analyze the data. The relationship between the expression of GBAS and prognosis was analyzed by Kaplan–Meier method, and the multivariate analysis was performed by Cox regression model. The values are expressed as mean ± SD. The analysis of differences between the two groups was done using Student's *t*-test. $P < .05$ was considered statistically significant.

Results

Immunohistochemistry

The results of immunohistochemistry showed that GBAS is highly expressed in epithelial ovarian cancer (EOC) tumor cells (Figure 1A and B). Table 1 lists the correlation between GBAS overexpression and clinicopathological characteristics. It was found that the overexpression of GBAS was significantly related to lymph node metastasis ($P = .029$), and was not related to age ($P = .671$), FIGO stage ($P = .055$), pathological type ($P = .056$), and histological grade ($P = .399$). Kaplan–Meier survival curve and logistic test (Figure 1E and F) showed that contrasted to patients with low GBAS expression, patients with overexpression of GBAS had markedly reduced DSF time and overall survival (OS) time. Supplementary Table S1 revealed the correlation between GBAS expression and clinicopathological parameters and prognosis. Cox regression analysis of survival rate (Supplementary Table S2) indicated that GBAS overexpression significantly increased the prognostic risk of OC. Overexpression of GBAS is an independent prognostic indicator of EOC patients.

Glioblastoma-amplified sequence lentiviral transfection silences the expression of GBAS

Lentivirus-mediated shRNA transfection was used to knock down the expression of GBAS in vitro. Figure 2A shows that the control and target lentiviruses infected the target cells by fluorescence observation under a microscope for 72 h. The results showed that the cell infection efficiency reached about 80% and the cell status was normal. To evaluate the expression of GBAS in OC cell lines after shGBAS and shctrl transfection, the levels of GBAS mRNA and protein were detected by QRT-PCR and Western blot. As shown in Figure 2B, compared with shctrl cells, the mRNA levels in HO-8910 and SK-OV-3 cells transfected with shGBAS were significantly reduced, and the difference was statistically significant ($P < .01$). Likewise, Western blot results showed that the protein expression level decreased significantly after GBAS knockdown (Figure 2C).

The Silence of GBAS Markedly Inhibits Cell Proliferation and Promotes Cell Apoptosis

The results of clone formation detection and MTT experiment showed that the cell proliferation ability was significantly inhibited after GBAS knockdown in vitro. As shown in Figure 3A, the number of cell clones in the shGBAS group was significantly reduced, and the difference

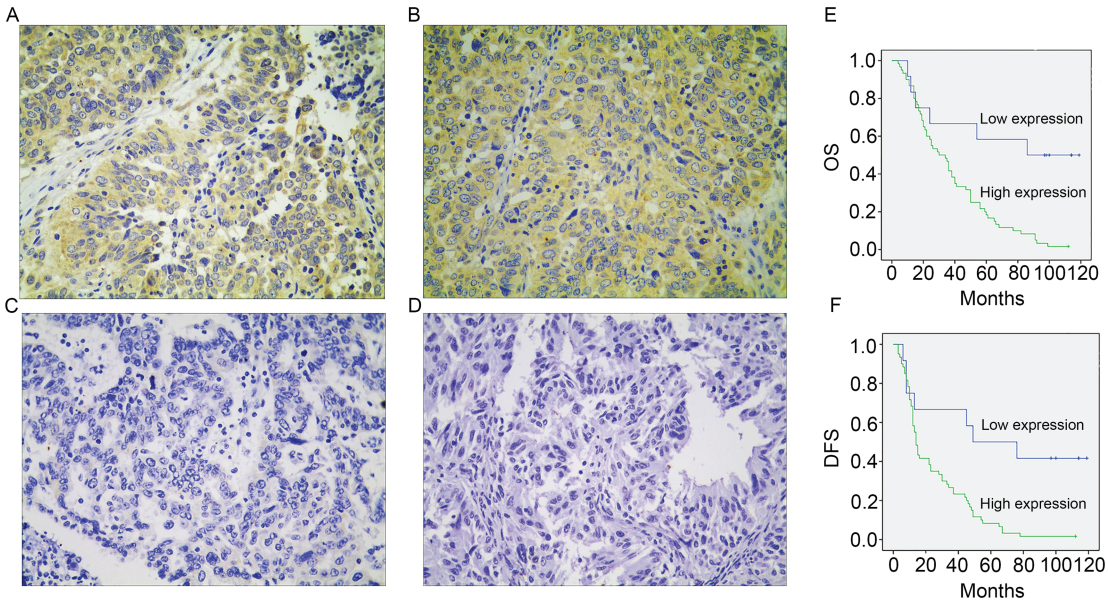

Figure 1. Immunohistochemical staining was performed to evaluate the expression level of GBAS in OC cells and its relationship with clinicopathological characteristics and prognosis. (A) and (B) are positive cases for GBAS with cytoplasmic immunohistochemical expression. (C) and (D) showed low expression of GBAS. Overall survival (E) and disease-free survival (F) of GBAS-positive patients were sharply declined compared with GBAS-negative patients by using the Kaplan-Meier method ($P < .01$).

Table 1. Association between overexpression of GBAS and clinicopathologic variables in epithelial ovarian cancer.

Variables	N	GBAS overexpression		*P*-value
		Negative[a]	Positive[b]	
All cases				
Age (years)				
>53	32	6	26	.671
≤53	40	6	34	
FIGO stage				
I-II	13	5	8	.055
III-IV	59	7	52	
Histological grade				
G1	9	4	5	.056
G2/G3	63	8	55	
Pathological type				
Serous	64	10	54	.399
Mucus	4	1	3	
Other types	4	1	3	
Lymph node metastasis				
No	52	12	40	.029
Yes	20	0	20	

Abbreviations: *n*, number of patients; FIGO, International Federation of Gynecology and Obstetrics; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated.
[a]Glioblastoma-amplified sequence immunohistochemical staining manifested as (–) or (±).
[b]Glioblastoma-amplified sequence immunohistochemical staining manifested as (+) or (++).

was statistically significant ($P < .01$). The cell growth curve drawn by detecting the OD value of the cells with a microplate reader shows that the cell proliferation ability after shGBAS transfection is significantly inhibited (Figure 3B). In addition, Annexin-V FITC staining was executed to investigate cell apoptosis. Compared with the control group, cell apoptosis increased significantly after shGBAS transfection (Figure 3C).

Downregulation of GBAS Expression Inhibits Cell Migration and Invasion Ability

To evaluate the migration and invasion capabilities of cells, we conducted transwell experiments and scratch experiments. It can be seen from Figure 4A that in the transwell assay without Matrigel, contrasted to the control group, the number of migration of shGBAS transfected cells was distinctly reduced ($P < .01$). Similarly, in the Matrigel assay, the invasive capacity of shGBAS cells was significantly reduced, and the difference was statistically significant ($P < .01$). What is more, scratch experiments showed that GBAS silencing could significantly reduce the migration length of cells (Figure 4C). In summary, the inhibition of GBAS expression can reduce the migration and invasion ability of OC cells.

Expression of GBAS In Vivo Promotes Tumor Development in Mice

The shGBAS-transfected cells were inoculated into nude mice to evaluate the effects of GBAS in vivo. The results showed that the tumor volume and weight of nude mice infected with GBAS knockdown cells were significantly smaller than those in the control group (Figure 5B), and the difference was statistically significant (Figure 5C). The weight of the two groups of mice was not statistically significant. In conclusion, the silence of GBAS expression in mice inhibited tumor development.

Glioblastoma-Amplified Sequence Exerts Its Control Ability by Combining with eEF1A1

Next, we wonder about the regulation mechanism of GBAS in OC. A lentivirus fused with 3× FLAG-GBAS was constructed to infect SKOV03 cells, and Co-IP was performed with an Anti-Flag antibody. Co-immunoprecipitation protein samples were subjected to SDS-PAGE electrophoresis and Coomassie brilliant blue staining. The obtained gel strips were decomposed into peptides with trypsin. Then, each peptide sample was identified by LC-MS and the library was searched by Proteome Discoverer/MASCOT software to obtain protein identification results. In the end, we obtained 253 candidate interaction proteins for bio-signal analysis and drew a gene interaction network diagram (Figure 6A). Since no relevant TCGA data was found, the expression levels of cancer and adjacent cancers could not be analyzed. Five top candidate proteins were selected for functional recovery experiments, including EIF3F, ACTN4, eEF1A1, ANXA2, and CDK1. As shown in Figure 6B, the Western blot results revealed that, in addition to ACTN4, EIF3F, CDK1, eEF1A1, and ANXA2, were pulled down by GBAS, indicating that they may interact with GBAS. Then, Celigo was performed to screen the functional recovery effect of the interacting protein gene on GBAS. Construct GBAS interference and interaction protein overexpression cells, use Celigo to monitor cell growth, and use the cell group infected with the empty virus vector as a control. The results showed (Figure 6D) that compared with other groups, the proliferation ability of the GBAS knockdown and eEF1A1 overexpression groups recovered better. Therefore, we chose eEF1A1 for MTT and Transwell experiments to verify cell proliferation and invasion capabilities, respectively. The MTT results are shown in Figure 6E. Contrasted to the pure GBAS knockdown group, the cell activity of the overexpression eEF1A1 group was increased ($P < .05$). Transwell results (Figure 6F) stated that the overexpression of eEF1A1 cells had a stronger cell invasion ability than the simple GBAS expression inhibition group ($P < .05$). Therefore, we believe that eEF1A1 overexpression can compensate for the decrease in cell proliferation and invasion caused by GBAS knockdown. At least to a certain extent, GBAS exerts its effect by binding to EEF1A, which is a potential downstream protein of GBAS.

Bioinformatics Analysis Was Used to Elucidate the Molecular Mechanisms by Which GBAS Contributes to the Carcinogenesis of Ovarian Cancer

Publicly available RNA-seq gene expression and miRNA expression data of 379 OC patients were downloaded from the UCSC Xena database (https://xena.ucsc.edu/). Biological targets of miRNAs were acquired from the TargetScan database (http://www.targetscan.org/.[22] Human protein–protein interaction network (PPIN) was retrieved from the STRING database (https://string-db.org/.[23] Functional enrichment analysis of GO and KEGG was performed using the cluster profiler.[24]

We calculated the Pearson correlation coefficient between expression levels of GBAS and other protein-coding genes

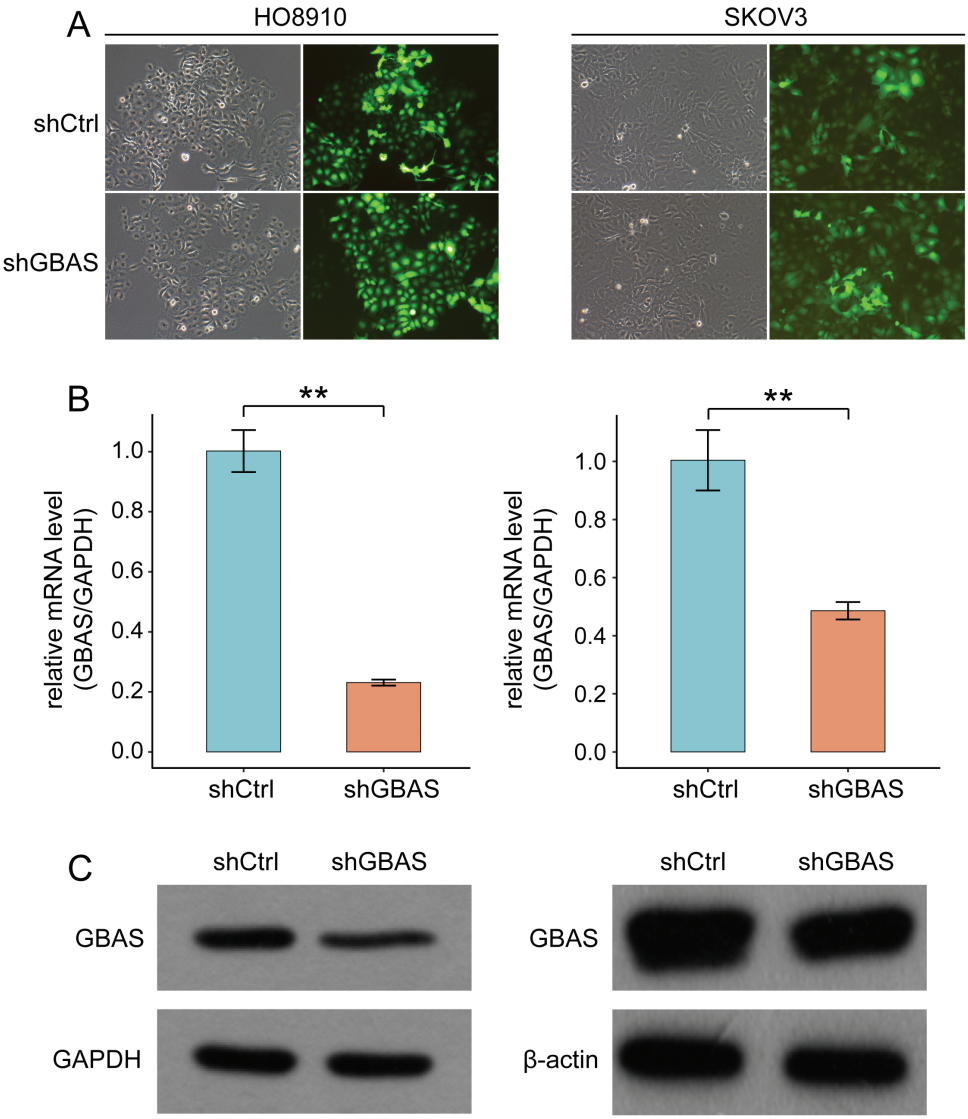

Figure 2. Glioblastoma-amplified sequence knockdown in ovarian cancer cells. (A) HO8910 and SKOV3 cells were infected with the shGBAS-carrying lentiviral vector, and were observed under a microscope for 72 h. The empty lentiviral vector was used as a control. The observation results showed that the cell infection efficiency was about 80% or more, the cell status was normal. The magnification was 100×. (B) Real-time RT-PCR was used to detect the expression of GBAS mRNA in cells after infection, and the results showed that the expression of GBAS in ovarian cancer was inhibited, and the difference was statistically significant ($P < .01$). (C) Western blot analysis showed that the expression of GBAS protein decreased in HO8910 cells after infection. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control.

(PCGs), and identified 86 PCGs co-expressed with GBAS ($P < .05$ and $|r| > .35$). After mapped to the human PPIN, we obtained an active GBAS-associated subnetwork including 57 nodes and 110 interactions (Supplementary Figure S1). Results of KEGG analysis showed that PCGs co-expressed with GBAS were significantly enriched in Thermogenesis, Spliceosome, Parkinson's disease, Prion disease, and Citrate cycle (TCA cycle) pathways. GO enrichment analysis showed that PCGs co-expressed with GBAS were significantly enriched in mitochondrial matrix, mitochondrial gene expression, and mitochondrial inner membrane pathways (Supplementary Figure S1).

To further examine whether there are miRNAs for regulating GBAS involved in OC, we also calculated the Pearson correlation coefficient between expression levels of GBAS and potential miRNAs, and found that three miRNAs (MicroRNA-27b, MicroRNA-23a, and MicroRNA-590) are significantly negatively co-expressed with GBAS ($P < .05$ and $r < -0.2$; Supplementary Figure S1).

Discussion

Metastasis is one of the main reasons for the poor prognosis of OC. Patients with OC in the middle and late stages will eventually experience systemic metastasis and gradually die.[25] Thence, it is indispensable to find a target for therapeutics of metastasis to extend the life of OC patients. Our findings indicate that the active performance of GBAS is positively related to the lymph node metastasis of OC patients, and encourages the proliferation and invasion of OC cells. Glioblastoma-amplified sequence has been reported to be related to a variety of tumors and may have broad prospects in the therapy of tumors.

According to the results of this study, we believe that GBAS can be involved in the progression and metastasis of OC as a

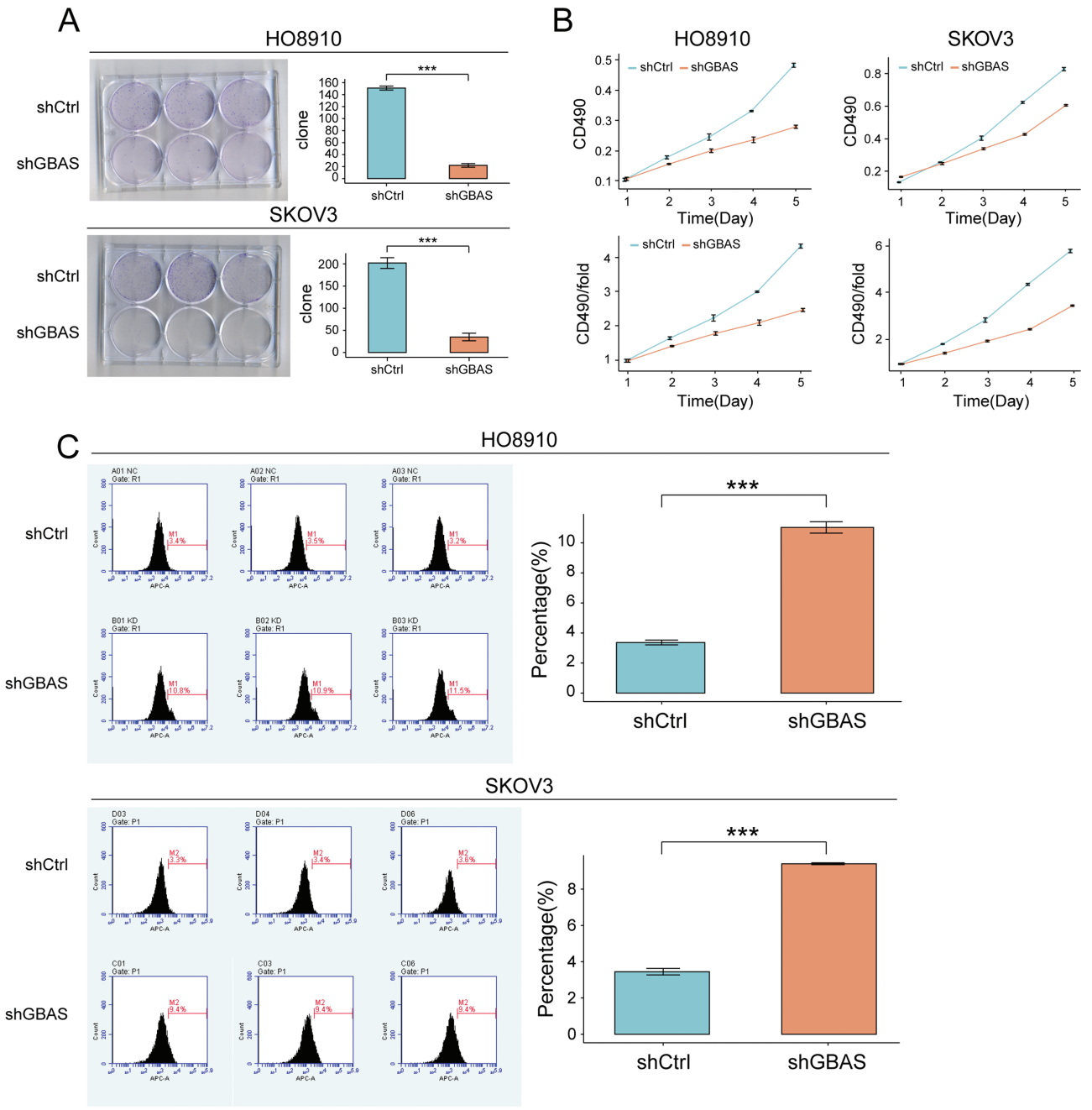

Figure 3. shGBAS transfection inhibits the proliferation of HO8910 cells and promotes apoptosis. (A) The results of the clone formation experiment showed that the cell proliferation ability was significantly reduced after GBAS knockdown ($P < .01$). (B) The transfected cells were cultured continuously for 5 days and treated with MTT for 4 h. Record the comparison of the absorbance of 490 nm light with a microplate reader every 24 h between the shGBAS group and the control group (shCtrl), and draw the cell proliferation curve. (C) Annexin V-APC single staining method detects GBAS expression inhibition and promotes cell apoptosis ($P < .01$). Values are expressed as mean ± standard deviation.

pro-oncogene. Glioblastoma-amplified sequence is highly expressed in OC cells and is associated with the malignant biological behavior and poor prognosis of OC. First, we evaluated the expression of GBAS in OC using immunohistochemistry experiments. The results showed that the overexpression of GBAS in OC was positively correlated with lymph node metastasis. Patients with overexpression of GBAS had poorer OS and disease-free survival. Glioblastoma-amplified sequence could be used as an independent prognostic factor for OC. To investigate the role of GBAS in the development and progression of OC, further experiments were conducted to observe the effect of GBAS expression on the biological behavior of OC cells. After shGBAS was designed to knock down GBAS expression in OC cells, qRT-PCR, and Western blot experiments were carried out to prove the silence efficiency. Then the clone formation experiment and MTT experiment were carried out. The results revealed that the proliferation ability of cells was obviously decreased after the down-regulation of GBAS expression. It can be observed in Transwell migration and invasion experiments that the number of cells in the shGBAS group was significantly less. Flow cytometry was used to detect cell apoptosis and the results stated that the proportion of cell apoptosis in the experimental group increased, and the difference was statistically significant. In short, we

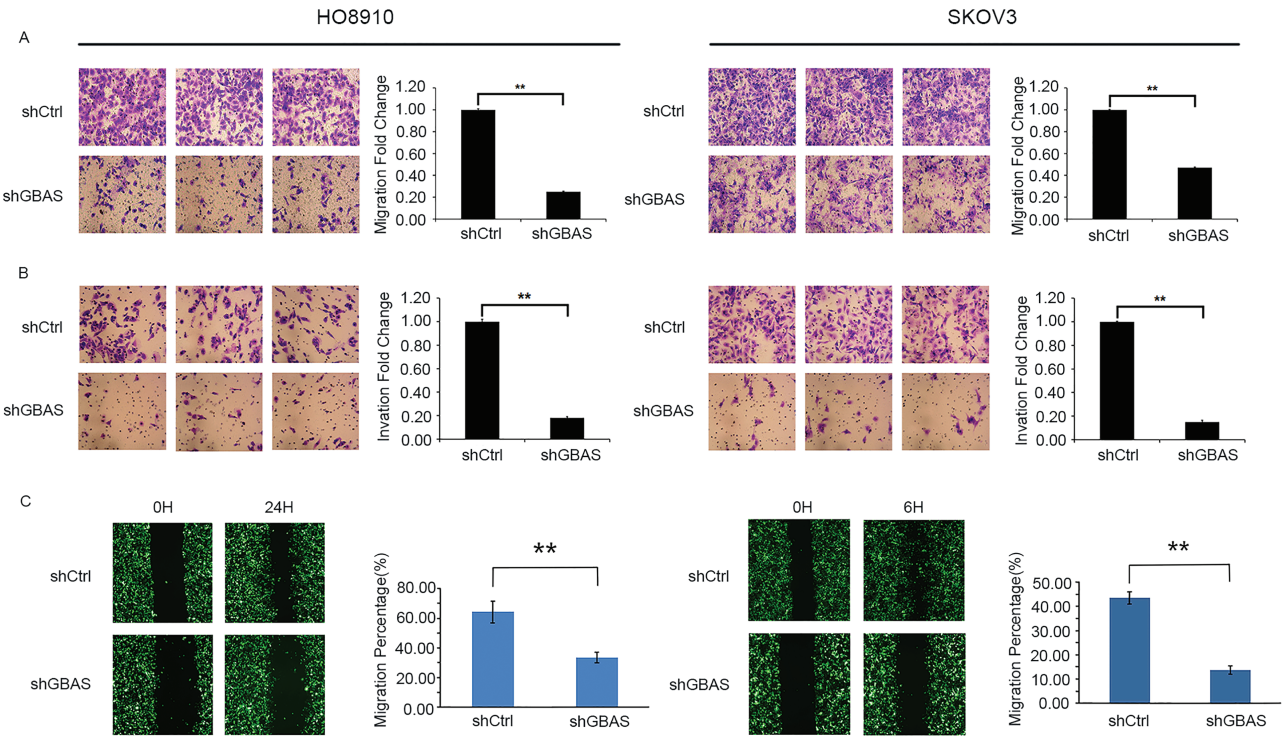

Figure 4. Glioblastoma-amplified sequence knockdown inhibits the migration and invasion ability of ovarian cancer cells. (A) Transwell analysis results without Matrigel showed that the migration ability of cells was reduced after the inhibition of GBAS expression ($P < .01$). (B) Transwell results with Matrigel showed that the invasion ability of cells was significantly reduced after transfection ($P < .01$). (C) Scratch experiment showed that the cell migration ability of shGBAS group decreased ($P < .01$). Values are expressed as mean ± SD.

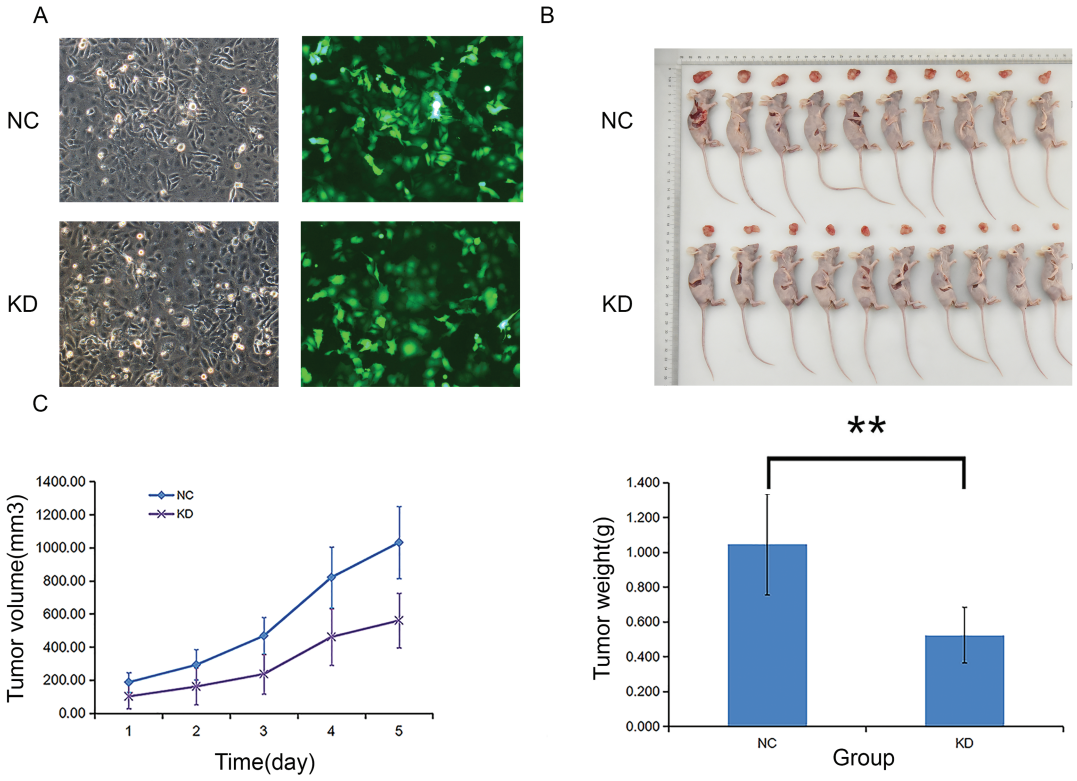

Figure 5. In vivo tumor formation experiment in mice was used to evaluate the effect of GBAS knockdown on tumor growth in vivo. (A) SKOV3 cells before inoculation of mice, magnification 200×. (B) Animal and tumor images. (C) The volume and weight of the tumor in the GBAS knockdown group were reduced compared with the control group, the difference was statistically significant ($P < .01$).

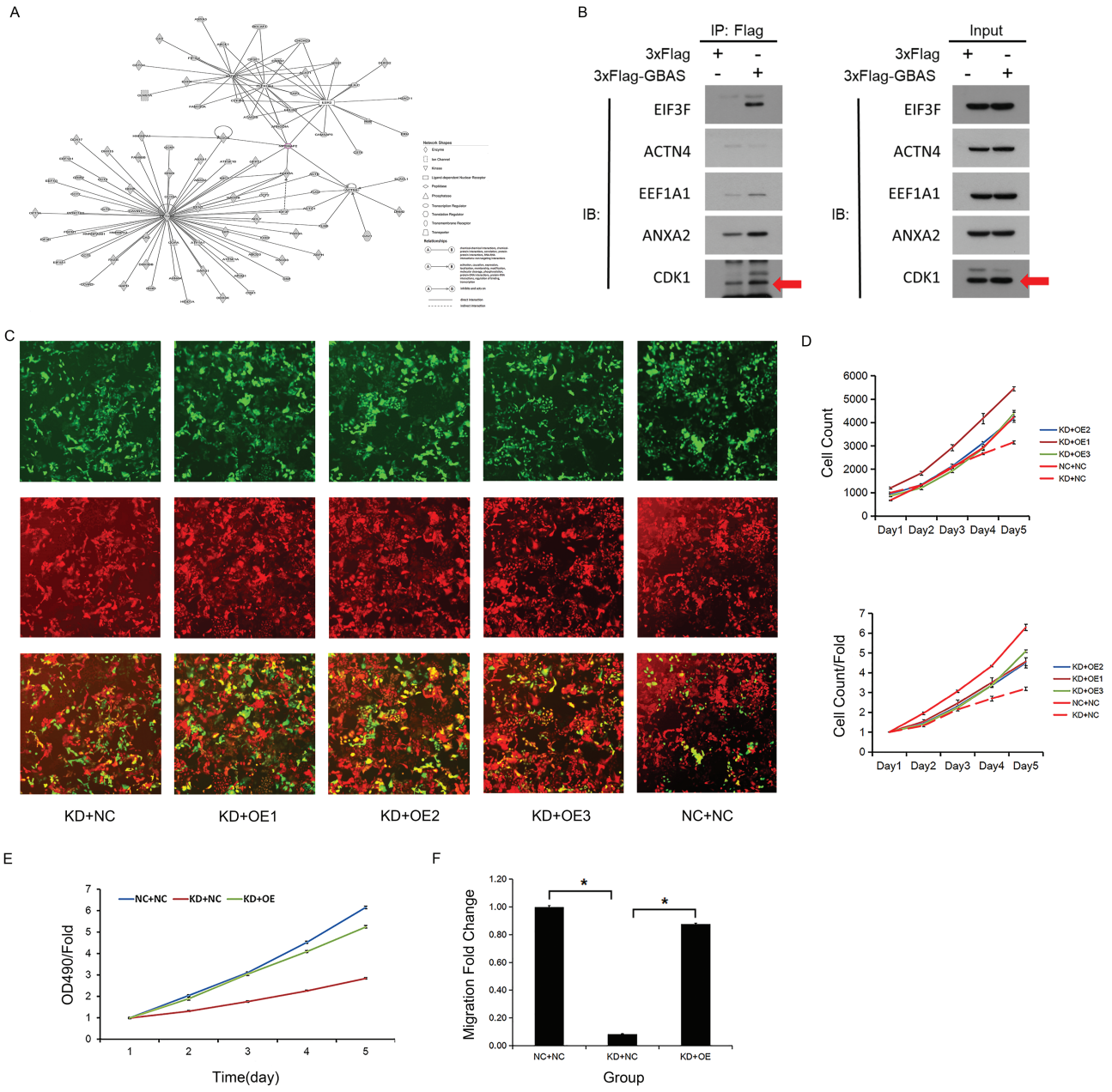

Figure 6. Glioblastoma-amplified sequence and EEF1A1 are interacting proteins. (A) Diagram of the GBAS gene interaction network. According to the gene list obtained by shotgun mass spectrometry analysis, predict their interaction with the target gene Nipsnap2, and draw a gene network diagram. (B) Select 5 best candidate proteins for functional recovery experiments. Western blot results showed that, except ACTN4, EIF3F, CDK1, EEF1A1, and ANXA2 can all be pulled down by GBAS. (C) and (D) The results of the HCS proliferation screening analysis showed that the proliferation of the KD+NC group was significantly slower than that of the NC+NC group; compared with the KD+NC group, the proliferation slowdown of the KD+OE3 group was significantly restored. NC+NC: negative control virus-infected cell group; KD+NC: GBAS knockdown + empty control virus-infected cell group; KD+OE1: GBAS knockdown+CDK1 overexpression cell group; KD+OE2: GBAS knockdown +EIF3F overexpression cell group; KD+OE3: GBAS knockdown + EEF1A1 overexpression cell group. The original magnification is 200×. (E) The results of the MTT test showed that the cell viability of the KD+NC group was decreased compared with the NC+NC group ($P < .05$); the cell viability of the KD+OE group was increased compared with the KD+NC group ($P < .05$). (F) Transwell test results showed that: compared with NC+NC group, the cell metastasis ability of KD+NC group was weakened ($P < .05$); compared with KD+NC group, cell metastasis ability of KD+OE group was enhanced ($P < .05$). (E) and (F) NC+NC: negative control virus-infected cell group; KD+NC: GBAS knockdown + empty control virus-infected cell group; KD+OE: GBAS knockdown + EEF1A1 overexpression cell group. The data are expressed as mean ± SD.

demonstrated that the downregulation of GBAS expression in vitro inhibits the pathological processes of OC cells, such as proliferation, invasion, and metastasis, and promotes the apoptosis of cancer cells. Then, we wonder whether GBAS can interfere with tumor growth in the body. The nude mouse xenograft tumor test came to verify this idea. Obviously, the growth of tumors in the GBAS downregulated group became slower. More, we wonder about the specific regulation mechanism of GBAS in OC. Therefore, CO-IP and LC-MS experiments were implemented, and the results showed that GBAS

can be combined with eEF1A1, and the inhibition of cell proliferation and invasion after GBAS knockdown can be rescued by overexpression of eEF1A1.

GBAS has previously been reported as a potential oncogene involved in the regulation of tumor development. Glioblastoma-amplified sequence has been shown to co-amplify with EGFR in a variety of cancer cell lines such as lung cancer. The study by Mi Jeong Hong and colleagues showed that EGFR and GBAS mRNA expression in NSCLC cells are significantly positively correlated, and the level of GBAS affects the prognosis of early NSCLC patients undergoing surgical resection.[12] Glioblastoma-amplified sequence has also been reported to regulate the proliferation and apoptosis of oral squamous cell carcinoma through the p53 signaling pathway.[11] Another report showed that GBAS overexpression affected the centrosomal amplification rate of bladder cancer cells and was related to adverse survival outcomes in bladder cancer patients.[12] Interestingly, GBAS was named because it is amplified in 40% of glioblastomas and was later confirmed as a mitochondrial matrix protein, which acts as a signal marker to participate in the process of mitochondrial apoptosis.[9] In addition, the protein encoded by GBAS has two tyrosine phosphorylation sites, indicating that it can be used as a substrate for tyrosine kinases. Related studies have also shown that GBAS plays a role in oxidative phosphorylation.[26,27] Glioblastoma-amplified sequence can also affect the production of inflammatory factors. Yamamoto and others pointed out that macrolide antibiotics can combine with NIPSNAP2 (GBAS) to inhibit the production of pro-inflammatory cytokines mediated via the NF-κB pathway.[10]

Our experiments show that eEF1A1 can act as an interaction protein of GBAS, and GBAS may participate in the regulation of malignant biological behaviors such as OC cell proliferation and metastasis by combining with eEF1A1. In SK-OV-3 cells, overexpression of eEF1A1 can rescue the decline in cell proliferation and metastasis caused by the downregulation of GBAS. We believe that GBAS can regulate the biological behavior of OC by combining with eEF1A1. Similarly, it has been reported that eEF1A1 can act as a new binding partner of PAK4 (P21-activated kinase 4) in gastric cancer cells, and coordinately mediate the migration and invasion of gastric cancer cells.[13] Studies by Li et al confirmed that eEF1A1 can be used as an interaction gene for MALAT1 (nuclear retention and transfer-related lung adenocarcinoma transcription 1). MALAT1 can directly bind to the eEF1A1 promoter to enhance the tumor progression of breast cancer cells.[28] Previous studies have shown that eEF1A1 is one of the pAkt-interacting proteins,[29] and eEF1A is also related to ERK.[30] Further studies by Bao et al have shown that eEF1A1 knockdown inhibits the phosphorylation of AKT and ERK, EEF1A may combine with AKT and ERK and affect their phosphorylation. In short, eEF1A1 may be a powerful chaperone protein that can regulate cell proliferation and migration by combining with other proteins, but its specific mode of action remains to be explored. eEF1A1 can also regulate the cell cycle of hepatocellular carcinoma induced by cyclin D1 through STAT1 (a member of the signal transducer and transcriptional activator family), so that the cells are arrested in the G1 phase.[31] In addition, Vera et al proved that eEF1A1 mRNA is the highest in breast cancer cells in the G1 phase. In breast cancer cells, eEF1A1 can also promote heat shock response and protect cancer cells from death caused by hypoxia or stress.[20] Specifically, eEF1A1 can initiate the HSP under stress conditions, recruiting heat shock factor 1 to key promoters, thereby stabilizing oncoproteins, such as mutant P53.[32] These data prove the ability of eEF1A1 to regulate cell cycle and apoptosis. In short, in addition to being a translation factor, eEF1A1 is also a multifunctional protein that is highly expressed in human solid tumors and hematological tumors. However, the combination of GBAS and eEF1A1 and its next action path still need to be explored.

Our research proves that eEF1A1 can rescue the decrease in cell proliferation and invasion caused by GBAS knockdown. We supposed that GBAS can play its regulatory role by regulating eEF1A1. Through immunohistochemical staining, we have reason to believe that GBAS is related to lymph node metastasis of OC, and cell morphology is very important in the process of cell metastasis. The binding of eEF1A1 to actin can affect the cytoskeleton and further change the morphology of cells. We constructed a cell model of eEF1A1 overexpression after GBAS knockdown, and verified the invasion ability of cells through the Transwell experiment with Matrigel. The results proved that the active expression of eEF1A1 promotes the invasion of OC cells. In the future, we plan to verify the specific combination of GBAS and eEF1A1, and explore whether the combination of GBAS and eEF1A1 activates the eEF1A1 regulatory pathway, or has its unique mode of action. On the other hand, eEF1A1 only plays a role as a binding protein of GBAS, or exists as a member of its downstream pathway.

In addition, bioinformatics analysis was used to elucidate the molecular mechanisms by which GBAS contributes to the carcinogenesis of OC. We obtained an active GBAS-associated subnetwork including 57 nodes and 110 interactions. We get the same conclusion that eEF1A1 can act as an interaction protein of GBAS. enrichment analysis showed that PCGs co-expressed with GBAS were significantly enriched in the mitochondrial matrix,[33] mitochondrial gene expression[34] and mitochondrial inner membrane pathways,[35,36] which were all cancer-associated signaling pathways. Mitochondria are a major source of intracellular reactive oxygen species, the production of which increases with cancer. The deleterious effects of reactive oxygen species may be responsible for the impairment of mitochondrial function observed during various pathophysiological states associated with cancer.[37] These data indicate that GBAS may be a key regulator of the mitochondrial matrix, mitochondrial gene expression and mitochondrial inner membrane, therefore controlling cancer progression. Increasing researches have demonstrated the critical functions of miRNAs in the progression of malignant tumors, including OC.[38] We also found that three miRNAs (MicroRNA-27b, MicroRNA-23a, and MicroRNA-590) are significantly negatively co-expressed with GBAS. It was reported that MicroRNA-27b, MicroRNA-23a, and MicroRNA-590 were all important oncogene in OC.[39-41] The above data indicate that MicroRNA-27b, MicroRNA-23a, and MicroRNA-590 may directly target GBAS affects the biological behavior of OC cells. Taken together, our findings regarding the role of GBAS in OC carcinogenesis, its application as a biomarker and the future perspectives of this research area.

All in all, we reported for the first time that GBAS is highly expressed in OC cells and is related to OC lymph node metastasis. Glioblastoma-amplified sequence regulates the proliferation and metastasis of OC cells at least to a certain extent by

combining with eEF1A1, and is related to the poor prognosis of OC. But its more specific mode of action still needs further experimental exploration.

Conclusion

In summary, GBAS is over-expressed in OC, which is involved in the proliferation and metastasis of OC to a certain extent, and is related to the poor survival outcome of OC patients. Our research confirms the potential role of GBAS in OC for the first time, and GBAS is expected to become a new target for the treatment of OC.

Acknowledgments

We thank all participants for their contributions to this research.

Funding

This work was supported by grants of the Liande Wu Science Foundation for Young Scholars of Harbin Medical University Cancer Hospital (WLD-QN1705) and the Jingying Foundation of the Harbin Medical University Cancer Hospital (JY2015-04). The Medical Award Foundation Project of Beijing (YXJL-2021-0577-0421); The Science and Technology Innovation Medical Development Foundation Project of Beijing (KC2021-JF-0055-01). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors indicated no financial relationships.

Author Contributions

Conception/design: F.M., R.M. Provision of study material or patients: G.S., R.Z., Q.X., W.X. Collection and/or assembly of data: G.S., J.D., S.L., L.L. Data analysis and interpretation: X.N., G.S., S. R. Manuscript writing: X.N., S.R., G.S.. Final approval of manuscript: All authors.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Harbin Medical University Cancer Hospital, and written informed consent was obtained for the use of all human specimens. The research was conducted in accordance with the Helsinki Declaration of the World Medical Association and the ethical standards of the Ethics Committee.

Data Availability

The data underlying this article will be shared at reasonable request to the corresponding author.

Supplementary material

Supplementary material is available at *The Oncologist* online.

References

1. Mathis J, Jellouli MA, Sabiani L, Fest J, Blache G, Mathevet P. Ovarian cancer screening in the general population. *Horm Mol Biol Clin Investig*. 2019;41(3). doi: 10.1515/hmbci-2019-0038
2. Ma J, Ren S, Ding J, et al. Expression of RRBP1 in epithelial ovarian cancer and its clinical significance. *Biosci Rep*. 2019;39(7):BSR20190656.
3. Kuroki L, Guntupalli SR. Treatment of epithelial ovarian cancer. *BMJ*. 2020;371:m3773.
4. Banerjee S, Gonzalez-Martin A, Harter P, et al. First-line PARP inhibitors in ovarian cancer: summary of an ESMO Open—Cancer Horizons round-table discussion. *ESMO Open*. 2020;5(6):e001110.
5. Marchetti C, Muzii L, Romito A, Benedetti Panici P. First-line treatment of women with advanced ovarian cancer: focus on bevacizumab. *Oncol Targets Ther*. 2019;12:1095-1103.
6. Wang XY, Smith DI, Liu W, James CD. GBAS, a novel gene encoding a protein with tyrosine phosphorylation sites and a transmembrane domain, is co-amplified with EGFR. *Genomics*. 1998;49(3):448-451.
7. Seroussi E, Pan HQ, Kedra D, Roe BA, Dumanski JP. Characterization of the human NIPSNAP1 gene from 22q12: a member of a novel gene family. *Gene*. 1998;212(1):13-20.
8. Brittain JM, Wang Y, Wilson SM, Khanna R. Regulation of CREB signaling through L-type Ca2+ channels by Nipsnap-2. *Channels (Austin)*. 2012;6(2):94-102.
9. Abudu YP, Pankiv S, Mathai BJ, Lamark T, Johansen T, Simonsen A. NIPSNAP1 and NIPSNAP2 act as "eat me" signals to allow sustained recruitment of autophagy receptors during mitophagy. *Autophagy*. 2019;15(10):1845-1847.
10. Yamamoto S, Ogasawara N, Yamamoto K, et al. Mitochondrial proteins NIP-SNAP-1 and -2 are a target for the immunomodulatory activity of clarithromycin, which involves NF-κB-mediated cytokine production. *Biochem Biophys Res Commun*. 2017;483(3):911-916.
11. Wang X, Bai Y, Han Y, Meng J, Liu H. Downregulation of GBAS regulates oral squamous cell carcinoma proliferation and apoptosis via the p53 signaling pathway. *Onco Targets Ther*. 2019;12:3729-3742.
12. Hong MJ, Lee SY, Choi JE, et al. Intronic variant of EGFR is associated with GBAS expression and survival outcome of early-stage non-small cell lung cancer. *Thorac Cancer*. 2018;9(8):916-923.
13. Li X, Li J, Li F. P21 activated kinase 4 binds translation elongation factor eEF1A1 to promote gastric cancer cell migration and invasion. *Oncol Rep*. 2017;37(5):2857-2864.
14. Joung EK, Kim J, Yoon N, et al. Expression of EEF1A1 is associated with prognosis of patients with colon adenocarcinoma. *J Clin Med*. 2019;8(11):1903.
15. Vera M, Pani B, Griffiths LA, et al. The translation elongation factor eEF1A1 couples transcription to translation during heat shock response. *Elife*. 2014;3:e03164.
16. Bao Y, Zhao TL, Zhang ZQ, et al. High eukaryotic translation elongation factor 1 alpha 1 expression promotes proliferation and predicts poor prognosis in clear cell renal cell carcinoma. *Neoplasma*. 2020;67(1):78-84.
17. Borradaile NM, Buhman KK, Listenberger LL, et al. A critical role for eukaryotic elongation factor 1A-1 in lipotoxic cell death. *Mol Biol Cell*. 2006;17(2):770-778.
18. Gross SR, Kinzy TG. Translation elongation factor 1A is essential for regulation of the actin cytoskeleton and cell morphology. *Nat Struct Mol Biol*. 2005;12(9):772-778.
19. Dahl LD, Corydon TJ, Ränkel L, Nielsen KM, Füchtbauer EM, Knudsen CR. An eEF1A1 truncation encoded by PTI-1 exerts its oncogenic effect inside the nucleus. *Cancer Cell Int*. 2014;14(1):17.
20. Lin CY, Beattie A, Baradaran B, Dray E, Duijf PHG. Contradictory mRNA and protein misexpression of EEF1A1 in ductal breast carcinoma due to cell cycle regulation and cellular stress. *Sci Rep*. 2018;8(1):13904.

21. Dapas B, Pozzato G, Zorzet S, et al. Effects of eEF1A1 targeting by aptamer/siRNA in chronic lymphocytic leukaemia cells. *Int J Pharm*. 2020;574:118895.
22. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife*. 2015;4:e05005.
23. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*. 2021;49(D1):D605-D612.
24. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284-287.
25. Bilbao M, Aikins JK, Ostrovsky O. Is routine omentectomy of grossly normal omentum helpful in surgery for ovarian cancer? A look at the tumor microenvironment and its clinical implications. *Gynecol Oncol*. 2021;161(1):78-82.
26. Martherus RS, Sluiter W, Timmer ED, VanHerle SJ, Smeets HJ, Ayoubi TA. Functional annotation of heart enriched mitochondrial genes GBAS and CHCHD10 through guilt by association. *Biochem Biophys Res Commun*. 2010;402(2):203-208.
27. Smits P, Rodenburg RJ, Smeitink JA, van den Heuvel LP. Sequence variants in four candidate genes (NIPSNAP1, GBAS, CHCHD1 and METT11D1) in patients with combined oxidative phosphorylation system deficiencies. *J Inherit Metab Dis*. 2010;33 Suppl 3:S13-S19.
28. Li X, Chen N, Zhou L, et al. Genome-wide target interactome profiling reveals a novel EEF1A1 epigenetic pathway for oncogenic lncRNA MALAT1 in breast cancer. *Am J Cancer Res*. 2019;9(4):714-729.
29. Pecorari L, Marin O, Silvestri C, et al. Elongation Factor 1 alpha interacts with phospho-Akt in breast cancer cells and regulates their proliferation, survival and motility. *Mol Cancer*. 2009;8:58.
30. Sanges C, Scheuermann C, Zahedi RP, et al. Raf kinases mediate the phosphorylation of eukaryotic translation elongation factor 1A and regulate its stability in eukaryotic cells. *Cell Death Dis*. 2012;3:e276.
31. Huang J, Zheng C, Shao J, Chen L, Liu X, Shao J. Overexpression of eEF1A1 regulates G1-phase progression to promote HCC proliferation through the STAT1-cyclin D1 pathway. *Biochem Biophys Res Commun*. 2017;494(3-4):542-549.
32. Lee CS, Montebello J, Rush M, Georgiou T, Wawryk S, Rode J. Overexpression of heat shock protein (hsp) 70 associated with abnormal p53 expression in cancer of the pancreas. *Zentralbl Pathol*. 1994;140(3):259-264.
33. Goard CA, Schimmer AD. Mitochondrial matrix proteases as novel therapeutic targets in malignancy. *Oncogene*. 2014;33(21):2690-2699.
34. Reznik E, Wang Q, La K, Schultz N, Sander C. Mitochondrial respiratory gene expression is suppressed in many cancers. *Elife*. 2017;6:e21592.
35. Chaudhary AK, Yadav N, Bhat TA, O'Malley J, Kumar S, Chandra D. A potential role of X-linked inhibitor of apoptosis protein in mitochondrial membrane permeabilization and its implication in cancer therapy. *Drug Discov Today*. 2016;21(1):38-47.
36. Sang M, Luo R, Bai Y, et al. Mitochondrial membrane anchored photosensitive nano-device for lipid hydroperoxides burst and inducing ferroptosis to surmount therapy-resistant cancer. *Theranostics*. 2019;9(21):6209-6223.
37. Bulteau AL, Bayot A. Mitochondrial proteases and cancer. *Biochim Biophys Acta*. 2011;1807(6):595-601.
38. Deb B, Uddin A, Chakraborty S. miRNAs and ovarian cancer: an overview. *J Cell Physiol*. 2018;233(5):3846-3854.
39. Liu W, Lv C, Zhang B, Zhou Q, Cao Z. MicroRNA-27b functions as a new inhibitor of ovarian cancer-mediated vasculogenic mimicry through suppression of VE-cadherin expression. *RNA*. 2017;23(7):1019-1027.
40. Zhuang RJ, Bai XX, Liu W. MicroRNA-23a depletion promotes apoptosis of ovarian cancer stem cell and inhibits cell migration by targeting DLG2. *Cancer Biol Ther*. 2019;20(6):897-911.
41. Salem M, O'Brien JA, Bernaudo S, et al. miR-590-3p promotes ovarian cancer growth and metastasis via a novel FOXA2-versican pathway. *Cancer Res*. 2018;78(15):4175-4190.