Implication of BAG5 Downregulation in Metabolic Reprogramming of Cisplatin-resistant Ovarian Cancer Cells via mTORC2 Signaling Pathway

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Abstract

Background: Ovarian cancer is the most frequent cause of death among gynecologic malignancies due to the absence of an early effective diagnostic approach. Although the majority of patients typically respond well to the first line of chemotherapy based on platinum compounds and taxanes, recurrence and chemoresistance limits its clinical utility. Remarkably, cancer stem cells (CSC) tend to form minimal residual disease after chemotherapy and exhibit recurrent potential. The ability of cancer cells to reprogram their metabolism has recently been related with resistance to chemotherapies.

Methods: BAG5 expression was studied in 16 cisplatin-sensitive and 8 cisplatin-resistant ovarian cancer tissues by Western blot. BAG5-induced cell proliferation, migration and invasion were investigated by CCK-8 assay, colony formation assay, wound healing and Transwell assay. To investigate whether BAG5 is implicated in metabolism regulation, mitochondrial function was monitored by real-time measurement of changes in the oxygen consumption rate (OCR) and glycolysis was also determined by measuring the extracellular acidification rate (ECAR). Immunohistochemical staining measured correlations between BAG5 and Bcl6, Rictor in most ovarian serous adenocarcinoma tissues.

Results: The current study found BAG5 expression was decreased in cisplatin-resistant ovarian cancer cells and clinical tissues. Our data demonstrated that BAG5 knockdown was implicated in metabolic reprogramming and maintenance of cancer stem cell (CSC)-like features of cisplatin-resistant ovarian cancer cells via regulation of Rictor and subsequent mTORC2 signaling pathway. In addition, the current study demonstrated that Bcl6 upregulation was responsible for repression of BAG5 transactivation via recruitment on the BAG5 promoter in cisplatin-resistant ovarian cancer. The current study also demonstrated reverse correlations between BAG5 and Bcl6, BAG5 and Rictor in ovarian serous adenocarcinoma tissues.

Conclusions: Collectively, the current study identified the implication of Bcl6/BAG5/Rictor-mTORC2 signaling pathway in metabolic reprogramming and maintenance of CSC-like features including cisplatin-resistance in cisplatin-resistant ovarian cancer cells. Therefore, further studies on the mechanism underlying regulation of metabolic reprogramming and CSC-like characteristics of cisplatin-resistant ovarian cancer cells may contribute to the establishment of novel therapeutic strategy for cisplatin-resistance.

Background

Ovarian cancer is the leading cause of death among gynaecologic malignancies, and its incidence rate is second only to that of cervical carcinoma and endometrial carcinoma[1]. It is characterized by relatively insidious symptoms and a lack of reliable detection methods in the early stage. The majority of patients are in the middle-late stage of the disease by the time they are identified and diagnosed in the clinical setting. Current standard therapy for ovarian cancer includes cytoreductive surgery plus platinum-based combined chemotherapy. The proposed traditional therapy has a certain positive effect on reducing

tumor volume and relieving clinical symptoms in these patients. However, it should be noted that it is a great challenge to the treatment of primary or acquired resistance to cisplatin in ovarian cancer patients clinically[2].

Cancer stem cells (CSCs) are rare chemotherapy-resistant cells in tumor tissues, with self-renewal ability, unlimited proliferation and multiplex differentiation potential, which are closely related to the occurrence, development, invasion, metastasis and drug resistance of ovarian cancer[3]. But the molecular events underlying regulation and maintenance of CSCs remain largely unclarified. Therefore, it is crucial to understand the biological characteristics and to explore the self-renewal mechanism of ovarian cancer stem cells.

The Bcl-2 associated athanogene (BAG) family of proteins are co-chaperones known to act in both cell survival and cell death pathways[4]. Each of the six BAG family members (BAG1 to BAG6) contains at least one copy of an evolutionarily conserved domain, the BAG-domain, which allows them to interact with Hsp70 family molecular chaperones[5, 6]. As a protein that accompanies multiple binding partners, BAG protein is a multifunctional protein that participates in a variety of cellular processes, such as stress signals, cell cycle and tumorigenesis[7-9]. BAG5 is exceptional in BAG family since it consists of 5 BAG domains structured in two and a half helices [10]. BAG proteins are implicated in cell proliferation, survival and stress responses, therefore contribute to cancer development[11, 12]. It has been reported that BAG5 regulates Parkin dependent mitophagy and cell death[13, 14]. BAG5 is also a regulator of ER stress and its expression correlates with cardiomyocyte viability via regulating cardiomyocyte oxidative stress, apoptosis activation and inflammation[15-17]. BAG5 inhibits ER stress through suppressing pancreatic EIF2- α kinase (PERK)-eukaryotic initiation factor 2 (eIF2)-activating transcription factor 4 (ATF4) activity[18]. Besides this, BAG5 attenuates the activity of molecular chaperone Hsp70 [19]. BAG5 is identified as an antagonist of cell tumorigenicity in breast cancer cells [20]. We recently reported BAG5 promotes invasion of papillary thyroid cancer cells[21]. Although these results suggest a potential function of BAG5 in tumorigenesis, its function in ovarian cancer and cisplatin resistance of ovarian cancer had not yet been investigated.

In this study, we identified that BAG5 was downregulated in cisplatin resistant ovarian cancer. Our data showed that BAG5 downregulation was implicated in metabolic reprogramming and maintenance of stem cell like features including cisplatin resistance in cisplatin-resistant ovarian cancer via mTORC2 signaling pathway. In addition, our data showed that transcriptional repressor Bcl6 inhibited BAG5 transactivation via recruitment on *BAG5* promoter. Our study sheds new light on the mechanisms underlying metabolism and cisplatin resistance of ovarian cancer.

Materials And Methods

Cell culture

Human ovarian cancer cell lines, SKOV3 and A2780, as well as their cisplatin resistant cohorts, SKOV3/DDP and A2780/DDP, were maintained in RPMI1640 (Life Technologies) supplemented with 10%

fetal bovine serum (FBS, Sigma), 100 IU/ml of penicillin, and 100 µg/ml of streptomycin (Sigma). The cells were maintained in a humidified atmosphere at 37°C with 5% CO₂.

Clinical samples

24 patients with 43 to 69 years old (58 on the 9 average) from June 2014 to July 2017 who underwent surgical resection at Shengjing Hospital of China Medical University were recruited in this study. The collected tissues were snap frozen in liquid nitrogen and stored in -80 °C freezer for further analysis. The patients were divided into two groups, platinum-sensitive (sixteen patients) and platinum resistant (eight patients) groups according whether or not recurrence 6 months after first cisplatin-based chemotherapy. None of the patients had received chemotherapy or radiotherapy prior to the operation. The project was approved by Institutional Review Board of China Medical University that informed consent was not needed to obtain from the patients or their family.

Tissue microarray and immunohistochemical staining

Tissue microarray sections (Shanghai Outdo Biotech Co., LTD) were used for immunohistochemical staining using an antibody against BAG5, Rictor or Bcl6. A semi-quantitative H-score was used for each specimen as previously reported[22]. Briefly, immunohistochemical intensity was assessed by multiplying the distribution areas (0-100%) at each staining intensity level by the intensities (0: negative; 1: weak staining; 2: moderate staining; 3: strong staining).

Colony formation assay

For the plate colony formation assay, 200 cells/well were seeded into six-well plates and cultured for 2 weeks. The cells were fixed with 4% paraformaldehyde for 15 min, then stained with 0.1% crystal violet. The colony number was determined under an optical microscopy.

Transwell assay

Cells were suspended in serum-free medium, seeded at 1×10^4 cells per well in the upper chamber with 100 µL. 600 µL of RPMI1640 containing 10% FBS was added into the bottom chamber. After incubation for 24 hours, the cells remaining in the upper chamber were removed. The cells invaded through the Matrigel matrix membrane were fixed with 4% paraformaldehyde for 15 min, then stained with 0.1% crystal violet.

Cell viability assay

Cells were seeded at 1.0×10^4 per well into 96-well plates. After adhesion, cells were treated with the indicated concentrations of cisplatin. The CCK-8 cell proliferation kit was used to measure cell viability after 2 days. For each test, 10 µL of the CCK-8 reagent was added to the cells, which were incubated at 37°C for 3 h. Absorbance at 450 nm was then measured using a microplate reader.

Measurement of glucose consumption

Cells were plated in 24-well plates at the density of 3×10^4 cells per well in 0.5 ml RPMI1640. A blank well without cells was included as the control. After 24 h of incubation, glucose in the culture medium was analyzed using a Glucose Assay Kit (Biovision, Milpitas, CA). The levels of glucose consumption were determined by subtracting the concentration of glucose. The numbers of cells in each well were counted and used to normalize the data.

Determination of the extracellular lactate

Cells were seeded into 24-well plates. Cells were washed with PBS, and then incubated with a KRPH buffer supplemented with 0.2% BSA and 10 mM glucose for 2 h. Next, supernatants were analyzed for their lactate content via an enzyme-coupled fluorescent assay using a L-Lactate Assay Kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. In the assay, lactate dehydrogenase catalyzes the oxidation of lactate to pyruvate, along with the concomitant reduction of NAD^+ to NADH. NADH then reacts with the fluorescent substrate to generate a highly fluorescent product, which is analyzed with an excitation wavelength of 530 nm and an emission wavelength of 585 nm. Cell numbers were accounted to normalize extracellular lactate production.

Oxygen consumption rate (OCR) and Extracellular acidification rate (ECAR)

15,000 cells were seeded into each well of a 24-well assay plate (Seahorse Bioscience) and grown overnight. OCR and ECAR was performed using the Extracellular Flux Analyzer XF24 from Seahorse Bioscience according to the manufacturer's protocol.

Label and capture nascent RNA

Newly synthesized RNA was labeled and isolated using Click-iT Nascent RNA Capture kit (Invitrogen). In brief, cells were incubated with 0.2 mM of 5-ethynyl uridine for 4 h to label nascent RNA. Total RNA was isolated using TRIzol reagent, and the 5-ethynyluridine-labeled nascent RNA was biotinylated in Click-iT reaction buffer with 0.5 mM of biotin azide. The biotinylated nascent RNA was subsequently captured on streptavidin magnetic beads.

Western blot and immunoprecipitation

Total cellular proteins were extracted using a lysis buffer containing 20 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 1% Triton-X100 and a protease inhibitor cocktail (Sigma-Aldrich, Saint Louis, MO). Extracted proteins were quantified using the BCA protein assay kit. Next, 20 μg total proteins were separated using 10% SDS-PAGE, and transferred to a PVDF membrane (Millipore Corporation, Billerica, MA). For immunoprecipitation, cell lysates were pre-cleared with protein A/G magnetic beads, which were treated with various antibodies, then incubated overnight at 4°C. The immunoprecipitants were washed three times with a lysis buffer, and analyzed using Western blot analysis, which was performed with primary antibodies against Flag, and Myc antibodies. The following antibodies were used in this study:

BAG5(Sigma); mTOR Pathway Antibody Sampler Kit, Rictor, Bcl6, Flag (Cell Signaling Technology), and GAPDH (Millipore).

Nude mouse xenograft experiments

BALB/c-nu/nu mice (4–5 weeks old, female) were purchased from Liaoning Changsheng Biotechnology Co., Ltd. All animal procedures were approved by and compiled with the guidelines of the Institutional Animal Care Committee of China Medical University. Mice were subcutaneously inoculated with the specified number of viable SKOV3/DDP cells. The status of the mice and tumor formation were observed over time, and the mice were sacrificed after 28 days. The subcutaneous tumors were removed and photographed.

Statistics

The statistical significance of the difference was analyzed by ANOVA and post hoc Dunnett's test. Statistical significance was defined as $P < 0.05$. All experiments were repeated three times, and data were expressed as the mean $\pm$ SD (standard deviation) from a representative experiment.

Results

BAG5 is implicated in maintenance of stem cell like features of cisplatin-resistant ovarian cancer cells

To explore the potential role of BAG5 in ovarian cancer, its expression was studied in 16 cisplatin-sensitive and 8 cisplatin-resistant ovarian cancer tissues. Western blot showed that BAG5 expression was decreased in cisplatin-resistant ovarian cancer tissues (Figure 1A). BAG5 expression was also decreased in cisplatin-resistant SKOV3/DDP and A2780/DDP cells (Figure 1B). Ectopic BAG5 was then transduced in SKOV3/DDP and A2780/DDP cells (Figure 1C). BAG5 overexpression increased the responsiveness of SKOV3/DDP and A2780/DDP cells to cisplatin treatment (Figure 1D-E). In addition, BAG5 overexpression significantly inhibited colony formation (Figure 1F) and invasion (Figure 1G) of SKOV3/DDP and A2780/DDP cells. Furthermore, spheroid formation assays demonstrated that BAG5 overexpression decreased spheroid formation capacity (Figure 1H) of cisplatin-resistant ovarian cancer cells. Western blot demonstrated that BAG5 overexpression decreased expression of ovarian cancer stem cell marker CD133 and CD44 in SKOV3/DDP and A2780/DDP cells (Figure 1I). *In vivo* tumor formation demonstrated that BAG5 overexpression significantly suppressed growth of SKOV3/DDP (Figure 1J) and A2780/DDP (Figure 1K) xenografted tumors in nude mice.

BAG5 was also knocked down using CRISP/Cas9 system in SKOV3 and A2780 cells (Figure 1L). Knockdown of BAG5 increased the survival of both SKOV3 (Figure 1M) and A2780 (Figure 1N) cells exposed to cisplatin treatment. Colony formation and spheroid formation assays demonstrated that BAG5 knockdown increased colony formation (Figure 1O) and invasiveness (Figure 1P) of SKOV3 and A2780 cells. Spheroid formation assays demonstrated that BAG5 knockdown increased spheroid formatting capacity of SKOV3 and A2780 cells (Figure 1Q). In addition, western blot demonstrated that

BAG5 knockdown increased expression of ovarian cancer stem cell marker CD133 and CD44 in SKOV3 and A2780 cells (Figure 1R). Collectively, these data indicated that BAG5 is implicated in the maintenance of stem cell like features of ovarian cancer cells.

High BAG5 expression is associated with favorable prognosis in patients with ovarian cancer

Immunohistochemical analysis in serous adenocarcinoma and mucinous adenocarcinoma demonstrated that BAG5 expression was variable in ovarian cancer tissues (Figure 2A). Compared with mucinous adenocarcinoma, BAG5 was often highly expressed in serous adenocarcinoma (Figure 2B). Survival time analysis demonstrated that low BAG5 intensity showed significantly poor overall survival of patients with serous adenocarcinoma (Figure 2C).

BAG5 overexpression suppresses glucose metabolism of cisplatin-resistant ovarian cancer cells

To investigate whether BAG5 is implicated in metabolism regulation, mitochondrial function was monitored by real-time measurement of changes in the oxygen consumption rate (OCR) after sequential treatment with oligomycin, the cyanide p-trifluoromethoxyphenyl-hydrazine (FCCP), or antimycin A. SKOV3/DDP and A2780/DDP cells demonstrated a significant rise of OCR, when compared with their cisplatin-sensitive partners SKOV3 and A2780 cells (Figure 3A). Glycolysis was also determined by measuring the extracellular acidification rate (ECAR) of media surrounding cells. There was a significant rise in the level of ECAR in SKOV3/DDP and A2780/DDP cells, indicating an increase of glycolysis (Figure 3B). In contrast, a significant drop in the level of OCR (Figure 3C) and ECAR (Figure 3D) was observed when BAG5 was ectopically overexpressed in SKOV3/DDP and A2780/DDP cells. In addition, a significant increase of glucose consumption and lactate release was observed in SKOV3/DDP and A2780/DDP cells (Figure 3E). In contrast, glucose consumption and lactate release were significantly decreased by ectopic BAG5 expression (Figure 3F). Flowcytometry confirmed that significant increase of glucose uptake in SKOV3/DDP and A2780/DDP cells, which was reversed by ectopic BAG5 expression (Figure 3G). Taken together, the above data suggested that BAG5 downregulation might be involved in metabolic reprogramming of cisplatin-resistant ovarian cancer cells.

BAG5 downregulation promotes glucose metabolism of cisplatin-sensitive ovarian cancer cells

To further confirm the potential involvement of BAG5 in aerobic glycolysis and mitochondrial respiratory, BAG5 was knocked down in SKOV3 and A2780 cells. Contrary to BAG5 overexpression, BAG5 knockdown resulted in a significant rise of OCR (Figure 4A) and ECAR (Figure 4B) in SKOV3 and A2780 cells. The rise of glucose metabolism was further confirmed by the higher level of glucose consumption and lactate release (Figure 4C). Flowcytometry also confirmed that significant increase of glucose uptake by BAG5 knockdown in SKOV3 and A2780 cells (Figure 4D).

BAG5 inhibits glucose metabolism of cisplatin-resistant ovarian cancer cells via mTOR signaling pathway

mTOR complex (mTORC) regulates mammalian cell growth in response to nutrients and growth factors. Western blot showed that the Raptor and Rictor, phosphorylation of mTOR at Ser2448, phosphorylation of mTOR at Ser2481, as well as phosphorylation of AKT at Ser473 were increased in cisplatin-resistant ovarian cancer cells, indicative of activation of mTOR signaling pathway (Figure 5A). BAG5 decreased Rictor, phosphorylation of mTOR at Ser2481, and phosphorylation of AKT at Ser473 (Figure 5B), indicative of mTORC2 inhibition by BAG5 overexpression. Thereby, these data indicated that BAG5 might suppress glucose metabolism of cisplatin-resistant ovarian cancer cells via reduction of Rictor expression and mTORC2 signaling pathway. Rictor was then ectopically overexpressed in cisplatin-resistant ovarian cancer cells with ectopic BAG5 expression (Figure 5C). Ectopic Rictor expression significantly weakened the suppressive effects of BAG5 overexpression on phosphorylation of mTOR at Ser2481 and AKT at Ser473 (Figure 5C). In addition, Rictor overexpression also reversed the inhibitory effect of BAG5 on OCR (Figure 5D) and ECAR (Figure 5E) of SKOV3/DDP and A2780/DDP cells. Rictor also weakened the inhibitory effects of BAG5 on glucose consumption (Figure 5F) and lactate release (Figure 5G). Meanwhile, Rictor increased survival of cisplatin-resistant ovarian cancer cells with BAG5 expression upon cisplatin exposure (Figure 5H). In addition, Rictor also promoted spheroid formation of cisplatin-resistant ovarian cancer cells with BAG5 expression (Figure 5I). These data indicated that BAG5 might inhibit glucose metabolism of cisplatin-resistant ovarian cancer cells via mTORC2 signaling pathway.

Bcl6 suppresses BAG5 transactivation and affects cisplatin sensitivity of ovarian cancer cells

The critical role of BAG5 in maintenance of cancer stem cell like features and glucose metabolism inspired us to explore the molecular mechanisms underlying BAG5 knockdown in cisplatin-resistant ovarian cancer. Real-time RT-PCR demonstrated that BAG5 mRNA expression was significantly decreased in cisplatin-resistant ovarian cancer cells (Figure 6A). Nascent RNA analysis also showed a significant reduction of novel synthesis of BAG5 mRNA in cisplatin-resistant ovarian cancer cells (Figure 6B). In addition, luciferase activity assays demonstrated luciferase activity of construct containing *BAG5* promoter was significantly decreased in cisplatin-resistant ovarian cancer cells (Figure 6C). These data indicated that BAG5 might be suppressed in cisplatin-resistant ovarian cancer cells at transcriptional activation level. Luciferase activity of reporter construct containing -1031~+25 fragment of BAG5 promoter significantly decreased in both SKOV3/DDP and A2780/DDP cells (Figure 6D). Similar luciferase activity of reporter construct containing -563~+25 fragment of BAG5 promoter was observed in SKOV3/DDP cells and its partner SKOV3 cells (Figure 6D). Although a reduction of luciferase activity of reporter construct containing -563~+25 fragment of BAG5 promoter was observed in A2780/DDP cells, which was much minor than that of construct containing -1031~+25 fragment (Figure 6D). These data indicated that -1031~-564 fragment of BAG5 promoter might contain the regulatory elements responsible for different transactivation in cisplatin-sensitive and cisplatin-resistant ovarian cancer cells. To explore the potential mechanisms underlying of BAG5 downregulation in cisplatin-resistant ovarian cancer cells, reporter constructs containing a series of truncated *BAG5* promoter region were generated. The luciferase reporter activity decreased significantly after the deletion of the -1031~+25 promoter fragment in SKOV3/DDP and A2780/DDP cells (Figure 6D). Bcl6 attracted our attention while searching on *BAG5* gene because of the following reasons: firstly, a potential BCL6 responsive elements located on -739~724

of *BAG5* gene just falls into the regulatory region responsible for different transactivation in cisplatin-resistant and cisplatin-sensitive ovarian cancer cells; secondly, it has been reported that Bcl6 is upregulated in cisplatin-resistant ovarian cancer[23], and Bcl6 functions as a oncogene and its high expression is a negative prognostic factor in patients with ovarian cancer [24-26]; thirdly, Bcl6 functions as a transcriptional repressor for a variety of genes [27-30]. Western blot confirmed an increase of Bcl6 expression in SKOV3/DDP and A2780/DDP cells (Figure 6E). Bcl6 was then knocked down in SKOV3/DDP and A2780/DDP cells (Figure 6E). Bcl6 knockdown significantly increased BAG5, while decreased Rictor expression in SKOV3/DDP and A2780/DDP cells (Figure 6F). Bcl6 knockdown also decreased survival upon exposure to cisplatin (Figure 6G) and spheroid formation (Figure 6H) of cisplatin-resistant ovarian cancer cells. Luciferase activity assays demonstrated that mutation of the potential Bcl6 binding site significantly minimized the differential expression of reporter gene between cisplatin-resistant and cisplatin-sensitive ovarian cancer cells (Figure 6I). ChIP was also performed and significant increases in recruitment of Bcl6 on the *BAG5* promoter was observed in SKOV3/DDP and A2780/DDP cells, when compared with their cisplatin-sensitive partners (Figure 6G). These data indicated that Bcl6 inhibits BAG5 transactivation and affects cisplatin sensitivity of ovarian cancer cells.

BAG5 was negatively correlated with Bcl6 and Rictor in ovarian serous adenocarcinoma tissues

Immunohistochemical staining demonstrated inverse correlations between BAG5 and Bcl6, Rictor in most ovarian serous adenocarcinoma tissues (Figure 7A). A significantly negative correlation between BAG5 and Bcl6 (Figure 7B), BAG5 and Rictor (Figure 7C) in 93 cases of ovarian serous adenocarcinoma tissues.

Discussion

The BAG family proteins are a multifunctional group of proteins that have diverse functions in many biological processes. A common feature of the BAG family protein is the existence of the BAG domain, which is a functional domain to modulate chaperone activity[31-33]. Among all BAG family members, BAG5 is the only protein that contains more than one BAG domains [34]. BAG5 is known as to inhibit the E3 ubiquitin ligase activity of Parkin [35]. The expression of BAG5 was higher in adjacent lung tissues than in cancer tissues[36]. In addition, BAG5 overexpression suppressed ER stress-induced apoptosis by inhibiting PERK-eIF2-ATF4 and increasing the IRE1-XBP1 activity[37]. These data revealed the implication of BAG5 in cancer cells, while its possible role in ovarian cancer cells remains unclarified. In this report, we found that BAG5 expression was decreased in cisplatin-resistant ovarian cancer tissues and high BAG5 expression was associated with profitable prognosis in patients with ovarian cancer. In addition, the current found that ectopic expression of BAG5 increased and other cancer stem cells (CSC) like features of cisplatin-resistant ovarian cancer cells. These results indicated that BAG5 might play a role in cisplatin resistance in ovarian cancer.

Chemoresistance of ovarian cancer is at least in part due to the presence of ovarian cancer stem cells (CSC) that have self-renewal and differentiation capabilities[38]. The existence of ovarian cancer stem cells (OCSCs) is one of the fundamental drivers of occurrence and recurrence in ovarian cancer[39-41]. In

our study, we demonstrated that BAG5 expression was decreased in cisplatin-resistant ovarian cancer tissues and cells. Importantly, ectopic BAG5 overexpression increased the responsiveness of SKOV3/DDP and A2780/DDP cells to cisplatin treatment. In addition, the current study found that high expression of BAG5 inhibited CSCs-like features in ovarian cancer cells, including expression of CSCs' markers, chemoresistance, and tumorigenesis. Our data indicated that BAG5 downregulation might contribute to chemo-resistance and CSC-like characteristics of ovarian cancer cells.

Cancers commonly have altered metabolism, which involves excessive glucose consumption and acid production. Increased glycolysis in cancer cells switches cellular metabolic flux to produce more biological building blocks, thereby sustaining rapid proliferation. Recent studies have identified altered metabolism as one of the major mechanisms underlying adaptive or acquired chemoresistance[42-45]. Our study demonstrated that BAG5 knockdown was implicated in metabolic reprogramming of cisplatin-resistant ovarian cancer cells, indicating that BAG5 might be a promising therapeutic target by drug repositioning targeting metabolic reprogramming of ovarian cancer cells.

It has been reported that overactivation of the mechanistic target of rapamycin (mTOR) is related with ovarian diseases such as polycystic ovarian syndrome and ovarian cancer [46]. mTOR increases ovarian cancer cells viability and decreases their apoptosis and autophagy[47]. It is also linked with cisplatin resistance in ovarian cancer cells[48]. The current study demonstrated that BAG5 downregulation was involved in metabolic reprogramming and maintenance of CSCs-like features via regulation of mTOR signaling pathway via Rictor in cisplatin-resistant ovarian cancer cells. As a cochaperone protein, we expected that BAG5 might regulate stability of Rictor protein, however, the current study demonstrated that BAG5 had no effects on stability of Rictor (data not shown). The exact mechanism underlying regulation of Rictor by BAG5 requires further investigation.

In conclusion, our data suggested that downregulation of BAG5 was at least partly ascribed to metabolic reprogramming and maintenance of CSCs-like features of cisplatin-resistant ovarian cancer via the mTOR signaling pathway. In addition, the current study demonstrated that upregulation of Bcl2 suppressed BAG5 transactivation in cisplatin-resistant ovarian cancer. Targeting BAG5 may provide a novel therapeutic opportunity for developing a relapse-free treatment for ovarian cancer patients.

Conclusions

In this study, we identified that BAG5 was downregulated in cisplatin resistant ovarian cancer. Our data showed that BAG5 downregulation was implicated in metabolic reprogramming and maintenance of stem cell like features including cisplatin resistance in cisplatin-resistant ovarian cancer via mTORC2 signaling pathway. In addition, our data showed that transcriptional repressor Bcl6 inhibited BAG5 transactivation via recruitment on *BAG5* promoter. The current study identified the implication of Bcl6/BAG5/Rictor-mTORC2 signaling pathway in metabolic reprogramming and maintenance of CSC-like features including cisplatin-resistance in cisplatin-resistant ovarian cancer cells. Therefore, further studies on the mechanism underlying regulation of metabolic reprogramming and CSC-like characteristics of

cisplatin-resistant ovarian cancer cells may contribute to the establishment of novel therapeutic strategy for cisplatin-resistance.

Abbreviations

CSC cancer stem cells

OCR oxygen consumption rate

ECAR extracellular acidification rate

PERK pancreatic EIF2- α kinase

eIF2: eukaryotic initiation factor 2

ATF4: activating transcription factor 4

OCSCs: ovarian cancer stem cells

BSA: Bovine serum albumin

FBS: Fetal bovine serum

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Committee of China Medical University.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare no conflict of interest

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Authors' contributions

W JM, L BQ, Y J, L C, W HQ were involved in the experimental design, acquisition of data, analysis and interpretation of data, and drafting of the manuscript. Z Q, H L, J JY, HY LY were involved in the experimental design and acquisition of xenografts. Z Q, H L, J JY, HY LY were involved in the study conception and design, analysis and interpretation of data, revision of the manuscript, and study supervision. The authors read and approved the final manuscript.

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References

1. Siegel RL, Miller KD, Jemal A: **Cancer statistics, 2019**. *CA Cancer J Clin* 2019, **69**(1):7-34.
2. Kuczynski EA, Sargent DJ, Grothey A, Kerbel RS: **Drug rechallenge and treatment beyond progression-implications for drug resistance**. *Nat Rev Clin Oncol* 2013, **10**(10):571-587.
3. Abubaker K, Latifi A, Luwor R, Nazaretian S, Zhu H, Quinn MA, Thompson EW, Findlay JK, Ahmed N: **Short-term single treatment of chemotherapy results in the enrichment of ovarian cancer stem cell-like cells leading to an increased tumor burden**. *Mol Cancer* 2013, **12**:24.
4. Rahmani P, Rogalski T, Moerman DG: **The C. elegans UNC-23 protein, a member of the BCL-2-associated athanogene (BAG) family of chaperone regulators, interacts with HSP-1 to regulate cell attachment and maintain hypodermal integrity**. *Worm* 2015, **4**(2):e1023496.
5. Doong H, Vrailas A, Kohn EC: **What's in the 'BAG'?—A functional domain analysis of the BAG-family proteins**. *Cancer Lett* 2002, **188**(1-2):25-32.
6. Takayama S, Reed JC: **Molecular chaperone targeting and regulation by BAG family proteins**. *Nat Cell Biol* 2001, **3**(10):E237-241.
7. Bonelli P, Petrella A, Rosati A, Romano MF, Lerosé R, Pagliuca MG, Amelio T, Festa M, Martire G, Venuta S *et al*: **BAG3 protein regulates stress-induced apoptosis in normal and neoplastic leukocytes**. *Leukemia* 2004, **18**(2):358-360.
8. Kabbage M, Dickman MB: **The BAG proteins: a ubiquitous family of chaperone regulators**. *Cell Mol Life Sci* 2008, **65**(9):1390-1402.
9. Behl C: **Breaking BAG: The Co-Chaperone BAG3 in Health and Disease**. *Trends in pharmacological sciences* 2016, **37**(8):672-688.
10. Briknarova K, Takayama S, Homma S, Baker K, Cabezas E, Hoyt DW, Li Z, Satterthwait AC, Ely KR: **BAG4/SODD protein contains a short BAG domain**. *J Biol Chem* 2002, **277**(34):31172-31178.
11. Townsend PA, Cutress RI, Sharp A, Brimmell M, Packham G: **BAG-1: a multifunctional regulator of cell growth and survival**. *Biochim Biophys Acta* 2003, **1603**(2):83-98.

12. Kermer P, Digicaylioglu MH, Kaul M, Zapata JM, Krajewska M, Stenner-Liewen F, Takayama S, Krajewski S, Lipton SA, Reed JC: **BAG1 over-expression in brain protects against stroke.** *Brain Pathol* 2003, **13**(4):495-506.
13. De Snoo ML, Friesen EL, Zhang YT, Earnshaw R, Dorval G, Kapadia M, O'Hara DM, Agapova V, Chau H, Pellerito O *et al*: **Bcl-2-associated athanogene 5 (BAG5) regulates Parkin-dependent mitophagy and cell death.** *Cell Death Dis* 2019, **10**(12):907.
14. Chung KK, Dawson TM: **Parkin and Hsp70 sacked by BAG5.** *Neuron* 2004, **44**(6):899-901.
15. Audia JP, Yang XM, Crockett ES, Housley N, Haq EU, O'Donnell K, Cohen MV, Downey JM, Alvarez DF: **Caspase-1 inhibition by VX-765 administered at reperfusion in P2Y12 receptor antagonist-treated rats provides long-term reduction in myocardial infarct size and preservation of ventricular function.** *Basic Res Cardiol* 2018, **113**(5):32.
16. Zhu H, Zhao M, Chen Y, Li D: **Bcl-2-associated athanogene 5 overexpression attenuates catecholamine-induced vascular endothelial cell apoptosis.** *J Cell Physiol* 2020.
17. Gaglianone RB, Bloise FF, Ortiga-Carvalho TM, Quirico-Santos T, Costa ML, Mermelstein C: **Comparative study of calcium and calcium-related enzymes with differentiation markers in different ages and muscle types in mdx mice.** *Histol Histopathol* 2020, **35**(2):203-216.
18. Nowak-Sliwinska P, Alitalo K, Allen E, Anisimov A, Aplin AC, Auerbach R, Augustin HG, Bates DO, van Beijnum JR, Bender RHF *et al*: **Consensus guidelines for the use and interpretation of angiogenesis assays.** *Angiogenesis* 2018, **21**(3):425-532.
19. Bacmeister L, Schwarzl M, Warnke S, Stoffers B, Blankenberg S, Westermann D, Lindner D: **Inflammation and fibrosis in murine models of heart failure.** *Basic Res Cardiol* 2019, **114**(3):19.
20. Ying Z, Haiyan G, Haidong G: **BAG5 regulates PTEN stability in MCF-7 cell line.** *BMB Rep* 2013, **46**(10):490-494.
21. Zhang DL, Wang JM, Wu T, Du X, Yan J, Du ZX, Wang HQ: **BAG5 promotes invasion of papillary thyroid cancer cells via upregulation of fibronectin 1 at the translational level.** *Biochim Biophys Acta Mol Cell Res* 2020, **1867**(9):118715.
22. Detre S, Saclani Jotti G, Dowsett M: **A "quickscore" method for immunohistochemical semiquantitation: validation for oestrogen receptor in breast carcinomas.** *Journal of clinical pathology* 1995, **48**(9):876-878.
23. Shen J, Hong L, Chen L: **Ubiquitin-specific protease 14 regulates ovarian cancer cisplatin-resistance by stabilizing BCL6 oncoprotein.** *Biochemical and biophysical research communications* 2020, **524**(3):683-688.
24. Zhu L, Feng H, Jin S, Tan M, Gao S, Zhuang H, Hu Z, Wang H, Song Z, Lin B: **High expressions of BCL6 and Lewis y antigen are correlated with high tumor burden and poor prognosis in epithelial ovarian cancer.** *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2017, **39**(7):1010428317711655.
25. Shan W, Li J, Bai Y, Lu X: **miR-339-5p inhibits migration and invasion in ovarian cancer cell lines by targeting NACC1 and BCL6.** *Tumour biology : the journal of the International Society for*

Oncodevelopmental Biology and Medicine 2016, **37**(4):5203-5211.

26. Wang YQ, Xu MD, Weng WW, Wei P, Yang YS, Du X: **BCL6 is a negative prognostic factor and exhibits pro-oncogenic activity in ovarian cancer.** *American journal of cancer research* 2015, **5**(1):255-266.
27. Wang D, Long J, Dai F, Liang M, Feng XH, Lin X: **BCL6 represses Smad signaling in transforming growth factor-beta resistance.** *Cancer research* 2008, **68**(3):783-789.
28. Suzuki H, Kurita M, Mizumoto K, Moriyama M, Aiso S, Nishimoto I, Matsuoka M: **The ARF tumor suppressor inhibits BCL6-mediated transcriptional repression.** *Biochemical and biophysical research communications* 2005, **326**(1):242-248.
29. Phan RT, Saito M, Basso K, Niu H, Dalla-Favera R: **BCL6 interacts with the transcription factor Miz-1 to suppress the cyclin-dependent kinase inhibitor p21 and cell cycle arrest in germinal center B cells.** *Nature immunology* 2005, **6**(10):1054-1060.
30. Phan RT, Dalla-Favera R: **The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells.** *Nature* 2004, **432**(7017):635-639.
31. Hohfeld J, Jentsch S: **GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1.** *EMBO J* 1997, **16**(20):6209-6216.
32. Gassler CS, Wiederkehr T, Brehmer D, Bukau B, Mayer MP: **Bag-1M accelerates nucleotide release for human Hsc70 and Hsp70 and can act concentration-dependent as positive and negative cofactor.** *J Biol Chem* 2001, **276**(35):32538-32544.
33. Nollen EA, Kabakov AE, Brunsting JF, Kanon B, Hohfeld J, Kampinga HH: **Modulation of in vivo HSP70 chaperone activity by Hip and Bag-1.** *J Biol Chem* 2001, **276**(7):4677-4682.
34. Arakawa A, Handa N, Ohsawa N, Shida M, Kigawa T, Hayashi F, Shirouzu M, Yokoyama S: **The C-terminal BAG domain of BAG5 induces conformational changes of the Hsp70 nucleotide-binding domain for ADP-ATP exchange.** *Structure* 2010, **18**(3):309-319.
35. Glasmacher E, Hoefig KP, Vogel KU, Rath N, Du L, Wolf C, Kremmer E, Wang X, Heissmeyer V: **Roquin binds inducible costimulator mRNA and effectors of mRNA decay to induce microRNA-independent post-transcriptional repression.** *Nature immunology* 2010, **11**(8):725-733.
36. Li Y, Liu H, Wang J, Li Y, Wu H, Du X, Wang W, Yue J, Zhou Q, Chen J: **[BAG family gene and its relationship with lung adenocarcinoma susceptibility].** *Zhongguo Fei Ai Za Zhi* 2010, **13**(10):942-946.
37. Bruchmann A, Roller C, Walther TV, Schafer G, Lehmusvaara S, Visakorpi T, Klocker H, Cato AC, Maddalo D: **Bcl-2 associated athanogene 5 (Bag5) is overexpressed in prostate cancer and inhibits ER-stress induced apoptosis.** *BMC Cancer* 2013, **13**:96.
38. Ip CK, Li SS, Tang MY, Sy SK, Ren Y, Shum HC, Wong AS: **Stemness and chemoresistance in epithelial ovarian carcinoma cells under shear stress.** *Sci Rep* 2016, **6**:26788.
39. Zavesky L, Jandakova E, Kohoutova M: **[Cancer stem cells and ovarian cancer Characteristics, importance and potential applications in clinical practice].** *Ceska Gynekol* 2013, **78**(2):169-174.
40. Wang X, Li X, Fu X, Bai M, Li X, Mei Q, Nie J, Wu Z, Han W: **Eliminating ovarian cancer stem cells: a potential therapeutic target for ovarian cancer chemoresistance.** *Curr Protein Pept Sci* 2015,

16(4):270-278.

41. Klemba A, Purzycka-Olewiecka JK, Wcislo G, Czarnecka AM, Lewicki S, Lesyng B, Szczalik C, Kieda C: **Surface markers of cancer stem-like cells of ovarian cancer and their clinical relevance.** *Contemp Oncol (Pozn)* 2018, **22**(1A):48-55.
42. Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, De Saedeleer CJ, Kennedy KM, Diepart C, Jordan BF *et al*: **Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice.** *The Journal of clinical investigation* 2008, **118**(12):3930-3942.
43. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, Casimiro MC, Wang C, Fortina P, Addya S *et al*: **The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma.** *Cell cycle* 2009, **8**(23):3984-4001.
44. Semenza GL: **Tumor metabolism: cancer cells give and take lactate.** *The Journal of clinical investigation* 2008, **118**(12):3835-3837.
45. Yoshida GJ: **Metabolic reprogramming: the emerging concept and associated therapeutic strategies.** *Journal of experimental & clinical cancer research : CR* 2015, **34**:111.
46. Liu AL, Liao HQ, Li Zh L, Liu J, Zhou CL, Guo ZF, Xie HY, Peng CY: **New Insights into mTOR Signal Pathways in Ovarian-Related Diseases: Polycystic Ovary Syndrome and Ovarian Cancer.** *Asian Pac J Cancer Prev* 2016, **17**(12):5087-5094.
47. Feng S, Jin Y, Cui M, Zheng J: **Lysine-Specific Demethylase 1 (LSD1) Inhibitor S2101 Induces Autophagy via the AKT/mTOR Pathway in SKOV3 Ovarian Cancer Cells.** *Med Sci Monit* 2016, **22**:4742-4748.
48. Xu S, Fu GB, Tao Z, OuYang J, Kong F, Jiang BH, Wan X, Chen K: **MiR-497 decreases cisplatin resistance in ovarian cancer cells by targeting mTOR/P70S6K1.** *Oncotarget* 2015, **6**(28):26457-26471.

Figures

Figure 1

Figure 1

BAG5 is down-regulated and inhibits the maintenance of stem cell like features in cisplatin-resistant ovarian cancer cells. A, 16 cisplatin-sensitive and 8 cisplatin-resistant ovarian cancer tissues were subject for Western blot to analyze the BAG5 protein expression. B, BAG5 protein expression was studied in control and cisplatin-resistant (DDP) SKOV3 and A2780 cells. C, immunoblot analysis of ectopic BAG5 in SKOV3/DDP and A2780/DDP cells infected with lentivirus containing empty or BAG5 construct. D-E,

control or BAG5 overexpression SKOV3/DDP (D) or A2780/DDP (E) cells were treated with the indicated concentration of cisplatin for 48 hours, and cell viability was valued using CCK8 analysis. *, $P < 0.01$, n.s., not significant. F, independent cell growth was analyzed using colony formation assays in the indicated cells in control or BAG5 overexpression SKOV3/DDP or A2780/DDP cells. G, cell invasion was examined by Corning Matrigel invasion assay. H, the number of spheroids was counted in control or BAG5 overexpression SKOV3/DDP or A2780/DDP cells. I, stem cell marker CD44 and CD133 was analyzed using Western blot analysis. J-K, the indicated control or BAG5 overexpression SKOV3/DDP(J) and A2780/(K) cells with serial dilution were injected intracutaneously into nude mice. Tumors were excised after mice sacrifice on day 28. L, BAG5 expression was knocked down using CRISPR-Cas9 system, and the efficiency of knockdown was verified by Western blot analysis. M-N, control or BAG5 knockdown (KD) SKOV3 (M) or A2780 (N) cells were treated with the indicated concentration of cisplatin for 72 hours, and cell viability was valued using CCK8 analysis. O, independent cell growth was analyzed using colony formation assays in the indicated cells in control or BAG5 knockdown SKOV3 and A2780 cells. P, cell invasion was examined by Corning Matrigel invasion assay in control or BAG5 knockdown (KD) SKOV3 or A2780 cells. Q, the number of spheroids was counted in control or BAG5 knockdown SKOV3 or A2780 cells. R, stem cell marker CD44 and CD133 was analyzed using Western blot analysis in BAG5 knockdown cells.

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Figure 2

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High BAG5 is predictive poor prognosis of patients with ovarian cancer. A, representative immunohistochemistry staining with BAG5 in serous adenocarcinoma and mucinous adenocarcinoma. B, violin plot of BAG5 score between serous adenocarcinoma and mucinous adenocarcinoma. C, Kaplan-Meier plot indicates the overall survival of patients with ovarian cancer categorized by BAG5 expression, P value is determined by log-rank test. *, $P < 0.01$, n.s., not significant.

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Figure 3

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BAG5 overexpression suppresses glucose metabolism of cisplatin-resistant ovarian cancer cells. A-B, OCR(A) and ECAR(B) were measured using seahorse instrument in cisplatin-resistant ovarian cancer cells SKOV3/DDP and A2780/DDP cells or cisplatin-sensitive ovarian cancer cells SKOV3 and A2780 cells. C- D, OCR (C) and ECAR (D) were measured using seahorse instrument in control or BAG5 overexpression SKOV3/DDP or A2780/DDP cells. E-F, Glucose consumption and extracellular lactate production were

determined using colorimetric method in cisplatin-resistant or cisplatin-sensitive ovarian cancer cells(E) and control or BAG5 overexpression cells(F).All values displayed are mean $\pm$ SD. *P< 0.01. G, Glucose uptake was measured using 2-NBDG staining followed by flowcytometry.

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Figure 4

Figure 4

Knockdown of BAG5 promotes glucose metabolism of cisplatin-resistant ovarian cancer cells. A, OCR was measured using seahorse instrument in control or BAG5 knockdown SKOV3 and A2780 cells. B, ECAR was measured using seahorse instrument in control or BAG5 knockdown SKOV3 and A2780 cells. C, Glucose consumption and extracellular lactate production were determined using colorimetric method in control or BAG5 knockdown SKOV3 and A2780 cells. D, Glucose uptake was measured using 2-NBDG staining followed by flowcytometry in control or BAG5 knockdown SKOV3 and A2780 cells.

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BAG5 inhibits glucose metabolism of cisplatin-resistant ovarian cancer cells via mTOR signaling pathway A, the expression of genes associated with mTOR pathway were detected in cisplatin-resistant or cisplatin-sensitive ovarian cancer cells. B, the expression of genes associated with mTOR pathway were detected in control or BAG5 overexpression cells. C, the expression of genes associated with mTOR pathway were detected in BAG5 overexpression cells after infected with lentivirus containing empty or Rictor construct. D, OCR was measured using seahorse instrument in control or Rictor-overexpression cells with control or BAG5-overexpression. E, ECAR was measured using seahorse instrument in control or Rictor-overexpression cells with control or BAG5-overexpression. F, Glucose consumption was determined using colorimetric method in control or Rictor-overexpression cells with control or BAG5-overexpression. G, Extracellular lactate production was determined using colorimetric method in control or Rictor-overexpression cells with control or BAG5-overexpression. H, control or Rictor-overexpression cells with control or BAG5-overexpression cells were treated with the indicated concentration of cisplatin for 72 hours, and cell viability was valued using CCK8 analysis. I, the number of spheroids was counted in control or Rictor-overexpression cells with control or BAG5-overexpression cells.

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Figure 6

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Bcl6 inhibits BAG5 transcription and cisplatin sensitivity of cisplatin-resistant ovarian cancer cells A, BAG5 mRNA was analyzed using RT-PCR in cisplatin-resistant or cisplatin-sensitive ovarian cancer cells. B, new synthetic RNA was labeled and isolated, and newly synthesized BAG5 mRNA was analyzed using RT-PCR in cisplatin-resistant or cisplatin-sensitive ovarian cancer cells. C, cisplatin-resistant or cisplatin-sensitive ovarian cancer cells were transfected with the BAG5 recombined firefly luciferase reporter vector

and a Renilla luciferase reporter vector. Luciferase activity was measured 48 hours after transfection, and firefly luciferase activity was normalized to Renilla luciferase activity. *, $P < 0.01$. Error bars indicate means $\pm$ SD. D, cisplatin-resistant or cisplatin-sensitive ovarian cancer cells were transfected with the indicated recombined firefly luciferase reporter vector and a Renilla luciferase reporter vector. Luciferase activity was measured 48 hours after transfection, and firefly luciferase activity was normalized to Renilla luciferase activity. E, Western blot analysis of Bcl6 in cisplatin-resistant or cisplatin-sensitive ovarian cancer cells. H, Western blot analysis of BAG5 and Rictor in control or BCL6-knockdown cells. F, Western blot analysis of BAG5 and Rictor in control or BCL6-knockdown cells. G, control and Bcl6 knockdown cells were treated with the indicated concentration of cisplatin for 72 hours, and cell viability was valued using CCK8 analysis. H, the number of spheroids was counted in control or Bcl6 knockdown cells. I, Luciferase activity was measured cisplatin-resistant or cisplatin-sensitive ovarian cancer cells transfected with the indicated recombined firefly luciferase reporter vector. J, ChIP analysis of BAG5 recruitment to the Bcl6 in cisplatin-resistant ovarian cancer cells.

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Figure 7

Figure 7

BAG5 was negatively correlated with Bcl6 and Rictor in ovarian serous adenocarcinoma tissues A, representative images of immunostaining of Bcl6, BAG5 and Rictor in ovarian cancer tissues. B-C, Scatter plots showing the negative correlation between BAG5 and Bcl6 (B), BAG5 and Rictor (C), Pearson's coefficient tests were performed to assess statistical significance.

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