Uric acid enhances tumor stemness in breast cancer cells by upregulating GMPS

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Abstract. Metabolic reprogramming is closely associated with the development and progression of cancer and is considered an emerging hallmark of cancer. To meet the bioenergetic and biosynthetic demands, tumor cells tend to initiate the metabolic reprogramming process, creating a tumor microenvironment that is adaptable for cancer cell growth and further promote oncogenic signaling, cell proliferation and metastasis. As a final byproduct of purine metabolism, the uric acid (UA) concentration rises in the quickly growing tumor microenvironment. However, whether elevated UA affects the development and prognosis of patients with tumors has not been demonstrated. In the present study, Cell Counting Kit-8 and scratch healing test were used to investigate the effects of UA on the proliferation and migration of breast cancer cells. The effects of UA on nucleotide *de novo* synthesis genes in breast cells were validated using quantitative PCR (qPCR) and western blotting (WB). The activation of stemness in breast cancer cells by UA was validated using colony formation assays, ELISA, qPCR and WB. Finally, clinical data and The Cancer Genome Atlas database were analyzed to examine the expression of *de novo* nucleotide synthesis genes in cancerous and adjacent tissues, as well as in different types of breast cancer tissues. The present study investigated the potential effect of UA on the function of breast cancer; it also determined how UA affected the genes involved in nucleotide *de novo* synthesis, as well as how it contributes to tumor stemness. It was discovered that UA could enhance tumor stemness in breast cancer cells through the upregulation of guanosine 5'-monophosphate synthase (GMPS). It was notable that GMPS expression in patients with breast cancer may be strongly associated with the course of the disease and the prognosis of patients. In conclusion, the present study demonstrated that elevated UA upregulates GMPS and stimulates the migration and proliferation of breast cancer cells through the cyclic guanosine monophosphate/protein kinase G/mitogen-activated protein kinase pathway axis.

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Introduction

Breast cancer is one of the most common cancer types and the leading cause of cancer-related illness, disability, and death among women globally (1). It is reported that the influence of lifestyle and environmental factors contributes greatly to an increase in morbidity and mortality of patients with breast cancer. In recent years, great advances have been made in the development of modern and more precise imaging methods; however, a large proportion of cancers cannot be identified during early diagnosis, and severe breast cancer with distant metastases remains difficult to cure. Therefore, it is critical to explore targeted medications and investigate the mechanisms underlying breast cancer's distant metastases in addition to providing early diagnosis. Changes in metabolic expression are important in breast cancer, and various subtypes show distinct metabolic variations (2). One study highlights the identification of an early event in metastasis in breast cancer cells: Aa shift from glycolysis to high levels of oxidative phosphorylation (3). Furthermore, glutamine metabolism contributes markedly to the growth of cancer cells and is closely linked to both tumor

immunity and programmed cell death in breast cancer (4). Metabolic reprogramming of cancer cells is one of the most typical features in cancer cell proliferation and tumor growth.

Alterations in nucleotide metabolism in cancer cells are one of the hot spots in metabolic reprogramming of tumors. One metabolic characteristic that has been observed in metastatic breast cancer cells is increased *de novo* nucleotide synthesis. Furthermore, by controlling signaling pathways, nucleotide metabolites can enhance breast cancer stemness and metastasis (5). Stemness-related genes are involved in embryogenesis and stem cell maintenance. Tumor cells commonly have an elevated expression of stemness genes compared with normal cells. This function protects the tumor's developmental potential, which is directly linked to tumor growth and metastasis. Among these genes for stemness are octamer-binding transcription factor 4 (OCT4), SRY-box transcription factor 2 (SOX2), myelocytomatosis viral oncogene homolog (c-MYC) and others (6-8).

Uric acid (UA) is the end product of purine catabolism. The majority of research has indicated that UA is linked to the development and spread of tumors (9,10). It has been observed that elevated UA levels are an independent risk factor for metabolic syndrome (11). As yet, however, the precise processes by which UA influences the formation of tumors remain unclear. It is reported that the last stage of the *de novo* synthesis of guanine nucleotides is mediated by guanosine 5'-monophosphate synthase (GMPS), which transforms xanthosine 5'-monophosphate into guanosine monophosphate (GMP) (12). The present study successfully established that UA might boost cell proliferation and migration in breast cancer cells by enhancing tumor stemness in two different types of breast cancer cells. Additionally, UA could upregulate GMPS expression, which might further support GMP-related metabolism. Therefore, it stimulates the cyclic guanosine monophosphate (cGMP)/protein kinase G (PKG)/mitogen-activated protein kinase (MAPK) pathway, which in turn promotes tumor stemness. The findings could provide information to clarify the pathological significance of UA in breast cancer and design of metabolic strategies for the effective intervention of cancer diseases.

Materials and methods

Cell culture. The MCF-7 cell line was purchased from ATCC (cat. no. HTB-22TM) and the MDA-MB-231 cell line was purchased from Pronosay Life Sciences and Technology Co., Ltd. (cat. no. CL-0150B). MCF-7 and MDA-MB-231 cells were cultured in DMEM medium (cat. no. C11995500BT; Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). All cell lines were maintained at 5% CO_2 and 37˚C. Cells were cultured to full confluency, and plates were prepared for UA (cat. no. U0881-10G; MilliporeSigma) treatment.

Cell Counting Kit-8 (CCK-8) assay for cell viability. MCF-7 and MDA-MB-231 cells were cultured to logarithmic growth phase and digested for counting. MCF-7 was diluted to $2x10^4$ cells/ml (equivalent to 2,000 cells/well after seeding), and MDA-MB-231 was diluted to $4x10^4$ cells/ml (equivalent to 4,000 cells/well after seeding). Each cell line was seeded into three 96-well plates, with six technical replicates per group and three independent biological replicates. After 24 h of wall affixation, cells were treated with different concentrations of UA for 24, 48 and 72 h, respectively. The medium was then aspirated and the wells were washed once with PBS (cat. no. 60152ES76; Shanghai Yeasen Biotechnology Co., Ltd.). CCK-8 assay reagent (cat. no. 40203ES80; Shanghai Yeasen Biotechnology Co., Ltd.) diluted in serum-free, low-glucose DMEM medium (cat. no. C11885500BT; Gibco; Thermo Fisher Scientific, Inc.) was added, and the cells were transferred to the incubator and continued to be incubated for an additional 2 h. The absorbance was detected by a zymography at 450 nm, and the results were analyzed statistically.

Scratch healing test. Using a marker pen, horizontal lines were evenly drawn on the back of a 6-well plate (703001, NEST) with a straightedge, one line every 0.5-1 cm across the wells and three lines per well. Cells grown in the exponential phase in culture flasks were digested and counted. Then, ~$1x10^6$ MCF-7 cells and ~$7x10^5$ MDA-MB-231 cells were added in each of the 6 wells, inoculated to achieve a 100% confluency after overnight incubation. The cells were scratched perpendicular to the cell plane with a pipette tip (200 μl) and washed 2-3 times using sterile PBS. Then, serum-free medium containing different concentrations (0, 2, 4, 8, 12, and 16 mg/dl) of UA was added, with six technical replicates per group and three independent biological replicates. Images of the width of the scratch at 0 h were captured under a microscope and the position was recorded. Subsequently, the cells were removed at 24, 48 and 72 h, and images of the same positions corresponding to 0 h were captured under the microscope. Finally, the healing rate was calculated using ImageJ (version 1.8.0_112) software (National Institutes of Health).

Reverse transcription-quantitative (RT-q) PCR. Cells were seeded at a density of $4x10^5$ cells/well in complete DMEM medium (6 wells total), and cell density was measured using a JIMBIO cell counter (Jiangsu Jimbio Technology Co., Ltd.). After both breast cancer cells were treated at the optimal UA concentration and time, the cell density in the 6-well plates for both cell types was 80-90%. Total RNA was extracted using RNAiso Plus Total RNA Extraction Reagent (cat. no. 9109; Takara Bio, Inc.) according to the manufacturer's instructions and then reverse-transcribed to cDNA using TransScript Reverse Transcription Reagent (cat. no. AT-341; TransGen Biotech Co., Ltd.) in accordance with the manufacturer's instructions. qPCR was performed using 2X RealStar Power Dye Method qPCR premix (cat. no. A314; GenStar) on an ABI QS6P fully automated fluorescence qPCR instrument under the following cycling conditions: 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec, followed by 72˚C for 30 sec. The relative expression levels of the genes were quantified using the $2^{-\Delta\Delta Cq}$ method, with β-actin as the internal reference gene for normalization. The quantification method was performed as described by Livak and Schmittgen (13). All reactions were repeated three times, with six biological replicates in each experiment. Data are presented as mean ± SD. Primer sequences used for qPCR are listed in Table I.

Table I. Primer information used in the present study.

Genes	Direction	Primers (5'-3')
β-actin	Forward	ATCCACGAAACTACCTTCAA
	Reverse	ATCCACACGGAGTACTTGC
PRPS2	Forward	GACAAGGTAGGAGAGAGTCGTG
	Reverse	TGCAGGTCCATGGTGATGAT
GMPS	Forward	AACTGTAGGTGTGCAGGGTG
	Reverse	AACGTTGTGACACATGCGAG
ADSS1	Forward	GTGTCCTTCATTGGCAACGG
	Reverse	CGACAGCCTGGTGAAAATC
c-MYC	Forward	CCAAGCAGAGGAGCAAAAGC
	Reverse	GCACAAGAGTTCCGTAGCTG
SOX2	Forward	CATGAAGGAGCACCCGGATT
	Reverse	ATGTGCGCGTAACTGTCCAT
NANOG	Forward	CAATGGTGTGACGCAGAAGG
	Reverse	TGCACCAGGTCTGAGTGTTC
OCT4	Forward	GATGCTTTGAGCTCCCTCTGG
	Reverse	GAGGGTTTCTGCTTTGCATATCTCC

Western blotting. To test whether UA can induce the expression of GMPS and tumor stemness-associated proteins in breast cancer, cells were inoculated in complete DMEM medium at $4x10^5$ cells/well (6 wells). Lysates were obtained from MCF-7 cells treated with UA for 48 h, and MDA-MB-231 treated with UA for 24 h. Each group consists of three technical replicates and three independent biological replicates. Lysates were prepared in ice-cold RIPA buffer (cat. no. P0013B; Beyotime Biotechnology) containing protease inhibitors (cat. no. ST507; Beyotime Biotechnology). Protein concentrations were determined using the BCA Protein Assay Kit (cat. no. P0012S; Beyotime Biotechnology) with BSA as the standard. Equal amounts of protein (30 μg per lane) were separated by 10% SDS PAGE gel and transferred onto PVDF membranes. The membranes were blocked with 5% (w/v) non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h at room temperature. Subsequently, the membranes were incubated overnight at 4˚C with primary antibodies at the indicated concentrations: GMPS (cat. no. 14602; 1:1,000), Kruppel-like factor 4 (KLF4; cat. no. 12173; 1:1,000), OCT4 (cat. no. 75463; 1:1,000), Nanog homeobox (NANOG; cat. no. 4903; 1:2,000), c-MYC (cat. no. 18583; 1:1,000), SOX2 (cat. no. 23064; 1:1,000), extracellular signal-regulated kinase (ERK; cat. no. 4695, 1:1,000), phosphorylated (p)-ERK (cat. no. 4370, 1:2,000) and β-actin (cat. no. 8457, 1:1,000) (all from CST Biological Reagents Co., Ltd.). After washing with TBST, the membranes were incubated with secondary antibody anti-rabbit IgG-HRP (cat. no. 7074; 1:3,000; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.; OriGene Technologies, Inc.) for 1 h at room temperature. The protein bands were visualized using ECL detection reagent (cat. no. P0018FS; Beyotime Biotechnology) according to the manufacturer's instructions and then imaged using the Tanon 5200 fully automated chemiluminescence imaging system (Tanon Science and Technology Co., Ltd.). Densitometric analysis was performed using ImageJ software, and the relative expression levels were normalized to β-actin as the loading control.

ELISA. Logarithmic growth phase cells were collected and counted. Then, the cell suspension was inoculated into 6-well plates (~$5x10^5$ cells/well for MCF-7 and $3x10^5$ cells/well for MDA-MB-231). The cell culture plates were removed after 24 h of incubation, and the two types of breast cancer cells were treated according to the optimal UA concentration and time, with four technical replicates per group and three independent biological replicates. Samples were extracted according to the instructions of the ELISA kit (cat. no. AE86215Mu; AMEKO), standards were prepared and samples were tested. The linear regression equation of the standard curve was calculated based on the concentration of the standard and the corresponding OD value, and then the corresponding sample concentration was calculated from the regression equation according to the OD value of the sample, followed by statistical analysis.

Plate colony assay. Confluent MDA-MB-231 cells were harvested and plates were pre-treated with UA after counting using Trypan blue to exclude dead cells (0.4% trypan blue solution at room temperature in the dark for 3-5 min). Thereafter, the cells were suspended in medium containing different concentrations of UA at 3,000 cells/well in 6-well plates for 3 days. Then, the medium was changed back to normal complete medium every 3 days, and the cells were incubated for 1 week to allow clusters to form for staining. Images were captured to count the number of clusters. Each group consisted of five technical replicates and three independent biological replicates.

Clinical specimens. Human tumor specimens were obtained from patients with breast cancer (ages 40-60, all female) who underwent surgical treatment for breast cancer and were diagnosed at the Affiliated Hospital of Guangdong

Medical University. All patients provided written informed consent to participate in this study. The study was conducted in accordance with recognized ethical guidelines and were approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical University on June 25, 2023 (approval no. YJY2023231). Patient information collected included pathology and age. Samples were collected within 10 min of *ex vivo* removal during surgery, placed in RNA preservation solution, and stored at 4˚C. Within 24 h, they were transferred to a -80˚C freezer for storage. A total of 56 samples were collected. Clinical samples were collected between September 2023 and February 2024. After total RNA extraction and concentration measurement, 48 samples remained after excluding degraded portions and were used for qPCR experiments.

The cancer genome atlas (TCGA) database analysis. In the TCGA database, the present study analyzed the expression levels of nucleotide *de novo* synthesis-related genes PRPS, GMPS and ADSS in patients with invasive breast cancer across dimensions including cancer vs. normal tissue, tumor stage, and tumor type. Finally, the present study also examined the effect of nucleotide *de novo* synthesis gene expression levels on breast cancer patient survival rates.

Statistical analysis. All results are expressed as mean ± SEM. Statistical analyses were performed and plotted using SPSS 20.0 (IBM Corp.), GraphPad Prism 8 software (Dotmatics), and each set of experiments was repeated at least three times. Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's test was performed to assess which treatment groups showed significant differences from the control group. $P<0.05$ was considered to indicate a statistically significant difference.

Results

UA promotes breast cell proliferation and migration. UA is a product of dietary or endogenous purine metabolism based on adenine, and it has been reported that the clinical incidence of hyperuricemia has markedly increased (14). Based on the fact that UA plays an antioxidant role in the extracellular environment and a pro-oxidant role in the intracellular environment, clinical data collected from patients with cancer have shown that UA has a protective effect against cancer (15). There are also data suggesting that UA can mediate tumor cell proliferation, migration and survival, and contribute to tumorigenesis (16-18). No studies have yet shown the exact mechanism by which UA acts on cancer cells. First, the present study explored the role of UA on proliferation of two types of breast cancer cells, MCF-7 and MDA-MB-231, using the CCK-8 method. After cells were treated by different concentration of UA for 24, 48, and 72 h respectively, the present study discovered that UA most prominently promoted MCF-7 cell proliferation at 48 h, and there was a statistically significant difference at the concentration of 4 mg/dl compared with the control (Fig. 1A-C). Next, the present study treated MCF-7 cells with this ideal duration and three optimal concentration gradients to investigate the impact of UA on cell migration. By using scratch assays, the present study also discovered that, in comparison with a concentration of 0 mg/dl, all three UA concentrations ≥4 mg/dl encouraged the migration of MCF-7 cells (Fig. 1D and E).

In MDA-MB-231 cells, the present study discovered that after a 24 h treatment, cell viability increased with the rise in UA concentration. Only at a dose of 8 mg/dl was there a stimulation of proliferation after 48 h treatment, while UA showed no regulatory effects on the cells after 72 h treatment (Fig. 1F-H). This might be associated with the high proliferation and metabolism properties of triple-negative breast cancer MDA-MB-231 cells. After 72 h, when the medium is depleted more quickly and an acidic environment is created, the highly proliferative wells may experience a significant number of cell deaths simultaneously, which reduces their cell viability. To avoid this, the present study treated MDA-MB-231 cells for a 24 h period in the subsequent experiments. During this period, MDA-MB-231 cells exhibited persistent and evident pro-proliferative effects at all UA doses higher than 2 mg/dl. Subsequent studies treated MDA-MB-231 cells with UA concentrations of 0, 4, 8 and 12 mg/dl to match the concentration used for MCF-7 cells. At this time and concentration, the present study found that UA also markedly promoted the migration of MDA-MB-231 cells (Fig. 1I and J). In conclusion, the present study hypothesized that UA may stimulate the growth and migration of breast cancer cells.

UA upregulates the nucleotide de novo synthesis gene GMPS. It has been demonstrated that patients with stage IV breast cancer who have distant metastases have markedly elevated UA levels, and that poor survival is linked to high expression of the genes responsible for UA production (5). First, the present study investigated how UA affected PRPS2, ADSS, and GMPS, three important genes for *de novo* nucleotide synthesis. Based on the qPCR results, the present study found that UA increased the expression of GMPS in both breast cancer cell lines (Fig. 2A and D), but not PRPS2 (Fig. 2B and E). UA showed different regulatory patterns on ADSS expression in two breast cancer cell lines, which might be due to compensation of other genes (Fig. 2C and F). Western blotting studies were carried out to identify the effect of UA on GMPS expression in both breast cancer cell lines. In MCF-7 cells, the present study discovered that UA markedly affected the protein expression of GMPS, particularly at 8 mg/dl. By contrast, it is evident that MDA-MB-231 cells also rose in a concentration-dependent manner, even though the effect was not as strong (Fig. 2G-J). It is probable that triple-negative breast cancer requires a larger dose to satisfy the disease's rapid metabolism and high proliferation rate. In conclusion, through qPCR and western blotting experiments, the present study found that UA can upregulate the expression of GMPS, the nucleotide *de novo* synthesis gene, in breast cancers.

UA promotes tumor stemness through the cGMP/PKG/MAPK pathway. GMPS mediates the final step in the *de novo* synthesis of guanine nucleotides, converting xanthosine 5'-monophosphate to GMP (12). Enhanced GMP metabolism is caused by upregulation of GMPS expression, and elevated GMP may subsequently raise intracellular levels of the second messenger cGMP. cGMP is an upstream regulator of the MAPK pathway (5,19-22). ELISA testing of the intracellular cGMP

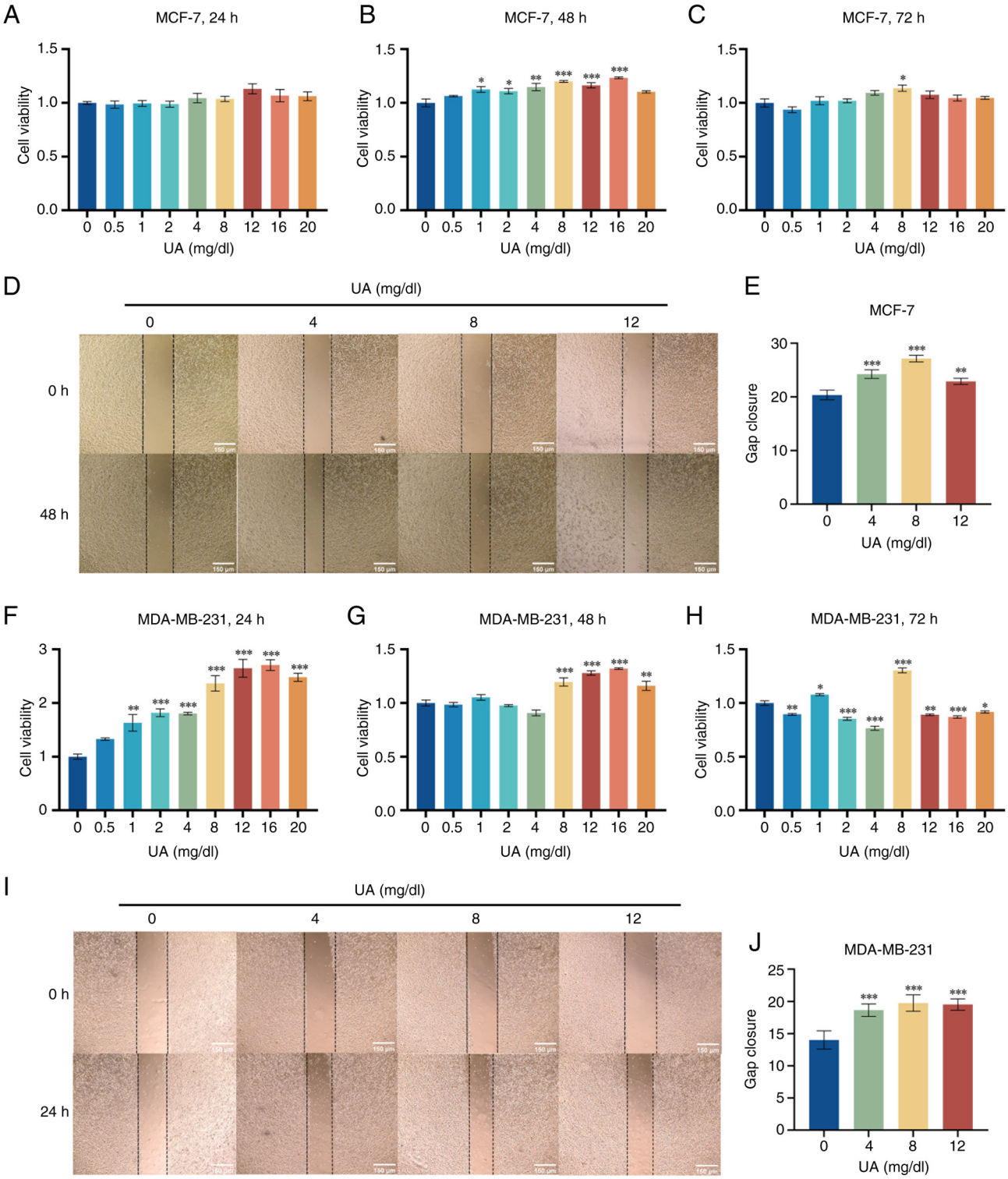

Figure 1. UA promotes proliferation and migration of breast cancer cells. (A-C) The effects of UA on proliferation of MCF-7 breast cancer cells were explored at a concentration ranging from 0 to 20 mg/dl for different time periods. (D and E) Effects of UA concentrations at 0, 4, 8 and 12 mg/dl on MCF-7 cell migration after 48 h of treatment. (F-H) The effect of UA on the proliferation of MDA-MB-231 in triple-negative breast cancer cells at a concentration ranging from 0 to 20 mg/dl for different time periods. (I and J) Effect of UA concentration at 0, 4, 8 and 12 mg/dl on the migration of MDA-MB-231 cells after 24 h of treatment. n=3 independent experiments, six technical replicates each. *P<0.05, **P<0.01, ***P<0.001 vs. 0 mg/dl UA. UA, uric acid.

content in both types of breast cancer cells treated with UA showed a rise in cGMP levels, particularly in MDA-MB-231 cells (Fig. 3A). By measuring the relative concentration of p-ERK by western blotting, the present study confirmed that elevated cGMP activates the downstream MAPK pathway. It was discovered that UA can cause different patterns of increase in p-ERK in the two types of breast cancer cells (Fig. 3B-E). In MCF-7 cells, UA strongly activated the MAPK pathway, and in MDA-MB-231 cells, it showed a concentration-dependent activation, similar to that of GMPS expression. Through

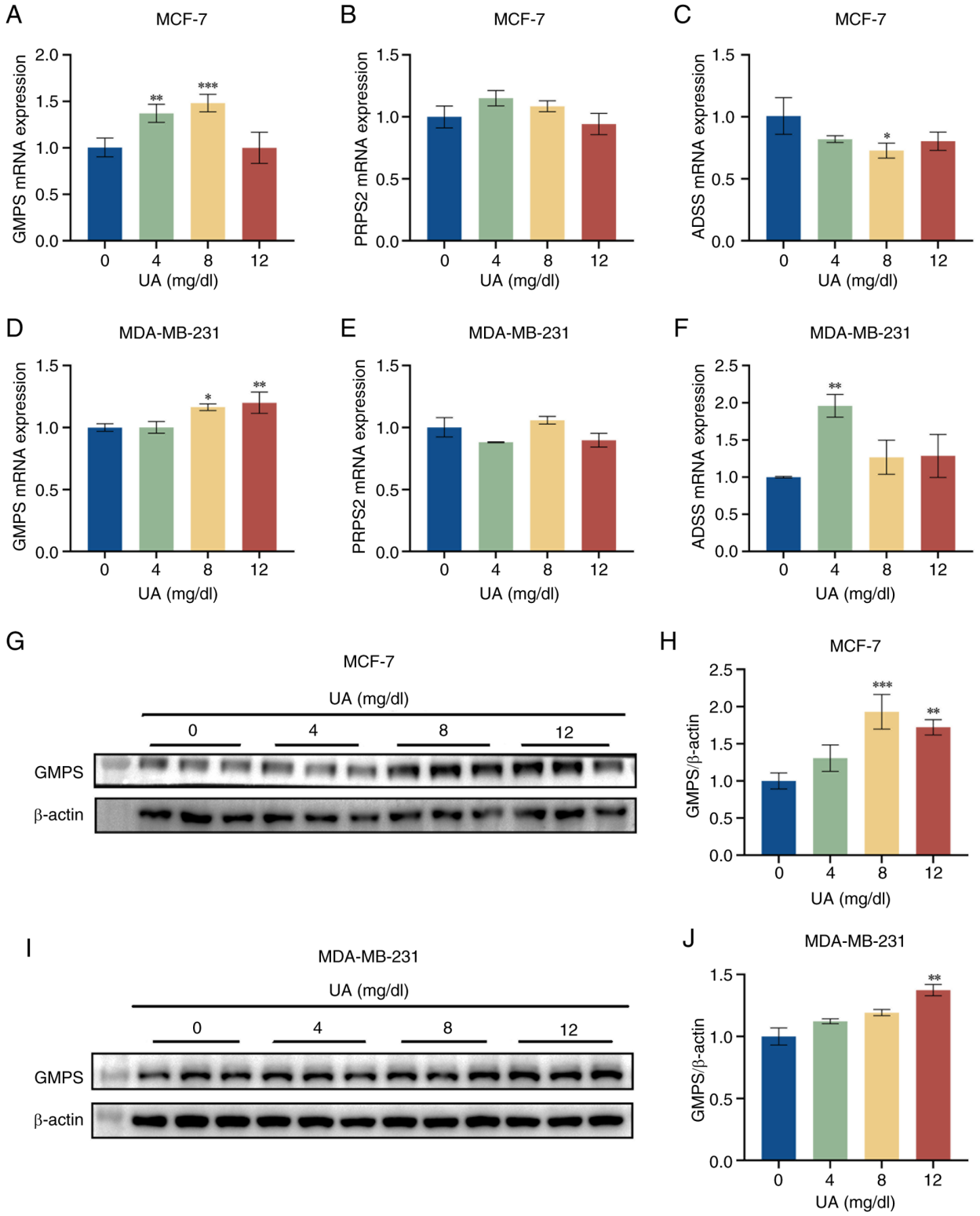

Figure 2. Effect of UA on genes related to nucleotide *ab initio* synthesis in breast cancer cells. (A-C) Effect of UA treatment of breast cancer cells MCF-7 on nucleotide *ab initio* synthesis gene expression. (D-F) Effect of UA treatment of triple-negative breast cancer cells MDA-MB-231 on nucleotide *ab initio* synthesis gene expression. n=3 independent experiments, six technical replicates each. (G and H) Western blotting of the effect of UA on the expression of GMPS protein, a nucleotide *ab initio* synthesis gene, in MCF-7 of breast cancer cells. (I and J) The effect of UA on GMPS protein expression of nucleotide *ab initio* synthesis gene of breast cancer cell MDA-MB-231. n=3 independent experiments, three technical replicates each. *P<0.05, **P<0.01, ***P<0.001 vs. 0 mg/dl UA. UA, uric acid; GMPS, guanosine 5'-monophosphate synthase.

plate colony studies of MDA-MB-231 cells, the present study discovered that UA treatment increased the number of cell colonies and enhanced tumor stemness (Fig. 3F and G). In conclusion, in both breast cancer cell lines, the upregulation of GMPS expression induced by UA could activate the cGMP/PKG/MAPK pathway by increasing the intracellular

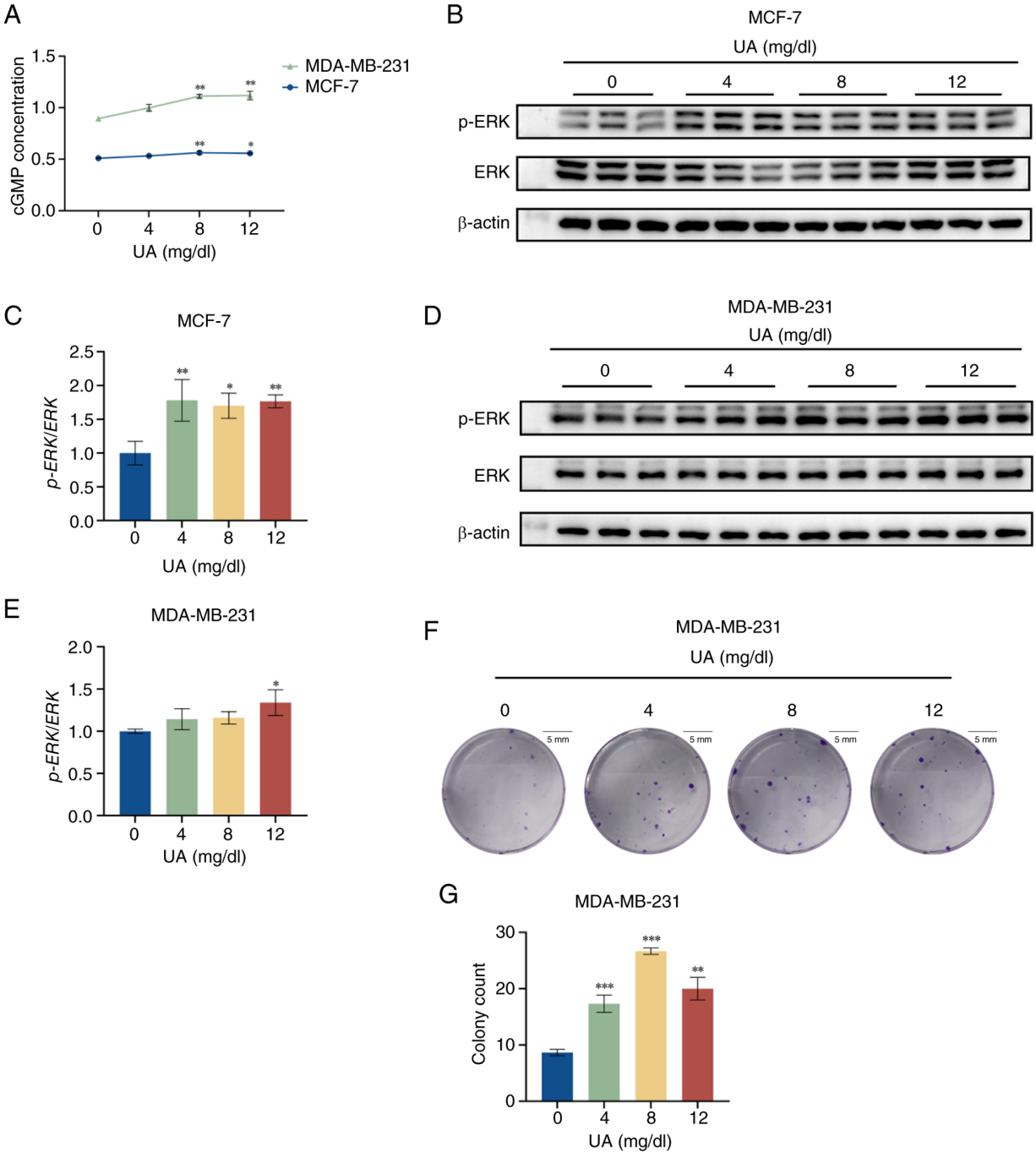

Figure 3. UA can activate the cGMP/PKG/MAPK pathway to promote tumor stemness. (A) Effect on intracellular cGMP content after UA treatment of breast cancer cells, detected by Elisa. (B and C) Effect on intracellular p-ERK after UA treatment of MCF-7 cells. (D and E) Effect of UA on intracellular p-ERK following treatment of MDA-MB-231 cells. n=3 independent experiments, three technical replicates each. (F and G) Results of plate colony experiments of MDA-MB-231 triple-negative breast cancer cells treated with UA. n=3 independent experiments, five technical replicates each. *P<0.05, **P<0.01, ***P<0.001 vs. 0 mg/dl UA. UA, uric acid; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; MAPK, mitogen-activated protein kinase; p-, phosphorylated.

cGMP content, which ultimately led to enhanced tumor stemness, thereby promoting cell proliferation and migration.

UA upregulates tumor stemness-related genes in breast cancer cells. Tumor stem cells (CSCs) are a small subpopulation of cells with the potential for self-renewal in cancer cells, and cancer development and progression is driven by CSCs (23). Tumor stemness-associated genes include OCT4, SOX2, KLF4, c-MYC, and NANOG, among others. A number of studies have shown that activation of the MAPK pathway promotes tumor stemness (5,24,25). The present study then validated the effects of UA on these tumor stemness-related genes by qPCR and western blotting to demonstrate that the MAPK pathway triggered by UA can promote the stemness of breast cancer cells. The findings demonstrated that stemness genes KLF4, c-MYC, and NANOG were markedly affected by UA in mRNA expression levels in MCF-7 cells (Fig. 4A). In addition, there were consistent results at the protein expression levels. In MCF-7

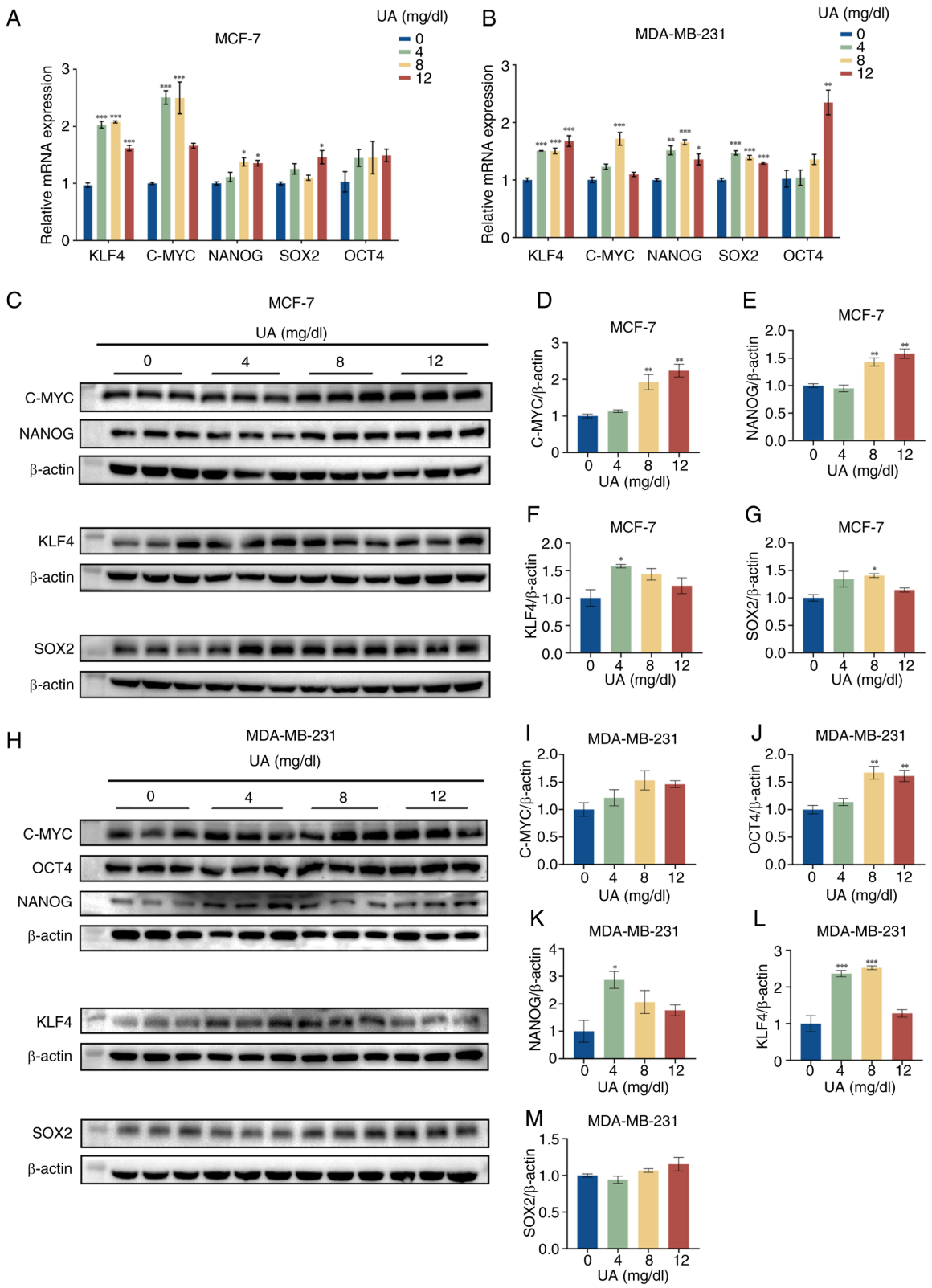

Figure 4. Effect of UA on tumor stemness-related genes in breast cancer cells. (A) Effect of UA on the expression of MCF-7 tumor stemness-related genes in breast cancer cells. (B) Effect of UA on the expression levels of MDA-MB-231 tumor stemness-related genes in breast cancer cells. (C-G) Effect of UA on the protein expression level of MCF-7 tumor stemness-related genes in breast cancer cells. (H-M) Effect on the protein expression levels of MDA-MB-231 tumor stemness-related genes in breast cancer cells. n=3 independent experiments, with three technical replicates each. *P<0.05, **P<0.01, ***P<0.001 vs. 0 mg/dl UA. UA, uric acid; KLF4, Krüppel-like factor 4; c-MYC, cellular myelocytomatosis oncogene; NANOG, Nanog homeobox; SOX2, SRY-box transcription factor 2.

cells, UA induced the highest level of protein expression for these three stemness genes (Fig. 4C-G). However, the OCT4 protein in MCF-7 cells was not detected by western blotting. Previous reports have indicated that faint cytoplasmic staining of OCT4 can be observed in MCF-7 cells, but OCT4 signals were not detected by western blotting (26,27). It is possible

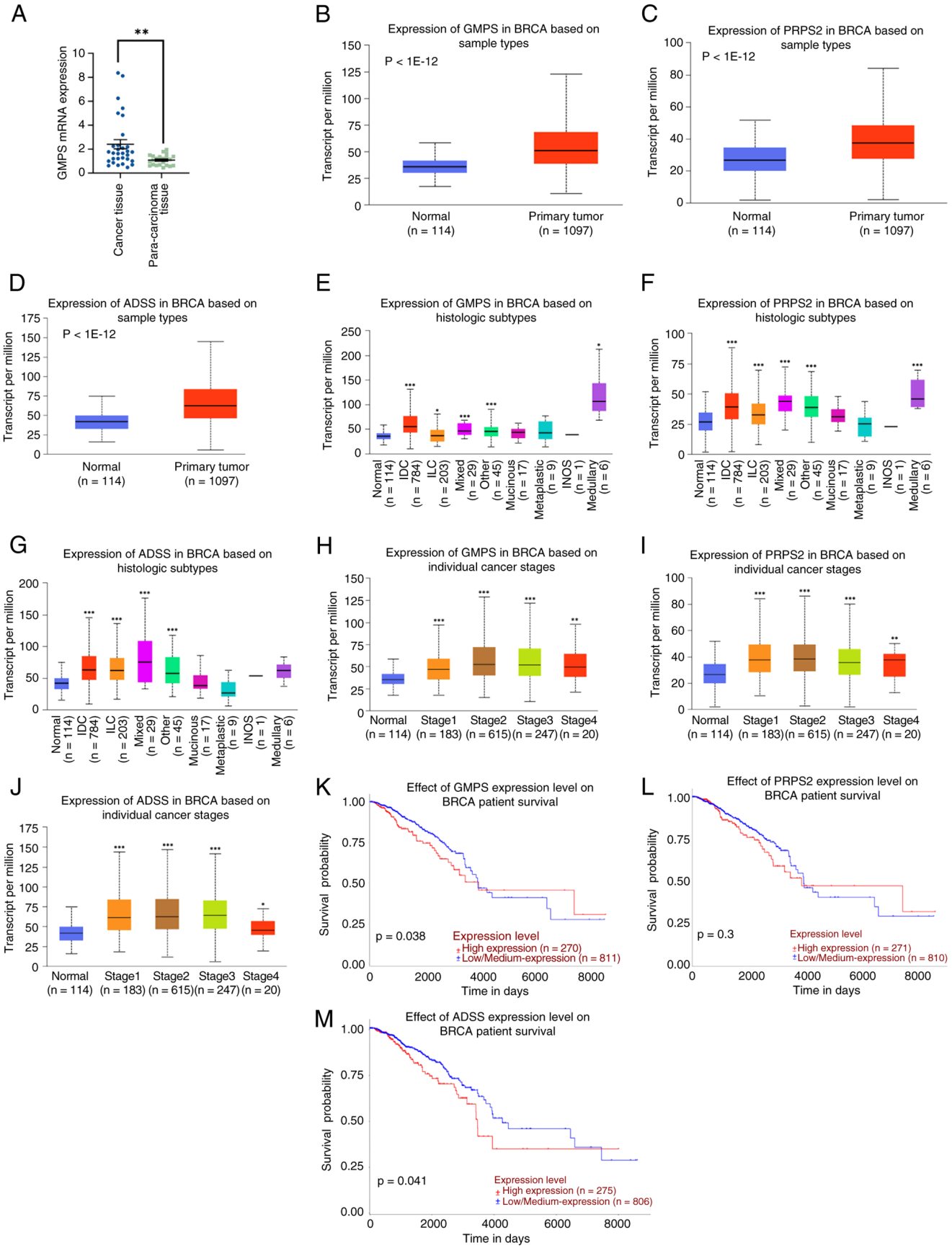

Figure 5. Expression of nucleotide *de novo* synthesis genes in breast cancer and paracancerous tissues. (A) GMPS expression in clinically collected cancer and paracancer tissues was detected by qPCR (n=25). (B-D) Differences in the expression of nucleotide *de novo* synthesis genes between carcinoma and paracancer of breast cancer were analyzed in the TCGA database. (E-G) Differences in expression of nucleotide *de novo* genes across pathological types of breast cancer analyzed in the TCGA database. (H-J) Differences in expression of nucleotide *de novo* synthesis genes across clinical stages of breast cancer analyzed in the TCGA database. (K-M) Analysis of the effect of nucleotide *de novo* synthesis genes in on the prognosis of patients with breast cancer in the TCGA database. *P<0.05, **P<0.01, ***P<0.001 vs. normal group. GMPS, guanosine 5'-monophosphate synthase; BRCA, breast cancer susceptibility gene; TCGA, The Cancer Genome Atlas.

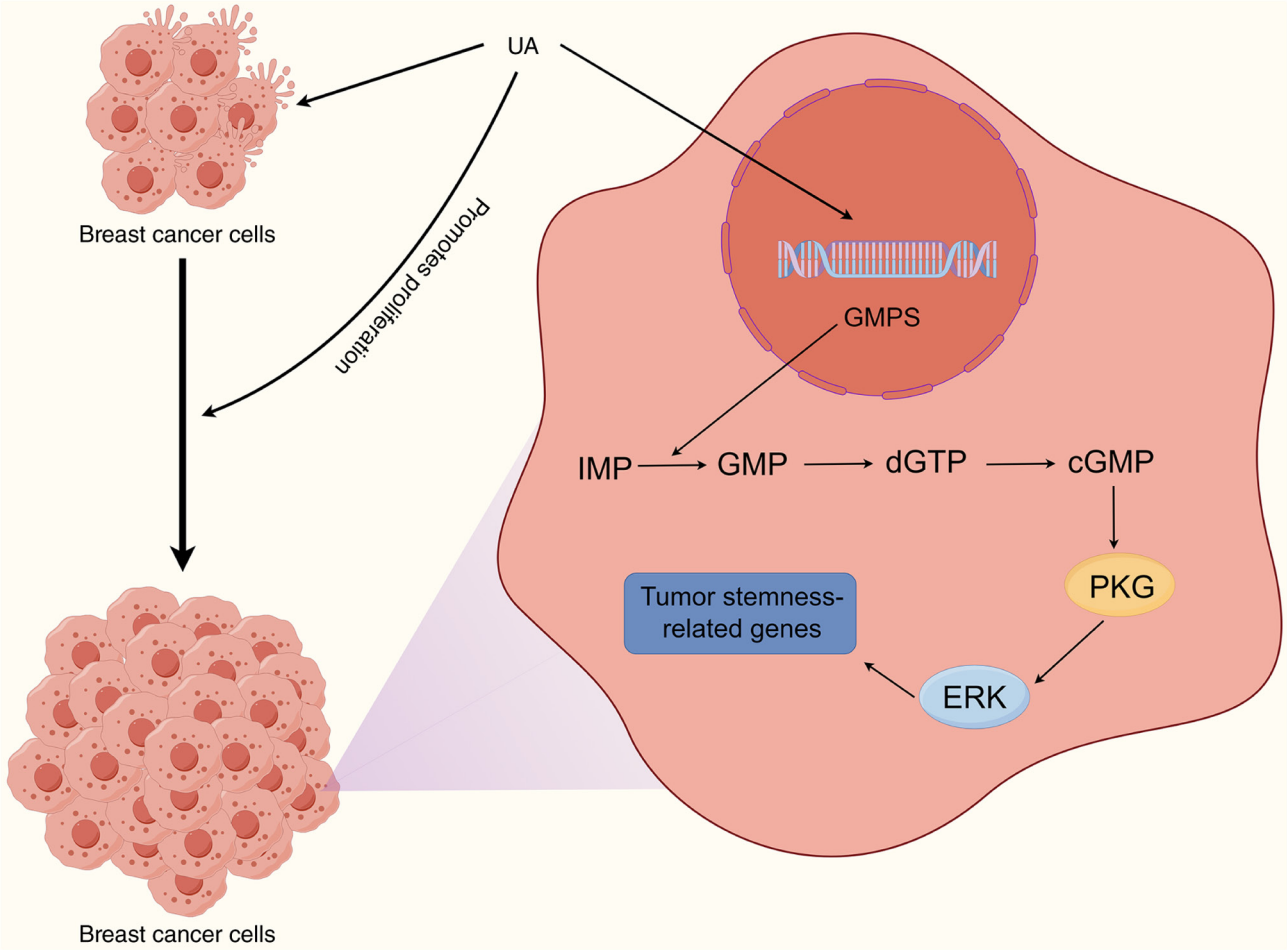

Figure 6. Schematic showing protection of UA-mediated tumor stemness in breast cancer cells by upregulating GMPS (Created with Figdraw). UA, uric acid; GMPS, guanine monophosphate synthase; IMP, inosine monophosphate; GMP, guanosine monophosphate; dGTP, deoxyguanosine triphosphate; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; ERK, extracellular signal-regulated kinase.

that the absence of OCT4 protein expression in MCF-7 cells is biologically relevant rather than a technical limitation. In MDA-MB-231 cells, UA markedly increased the mRNA expression of the five stemness-related genes (Fig. 4B). KLF4 and OCT4 were the stemness genes that greatly elevated by UA in protein expression levels (Fig. 4H, J and L). western blotting results demonstrate that KLF4 can be considerably elevated at a concentration of 4 mg/dl, and OCT4 was not statistically elevated until UA concentration reached 8 mg/dl. These results are also consistent with the qPCR results. Although the protein expression of other stemness genes in MDA-MB-231 did not show the same significant difference as mRNA expression, there was an overall trend of upregulation (Fig. 4H-M). In conclusion, the experimental results suggest that UA was able to differentially upregulate tumor stemness-related genes in both breast cancer cell lines.

Expression of nucleotide de novo synthesis genes in patients with breast cancer. Breast cancer patient tissues and paraneoplastic tissues were collected from the Affiliated Hospital of Guangdong Medical University, the cancer tissues showed much higher expression of GMPS than in paraneoplastic tissues (Fig. 5A). Three genes linked to nucleotide *de novo* synthesis, including PRPS2, ADSS and GMPS, were shown to have considerably higher expression levels in invasive breast cancer tissues as compared to normal tissues, according to TCGA database analysis (Fig. 5B-D). This also suggests that nucleotide synthesis from scratch is enhanced in breast cancer tissues to meet the demands of high metabolism and proliferation rates. Additionally, in most histopathologic forms of breast cancer, the genes linked to nucleotide *de novo* synthesis were upregulated (Fig. 5E-G). Genes linked to nucleotide *de novo* synthesis were also up-regulated at various stages of the clinical process (Fig. 5H-J). Although nucleotide *de novo* synthesis genes were highly expressed in tumors of patients with breast cancer, there was no consistent association with tumor types or clinical stages of patients with breast cancer. However, in the prognostic survival curves, upregulation of GMPS and ADSS expression was associated with poor prognosis in patients with breast cancer, whereas there was no statistically significant difference for PRPS2 (Fig. 5K-M). In conclusion, upregulation of GMPS expression is associated with the occurrence and prognosis of breast cancer.

Discussion

Individuals with gout and excessive UA are becoming more common (14). Additionally, there is a rising proportion of

cancer patients who also have elevated UA levels. Clinical evidence indicates that hyperuricemia may affect the prognosis of patients with breast cancer (9,10). It has also been suggested that UA may be a protective factor for patients with breast cancer (15). Additionally, there has not been any published research on the connection between UA and breast cancer. The present study demonstrated that UA could stimulate the migration and proliferation of breast cancer cells, and this implies that serum SUA, or elevated UA, is harmful to individuals with breast cancer (Fig. 6).

High tumor metabolism and proliferation are reflected in increased nucleotide *ab initio* synthesis, which is also associated with tumor stemness (5). PRPS2 catalyzes the formation of ribulose pentaphosphate pyrophosphate from ribulose pentaphosphate, and ADSS and GMPS are key enzymes in the generation of AMP and GMP from inosinic acid, respectively. Following their metabolic use, AMP and GMP are initially transformed into xanthine in the body, then xanthine oxidase catalyzes the production of UA. UA, as the byproduct of purine metabolism, has a regulatory effect on the major enzymes involved in the ab initio synthesis of these nucleotides; among these genes, UA markedly increases GMPS expression.

Using CCK-8 assays, the present study confirmed that UA stimulates proliferation in two different subtypes of breast cancer cells, determining and confirming the UA concentration that has the strongest proliferative effect. The present study also found that UA increases the migration of breast cancer cells at three uric acid treatment dosages. In order to stimulate purine synthesis in breast cancer cells, UA, a byproduct of purine breakdown, upregulates GMPS, an essential enzyme in *de novo* purine synthesis. The present study next found that UA could promote GMP-related metabolism by upregulating GMPS expression, which resulted in increased intracellular cGMP content. The cGMP-PKG pathway is further activated by elevated cGMP, which encourages tumor stemness in breast cancer cells (5). Further research verified that in two breast cancer cell lines, UA stimulated the overexpression of tumor stemness-related genes KLF4, c-MYC, NANOG, SOX2, and OCT4. Elevated GMPS expression is associated with the development and prognosis of breast cancer, as shown by both the institutional data of the present study and the TCGA database study.

In conclusion, UA encourages breast cancer cells to proliferate and migrate. It was discovered that UA increases the tumor stemness of breast cancer cells using a plate colony experiment. GMP-related metabolism is promoted by upregulating GMPS expression, which raises intracellular cGMP levels. The cGMP/PKG/MAPK pathway may be triggered by an increase in cGMP, which would encourage tumor progression. The present study further examined the effect of UA on tumor stemness-related genes in order to provide more evidence that UA encourages tumor stemness in breast cancer. It was demonstrated that UA could increase the expression of most genes linked to tumor stemness. Research has demonstrated that UA stimulates the growth of renal and cardiac fibroblasts. In animal experiments, hyperuricemia activates the TGF-β/SMAD signaling pathway, which causes aberrant myofibroblast activation in the myocardial infarction zone (28). According to some research, hyperuricemia can cause renal fibroblasts to proliferate, along with decreased podocyte function and increased inflammatory mediator levels (29). Furthermore, renal fibroblast activation and the advancement of renal fibrosis are markedly influenced by hyperuricemia-induced autophagy (30).

When combined with clinical data, UA can improve tumor stemness and stimulate the migration and proliferation of breast cancer cells by upregulating the expression of GMPS, which is linked to an unfavorable prognosis for patients with breast cancer. As a result, in the clinic, patients with elevated or fluctuating UA values should receive special attention. Normal tissues acquire most nucleotides by salvage synthesis, so targeting nucleotide *ab initio* synthesis-related gene GMPS not only reduces the effect of high UA on patients with breast cancer but may also have fewer side effects on these patients and may be a new therapeutic option.

Due to time and reagent constraints, the present study did not conduct GMPS knockdown/restoration experiments. In the future, the authors will refine this approach and carry out the GMPS knockdown experiments using short hairpin RNA, as well as rescue experiments by re-expressing GMPS to establish the link between UA and GMPS upregulation. No interaction experiments were conducted with non-tumor cell lines, such as fibroblasts. Therefore, whether the observed effects are specific to cancer cells or also occur in normal cells remains unknown. The clinical sample size is constrained by time and location, and the quality of the data may be biased and incomplete. Given the differences between mouse and human UA metabolism, establishing stable mouse models of hyperuricemia remains a challenge, necessitating further refinement of animal experiments.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XS and WL designed the study and provided guidance on interpreting the results. ZW and MH carried out the experiments, collected the data and analyzed the results. SH performed data collection and statistical analysis. YZ performed the clinical specimen analysis and data collection. ZW finished the original draft of the manuscript. XS and WL revised the manuscript. XS and WL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All patients included in this study signed a written informed consent form agreeing to participation in the study. The research was conducted in strict accordance with universally acknowledged ethical principles and guidelines and received approval from the Ethics Committee of the Affiliated Hospital of Guangdong Medical University on June 25, 2023 (approval no. YJY2023231).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. El Ansari R, McIntyre A, Craze ML, Ellis IO, Rakha EA and Green AR: Altered glutamine metabolism in breast cancer; subtype dependencies and alternative adaptations. Histopathology 72: 183-190, 2018.
3. Liu YM, Ge JY, Chen YF, Liu T, Chen L, Liu CC, Ma D, Chen YY, Cai YW, Xu YY, *et al*: Combined single-cell and spatial transcriptomics reveal the metabolic evolvement of breast cancer during early dissemination. Adv Sci (Weinh) 10: e2205395, 2023.
4. Li S, Zeng H, Fan J, Wang F, Xu C, Li Y, Tu J, Nephew KP and Long X: Glutamine metabolism in breast cancer and possible therapeutic targets. Biochem Pharmacol 210: 115464, 2023.
5. Lv Y, Wang X, Li X, Xu G, Bai Y, Wu J, Piao Y, Shi Y, Xiang R and Wang L: Nucleotide de novo synthesis increases breast cancer stemness and metastasis via cGMP-PKG-MAPK signaling pathway. PLoS Biol 18: e3000872, 2020.
6. Munro MJ, Wickremesekera SK, Peng L, Tan ST and Itinteang T: Cancer stem cells in colorectal cancer: A review. J Clin Pathol 71: 110-116, 2018.
7. van Schaijik B, Davis PF, Wickremesekera AC, Tan ST and Itinteang T: Subcellular localisation of the stem cell markers OCT4, SOX2, NANOG, KLF4 and c-MYC in cancer: A review. J Clin Pathol 71: 88-91, 2018.
8. Gao FY, Li XT, Xu K, Wang RT and Guan XX: c-MYC mediates the crosstalk between breast cancer cells and tumor microenvironment. Cell Commun Signal 21: 28, 2023.
9. Yue CF, Feng PN, Yao ZR, Yu XG, Lin WB, Qian YM, Guo YM, Li LS and Liu M: High serum uric acid concentration predicts poor survival in patients with breast cancer. Clin Chim Acta 473: 160-165, 2017.
10. Feng Y, Fu M, Guan X, Wang C, Yuan F, Bai Y, Meng H, Li G, Wei W, Li H, *et al*: Uric acid mediated the association between BMI and postmenopausal breast cancer incidence: A bidirectional mendelian randomization analysis and prospective cohort study. Front Endocrinol (Lausanne) 12: 742411, 2022.
11. Bowden RG, Richardson KA and Richardson LT: Uric acid and metabolic syndrome: Findings from national health and nutrition examination survey. Front Med (Lausanne) 9: 1039230, 2022.
12. Welin M, Lehtiö L, Johansson A, Flodin S, Nyman T, Trésaugues L, Hammarström M, Gräslund S and Nordlund P: Substrate specificity and oligomerization of human GMP synthetase. J Mol Biol 425: 4323-4333, 2013.
13. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
14. Dehlin M, Jacobsson L and Roddy E: Global epidemiology of gout: Prevalence, incidence, treatment patterns and risk factors. Nat Rev Rheumatol 16: 380-390, 2020.
15. Kühn T, Sookthai D, Graf ME, Schübel R, Freisling H, Johnson T, Katzke V and Kaaks R: Albumin, bilirubin, uric acid and cancer risk: Results from a prospective population-based study. Br J Cancer 117: 1572-1579, 2017.
16. Ames BN, Cathcart R, Schwiers E and Hochstein P: Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: A hypothesis. Proc Natl Acad Sci USA 78: 6858-6862, 1981.
17. Fini MA, Elias A, Johnson RJ and Wright RM: Contribution of uric acid to cancer risk, recurrence, and mortality. Clin Transl Med 1: 16, 2012.
18. Yan S, Zhang P, Xu W, Liu Y, Wang B, Jiang T, Hua C, Wang X, Xu D and Sun B: Serum uric acid increases risk of cancer incidence and mortality: A systematic review and meta-analysis. Mediators Inflamm 2015: 764250, 2015.
19. Bhargava P, Janda J and Schnellmann RG: Elucidation of cGMP-dependent induction of mitochondrial biogenesis through PKG and p38 MAPK in the kidney. Am J Physiol Renal Physiol 318: F322-F328, 2020.
20. Guo D, Tan YC, Wang D, Madhusoodanan KS, Zheng Y, Maack T, Zhang JJ and Huang XY: A Rac-cGMP signaling pathway. Cell 128: 341-355, 2007.
21. Guo D, Zhang JJ and Huang XY: A new Rac/PAK/GC/cGMP signaling pathway. Mol Cell Biochem 334: 99-103, 2010.
22. Wang X, Huang Z, Wu Q, Prager BC, Mack SC, Yang K, Kim JLY, Gimple RC, Shi Y, Lai S, *et al*: MYC-regulated mevalonate metabolism maintains brain tumor-initiating cells. Cancer Res 77: 4947-4960, 2017.
23. Reya T, Morrison SJ, Clarke MF and Weissman IL: Stem cells, cancer, and cancer stem cells. Nature 414: 105-111, 2001.
24. Hui K, Gao Y, Huang J, Xu S, Wang B, Zeng J, Fan J, Wang X, Yue Y, Wu S, *et al*: RASAL2, a RAS GTPase-activating protein, inhibits stemness and epithelial-mesenchymal transition via MAPK/SOX2 pathway in bladder cancer. Cell Death Dis 8: e2600, 2017.
25. Cui JX, Xu XH, He T, Liu JJ, Xie TY, Tian W and Liu JY: L-kynurenine induces NK cell loss in gastric cancer microenvironment via promoting ferroptosis. J Exp Clin Cancer Res 42: 52, 2023.
26. Cantz T, Key G, Bleidissel M, Gentile L, Han DW, Brenne A and Schöler HR: Absence of OCT4 expression in somatic tumor cell lines. Stem Cells 26: 692-697, 2008.
27. Schreiber C, Kuch V, Umansky V and Sleeman JP: Autochthonous mouse melanoma and mammary tumors do not express the pluripotency genes Oct4 and Nanog. PLoS One 8: e57465, 2013.
28. Zhuang Z, Liu A, Zhang J, Han S, Tang L, Yu T, Shi Y, Li H, Yang H, Bai P and Tang Y: Hyperuricemia suppresses lumican, exacerbating adverse remodeling after myocardial infarction by promoting fibroblast phenotype transition. J Transl Med 22: 983, 2024.
29. Romi MM, Arfian N, Tranggono U, Setyaningsih WAW and Sari DCR: Uric acid causes kidney injury through inducing fibroblast expansion, endothelin-1 expression, and inflammation. BMC Nephrol 18: 326, 2017.
30. Bao J, Shi Y, Tao M, Liu N, Zhuang S and Yuan W: Pharmacological inhibition of autophagy by 3-MA attenuates hyperuricemic nephropathy. Clin Sci (Lond) 132: 2299-2322, 2018.