Original Article

Up-regulation of PUM1 by miR-218-5p promotes colorectal tumor-initiating cell properties and tumorigenesis by regulating the PI3K/AKT axis

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Background: Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer-related death worldwide. Advanced stage CRC, during the recent past, had a dismal prognosis and only a few available treatments. Pumilio homologous protein 1 (PUM1) is reportedly aberrant in human malignancies, including CRC. However, the role of PUM1 in the regulation of tumor-initiating cells (T-ICs) remains unknown.

Methods: The levels of messenger RNAs (mRNAs) were determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and immunoblot analyses. Statistical analyses were performed to determine the associations between the levels of PUM1 and tumor features and patient outcomes. Whether PUM1 is a downstream target of miR-218-5p was verified by bioinformatics target gene prediction and qRT-PCR.

Results: Herein, it was found that T-ICs, chemoresistance, and recurrent CRC samples all manifest increased PUM1 expression. Functional investigations have shown that PUM1 increased the self-renewal, tumorigenicity, malignant proliferation, and chemoresistance of colorectal cells. PUM1 activates the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) signaling pathway biochemically. Furthermore, it was discovered that miR-218-5p specifically targets T-ICs' PUM1 3'-untranslated region (3'-UTR). More importantly, the PUM1/PI3K/AKT axis regulates CRC cells' responses to treatment with cetuximab, and PUM1 overexpression increased cetuximab resistance. More evidence points to the possibility that low PUM1 may predict cetuximab benefits in CRC patients after analysis of the patient cohort, patient-derived tumor organoids, and patient-derived xenografts (PDXs).

Conclusions: Taken together, the result of this work points to the critical function of the miR-218-5p/PUM1/PI3K/AKT regulatory circuit in regulating T-ICs characteristics and thus suggests possible therapeutic targets for CRC.

Keywords: Pumilio homologous protein 1 (PUM1); colorectal cancer (CRC); tumor-initiating cells (T-ICs); cetuximab; biomarker

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Introduction

The prevalence of colorectal cancer (CRC) has increased globally (1). Advanced stage CRC, during the recent past, had a dismal prognosis and only a few available treatments. Patients with CRC who are ineligible for surgery are currently treated first with targeted medications (2-4). A minor percentage of CRC patients react to targeted treatments because of the genetic heterogeneity of the disease, whereas most of the patients experience no therapeutic outcomes, rather they experience highly dangerous side effects. Understanding the underlying causes of treatment resistance is therefore critical to understand. Similarly, the identification of reliable biomarkers capable of forecasting a patient's response to medication for CRC is of key importance.

First discovered in hematological malignancies, cancer stem cells (CSCs) or tumor-initiating cells (T-ICs) have now been found in solid tumors, for instance, rectal, breast, and brain cancers (5,6). The multidirectional differentiation, self-renewal, and unlimited proliferation abilities of T-ICs, which make up a small part of the tumor tissue cells, are crucial for the development of tumors as well as for metastasis, recurrence, and chemo-resistance (7,8). T-ICs have been developed, which not only offer new theories for the clinical diagnosis and management of malignancies but also fresh perspectives on the pathophysiology of tumor growth and recurrence. Patients with CRC may experience a poor clinical outcome if their tumors contain a large T-IC population (9). Hence, thorough research on T-ICs' regulatory mechanism and the identification of suitable intervention targets are anticipated to yield novel CRC therapeutic approaches.

PUM1, a sequence-specific RNA binding protein, participates in quite a few physiological events, for instance, the cell cycle, cell renewal, and DNA repair (10-16). Non-small-cell lung carcinoma (NSCLC), lymphocyte leukemia, ovarian cancer, and other malignancies have all been shown to involve PUM1 as an oncogene (17-21). In our earlier research, we also discovered that colon cancer cells with acquired resistance to cetuximab overexpress PUM1 (21). The findings from this work show that PUM1 exists as a novel biomarker for liver T-ICs and is thus a possible target for CRC therapy. In T-ICs, PUM1 is elevated and has a crucial role in colorectal cells' capacity for tumorogenicity, malignant proliferation, self-renewal, and chemoresistance. We present the following article in accordance with the ARRIVE reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-6/rc).

Highlight box

Key findings
- PUM1 was upregulated in T-ICs and played an essential role in the self-renewal of colorectal cells.

What is known and what is new?
- PUM1 is overexpressed in colon cancer cells with acquired resistance to cetuximab.
- Through miR-218-5p, PUM1 promotes colorectal T-IC properties and tumorigenesis by regulating the PI3K/AKT axis.

What are the implications, and what should change now?
- PUM1 is a novel biomarker of liver T-ICs and a potential target for CRC therapy.

Methods

Patients and analysis

The specimens utilized in the current work were acquired from the Shanghai Fourth People's Hospital (Shanghai, China). The Ethical Committee of the Shanghai Fourth People's Hospital gave its approval to each of the aforementioned studies (No. 2022018-001), and they were all performed in conformity with the Declaration of Helsinki (as revised in 2013), and informed consent was obtained from all participants.

Cell lines and lentivirus

Chinese Academy of Sciences Cell Bank of Type Culture Collection (Shanghai Institute of Cell Biology of the Chinese Academy of Sciences) provided the Normal and CRC cell lines. The CRC cells were allowed to incubate at 37 ℃ with 5% CO_2 and subsequently cultured separately in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% or 15% fetal bovine serum (FBS), 25 g/mL of gentamicin, 2 mM L-glutamine. GenePharma

(Shanghai, China) was used to purchase all lentiviruses.

Mice and CRC induction

Shanghai Nanfang Model Biotechnology Co., Ltd. produced PUM1 TG mice (Shanghai, China). All mice were kept in ambient temperatures of 25 ℃ in a 12/12 h light-dark cycle and unrestrained supply of water and food. A single dose of diethylnitrosamine (DEN) as an intraperitoneal injection was administered to the male mice at the age of 14 days (n=6 per group, randomly assigned) in order to induce CRC. Mice were administered intraperitoneal injections of CCl_4 (0.5 mL/kg in olive oil, Shanghai Macklin Biochemical Co., Ltd., Shanghai, China) at 4 weeks old. They continued this treatment for up to 12 weeks before being euthanized at 5 months. For upcoming investigations, mice livers and serum were obtained. A protocol was prepared before the study without registration.

In vivo xenograft and patient-derived xenograft (PDX) model

Male BALB/c nude mice (male, 4 weeks old) were taken from Shanghai Nanfang Model Biotechnology Co., Ltd. (Shanghai, China). As previously stated, an *in vivo* tumor growth experiment was carried out (22). Animal experiments were performed under a project license (No. TJBH0122101) granted by the Ethics Committee of Shanghai Tongji university, in compliance with Shanghai Fourth People's Hospital guidelines for the care and use of animals.

Tissue dissociation and organoid culture

An organoid culture was performed as previously described (23). Different concentrations of cetuximab were added to the organs when they grew to a certain amount and size.

Spheroids assay

Three hundred single cells were seeded into 96-well ultra-low attachment microplates (Corning, USA) in serum-free DMEM/F12 (Invitrogen, USA), supplemented with B27 (1:50, Invitrogen), 20 ng/mL epidermal growth factor (EGF; Peprotech, CA, USA), 10 ng/mL basic fibroblast growth factor (bFGF; Invitrogen), and 4 mg/mL insulin (Sigma, CA, USA). The spheres were photographed and counted 7 days after seeding (primary spheres).

In vitro limiting dilution assay

Seeding of the indicated cells at various cell densities was made into 96-well ultra-low attachment culture plates and incubated for a period of 7 days. Using the LCalc version 1.1 software (Stem Cell Technologies, Inc., Vancouver, Canada) and Poisson distribution statistics, T-ICs were quantified on the basis of the frequency of wells with developing spheres

Flow cytometric analysis

To analyze the proportion of epithelial cell adhesion molecule (EpCAM) or CD24-positive liver CSCs, the colorectal cells were harvested and resuspended in the staining buffer, and then incubated with allophycocyanin (APC)-conjugated EpCAM antibody (BioLegend, Shanghai, China) or CD24 antibody (BioLegend) for 30 minutes at 4 ℃ in the dark. The cells were further washed with cold staining buffer twice and resuspended in the staining buffer containing 1 μg/mL propidium iodide (PI; BioLegend) followed by flow cytometry analysis using a Moflo XDP flow cytometer from Beckman Coulter, CA, USA. For $CD24^+$ or $EPCAM^+$ cell sorting, the colorectal cells (5×10^7) were harvested and resuspended in cold staining buffer, then incubated with antibodies against human CD24 (BioLegend) or EpCAM (BioLegend), respectively. Positively and negatively stained cells were then sorted using the Moflo XDP flow cytometer. The sorted cells from three independent experiments were subjected to a real-time polymerase chain reaction (PCR) assay.

RNA sequencing (RNA-seq)

Total RNA (PUM1 and control spheroids) was extracted using the mirVana microRNA (miRNA) Isolation Kit (Ambion, CA, USA) according to the manufacturer's protocol. Gene ontology analysis was respectively performed using R based on the hypergeometric distribution.

Real-time PCR analysis

Total RNA was extracted from tissues or cells using TRIzol (Invitrogen) and was reverse transcribed using a Reverse Transcription System (Promega) to synthesize

complementary DNA (cDNA). In the Roche Light Cycler 96 System (Roche, USA), the cDNA was mixed with SYBR Green PCR Kit (Roche) and specific primers to actuate the real-time PCR. The PCR conditions were as follows: 1 cycle at 95 ℃ for 5 minutes, followed by up to 40 cycles at 95 ℃ for 15 seconds (denaturation), 60 ℃ for 30 seconds (annealing), and 72 ℃ for 30 seconds (extension). Each experiment was repeated at least three times and the representative results were shown.

Phosphatidylinositol-3-kinase (PI3K) activity assay

PI3K activity in the immunoprecipitates was analyzed with a PI3K enzyme-linked immunosorbent assay (ELISA) kit (Echelon Biosciences, Salt Lake City, UT, USA) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS V.18.0 (IBM, CA, USA). Data have been presented as mean ± standard deviation (SD). Comparison of three or more variables was carried out by analysis of variance (ANOVA), whereas Student's *t*-test or the Mann-Whitney U-test was carried out for comparing two variables. The log-rank analysis and Kaplan-Meier method were used for the comparison of patient survival rates in various subgroups. The correlation between two variables was examined by making use of Pearson's correlation analysis. Individual data sets underwent a different analysis. A P value <0.05 defines statistical significance.

Results

PUM1 enhances CRC oncogenesis by promoting the generation of T-ICs

PUM1 was over-expressed in a normal colorectal cell line (FHC) for elucidating the tumor driver function of PUM1 in CRC (*Figure 1A*). It's interesting to note that PUM1 significantly promoted the production of T-ICs and increased the degree of expression of markers associated with T-IC in FHC cells (*Figure 1A*). Additionally, PUM1 caused tumor growth in a model comprising murine subcutaneous tumor and transformed colorectal cells *in vitro* (*Figure 1B*), but control cells did not (*Figure 1C*). PUM1 transgenic (TG) mice with specific colorectal mutations were created to further clarify the functions of PUM1 in colorectal carcinogenesis. Additionally, it was noticed that TG mice's colorectal PUM1 expression was higher than that of wild-type (WT) mice. The control mice and PUM1-TG mice were given DEN to start the formation of tumors before receiving CCl_4 twice weekly for 2 months to accelerate tumor growth. Both the control and PUM1-TG groups both produced clear colorectal tumor foci in all of the male animals. The number of tumors and maximum tumor diameters were considerably raised by PUM1's colorectal-specific mutation (*Figure 1D*). Importantly TIC-associated markers, for instance, EpCAM, CD90, CD133, and CD24 had a considerably higher level of the expression in CRCs from PUM1 overexpression animals (*Figure 1E*), supporting the notion that PUM1 accelerated T-ICs production and facilitated liver oncogenesis.

PUM1 enhances T-IC expansion in CRC

T-IC markers CD24 and EpCAM are widely used (24). PUM1 levels were favorably linked with CD24 and EpCAM expression within tumor cells derived from primary CRC tissues as demonstrated by Pearson correlation analysis (*Figure 2A*). We used flow cytometry sorting or sphere creation to enrich T-ICs in order to analyze PUM1 expression in T-ICs. PUM1 expression was increased in sorted EpCAM+ or CD24+ generated primary CRC cells, as seen in *Figure 2B*. Notably, the level of PUM1 was elevated in CRC spheres. Nevertheless, upon reattachment of the spheres, it returned to its original level (*Figure 2C,2D*). The outcomes from the two CRC cell lines were comparable (*Figure 2E-2H*). Furthermore, compared to cetuximab sensitive CRC patient tissues, the expression of PUM1 was substantially high in cetuximab resistant CRC patient tissues (*Figure 2I*). By chance, the expression of PUM1 was established to be considerably higher in recurrent CRC in comparison to the initial lesion (*Figure 2J*). PUM1 was preferentially increased in T-ICs, according to these data.

PUM1 enhances T-IC expansion by promoting PI3K/protein kinase B (AKT) activation

In an attempt to explain the mechanistic pathway regulating the function of PUM1 in the expansion of T-ICs, gene expression in sphere-derived PUM1 overexpression or control cells was analyzed by employing RNA-seq. Analysis of gene ontologies showed that PUM1 overexpression enriched gene sets associated with the PI3K/AKT pathway (*Figure 3A*). Consistently, PUM1 down expression spheroids that were generated as xenografts showed lower

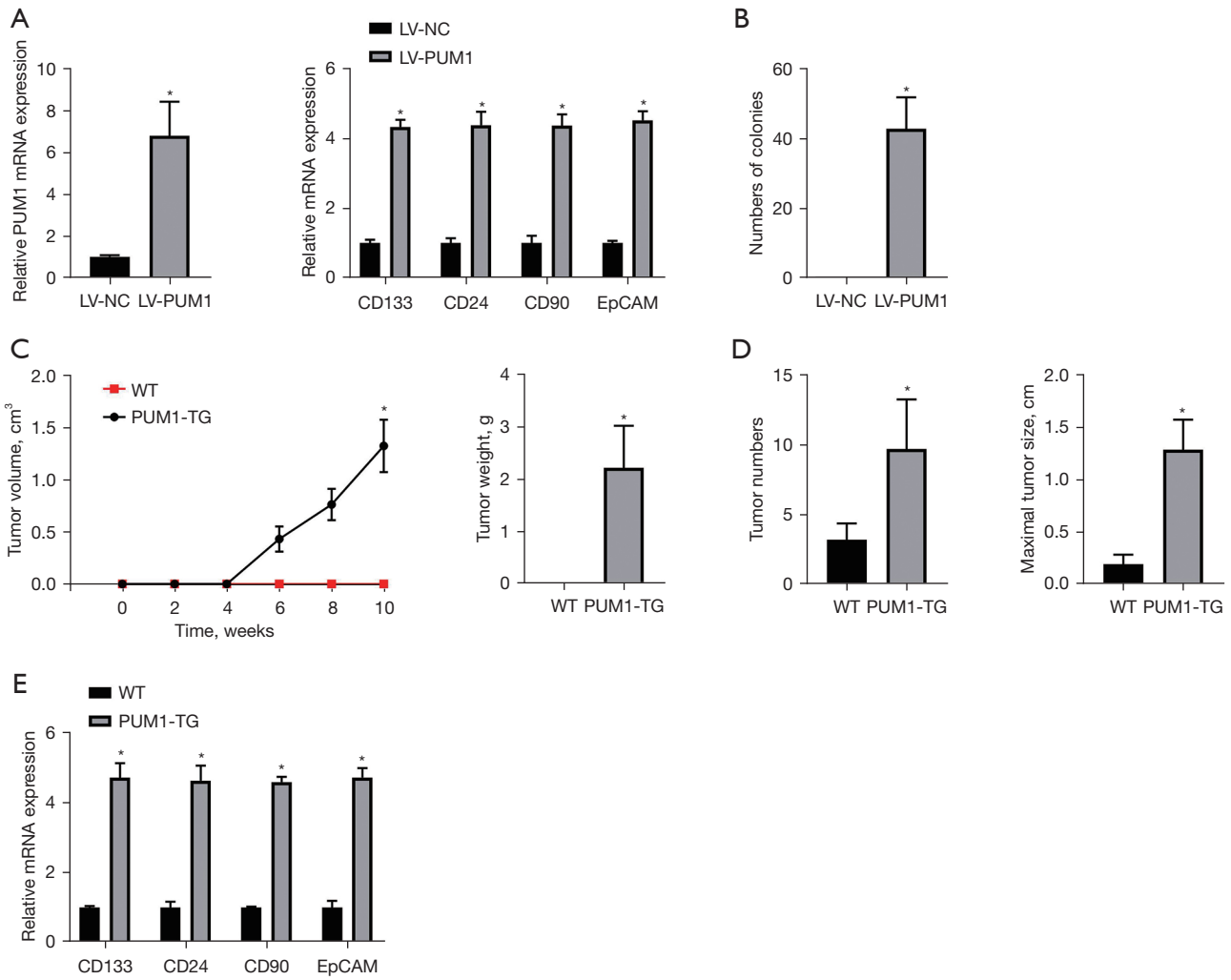


Figure 1 PUM1 enhances CRC oncogenesis by promoting the generation of T-ICs. (A) Real-time PCR testing was performed on PUM1 overexpression virus-infected FHC cells. (B) Spheroid formation tests were conducted on PUM1 and control colorectal cells. (C) NOD-SCID mice were given subcutaneous injections of 1×10^3 FHC PUM1 and control cells. The weight of the xenografted tumor was assessed 10 weeks after it had grown. (D) Five months following DEN injection, the tumor incidence and maximum tumor diameters in PUM1-TG and WT mice were assessed. (E) Real-time PCR analysis was used to look at the mRNA expression of TIC-associated markers in tumors from PUM1-TG and WT mice, 5 months following DEN injection. All results are presented as the mean ± SD, and statistical significance was assessed using a two-tailed Student's *t*-test. *P<0.05. PUM1, pumilio homologous protein 1; mRNA, messenger RNA; LV, lentiviral vector; NC, negative control; EpCAM, epithelial cell adhesion molecule; WT, wild-type; TG, transgenic; CRC, colorectal cancer; T-IC, tumor-initiating cell; PCR, polymerase chain reaction; FHC, normal colorectal cell line; NOD-SCID, non-obese diabetes-server combined immune-deficiency; DEN, diethylnitrosamine; SD, standard deviation.

p-AKT levels (*Figure 3B*). Additionally, in colorectal T-ICs PUM1 overexpression also resulted in PI3K elevation, as demonstrated by kinase activity assays (*Figure 3C*). Additionally, by inhibiting AKT, PUM1 colorectal cells' potential for self-renewal, the frequency of colorectal T-ICs, and tumorigenesis can all be improved (*Figure 3D*).

miR-218-5p inhibited T-ICs expansion by targeting PUM1 in CRC

The candidate miRNA miR-218-5p that was found after searching the TargetScan database was used to further examine the upstream regulation mechanism of PUM1

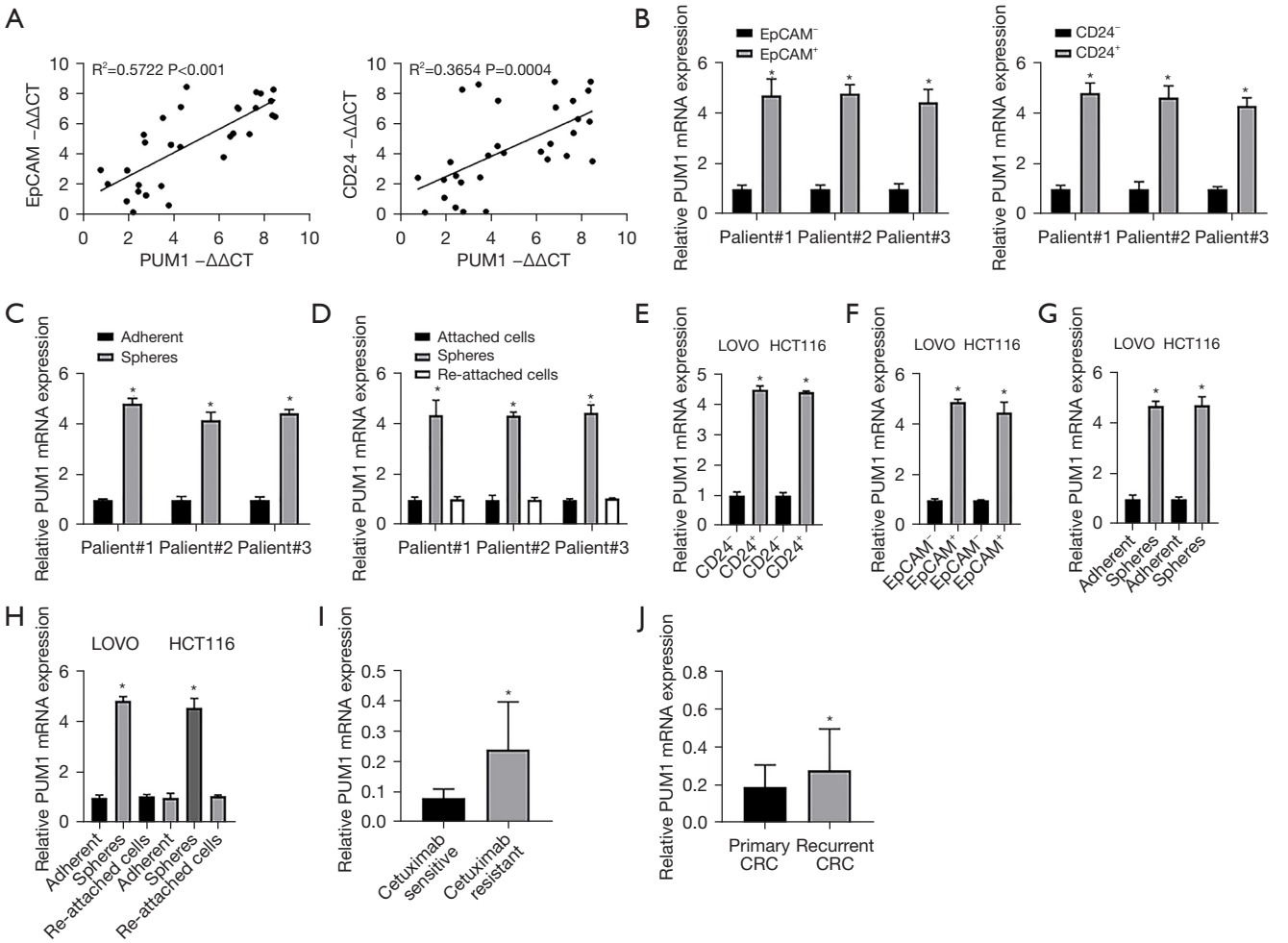
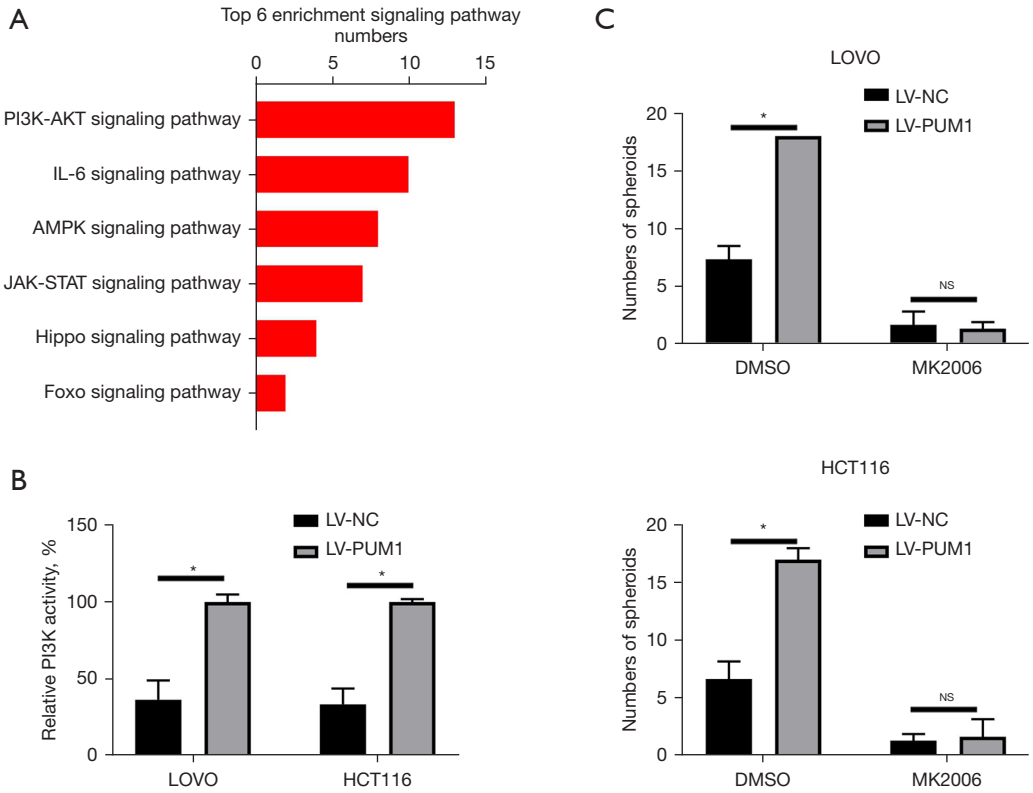


Figure 2 PUM1 enhances T-ICs expansion in CRC. (A) Real-time PCR analysis was used to ascertain the relationship between PUM1 level and CD24 (right) or EpCAM (left) in primary CRC cells (n=30). (B) Comparison of PUM1 expression in sorted CD24 (right) or EpCAM (left) primary CRC cells to negative cells using real-time PCR. (C) PUM1 expression in primary CRC adherent cells and spheres was examined using real-time PCR. (D) PUM1 expression in primary CRC adherent, sphere-like, and re-adherent cells examined using real-time PCR. (E) PUM1 expression in sorted $CD24^+$ CRC cells as compared to negative cells as determined by real-time PCR. (F) Real-time PCR analysis PUM1 expression in sorted $EpCAM^+$ CRC cells relative to negative cells. (G) PUM1 expression in CRC adherent cells and spheres determined using real-time PCR analysis. (H) PUM1 expression in CRC adherent, spheres and re-adherent cells determined using real-time PCR analysis. (I) PUM1 expression in cetuximab-sensitive or cetuximab-resistant CRC tissues using real-time PCR analysis. (J) Real-time PCR analysis of PUM1 in recurrent CRC and primary lesions of the patient. All results are presented as the mean ± SD, and statistical significance was assessed using a two-tailed Student's *t*-test. *P<0.05. EpCAM, epithelial cell adhesion molecule; CT, cycle threshold; PUM1, pumilio homologous protein 1; mRNA, messenger RNA; CRC, colorectal cancer; T-IC, tumor-initiating cell; PCR, polymerase chain reaction; SD, standard deviation.

in T-ICs. WT or mutant (MUT) PUM1 3'-untranslated region (3'-UTR)-coupled luciferase reporters were tested to demonstrate the binding among the PUM1 3'-UTR and miR-218-5p (*Figure 4A*). In CRC cell lines that had been transfected with mimics or inhibitors of this miRNA, resulting in its corresponding overexpression or downregulation, we performed functional studies of miR-218-5p (Figure S1A). Functional analysis revealed that miR-218-5p practically impedes CRC cells from proliferation, migration, as well as an invasion



D

Cells injected	LV-NC DMSO	LV-PUM1 DMSO	LV-NC MK2006	LV-PUM1 MK2006
1×1,000	2/8	5/8	0/8	0/8
5×1,000	4/8	6/8	0/8	1/8
1×10,000	5/8	8/8	1/8	0/8
5×10,000	5/8	8/8	1/8	2/8
Tumor incidence	16/32 (50%)	27/32 (84.4%)	2/32 (6.3%)	3/32 (9.4%)

Figure 3 PUM1 enhances T-ICs expansion by promoting PI3K/AKT activation. (A) Differentially expressed genes from RNA-seq of sphere-derived PUM1 overexpression or control cells analyzed via pathway enrichment analysis. (B) The PI3K kinase activity was ascertained as elaborated in the Methodology. (C) Spheres formation assay of indicated CRC cells infected with AKT overexpression virus. Counting and comparison of the number of spheroids were carried out. (D) *In vivo* limiting dilution assay was carried out for the indicated CRC cells infected with the AKT overexpression virus. Tumors were observed over 2 months, for each group n=8. All results are presented as the mean ± SD, and statistical significance was assessed using a two-tailed Student's *t*-test. *P<0.05; ^{NS}P>0.05. PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; IL-6, interleukin-6; AMPK, AMP-activated protein kinase; JAK, Janus kinase; STAT, signal transducer and activator of transcription; LV, lentiviral vector; NC, negative control; PUM1, pumilio homologous protein 1; DMSO, dimethyl sulfoxide; T-IC, tumor-initiating cell; RNA-seq, RNA sequencing; CRC, colorectal cancer; SD, standard deviation.

(Figure S1B-S1D). In CRC spheres transfected with WT PUM1 3'-UTR, miR-218-5p caused inhibition of luciferase activity, whereas a mutation in PUM1 rendered the inhibition ineffective (*Figure 4B*). PUM1 was further shown to be suppressed in miR-218-5p-overexpressing CRC spheres by real-time PCR (*Figure 4C*). In line with this, the level of expression of PUM1 and miR-218-5p in isolated primary CRC cells were inversely linked (*Figure 4D*). Significantly low miR-218-5p levels were observed in CRC tumor specimens in comparison

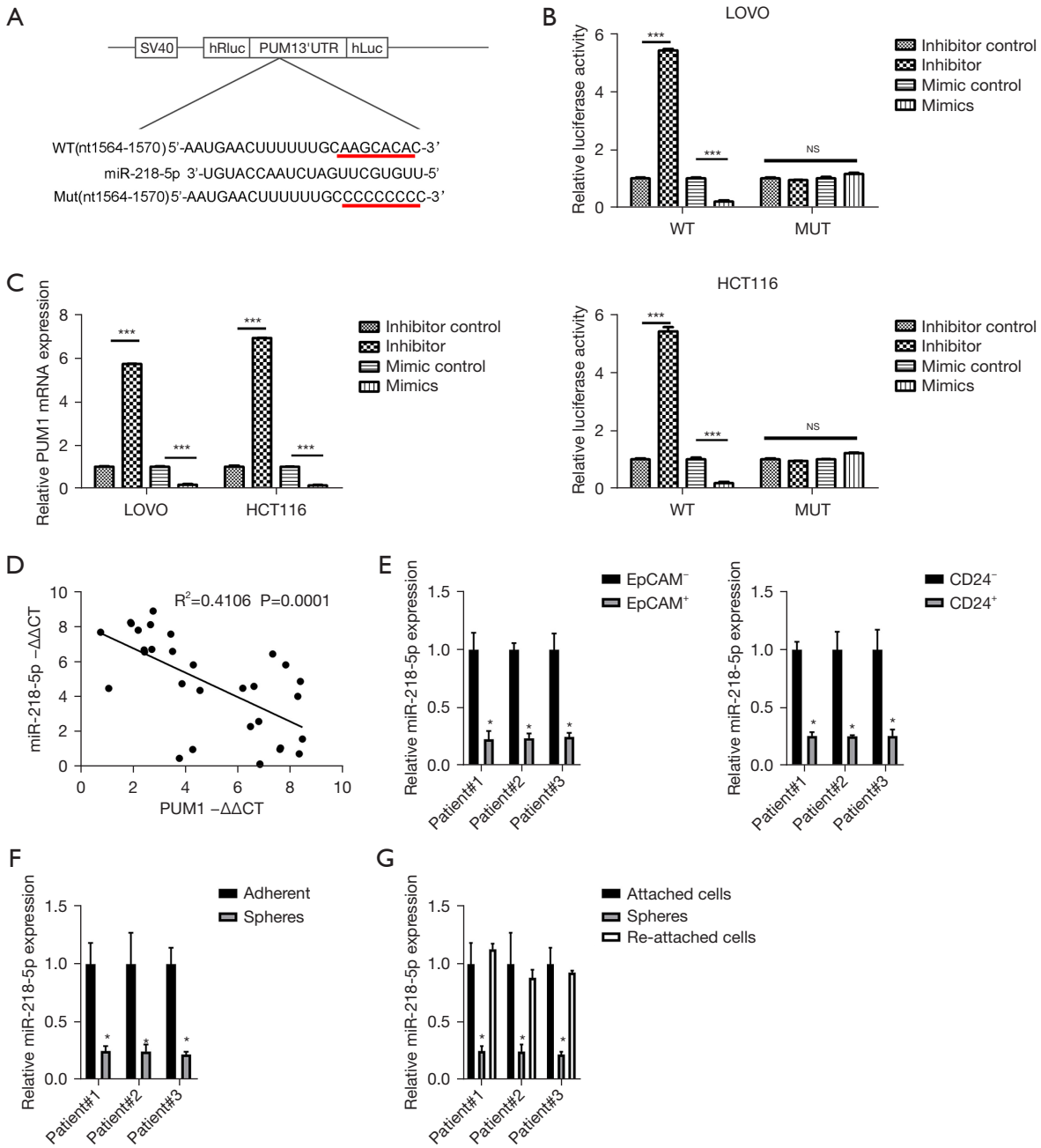


Figure 4 miR-218-5p inhibited T-IC expansion by targeting PUM1 in CRC. (A) Potential target site predicted by TargetScan for miR-218-5p in the 3'-UTR of human PUM1 mRNA. The target site was mutated for disrupting the interaction between PUM1 mRNA and miR-218-5p. (B) Luciferase reporter assays conducted on MUT PUM1 3'-UTR or WT constructs. (C) Real-time PCR analysis of PUM1 expression in indicated CRC cells. (D) Real-time PCR analysis was used to determine the correlation between the level of PUM1 and miR-218-5p in primary CRC cells (n=30). (E) Real-time PCR analysis of miR-218-5p expression in sorted EpCAM$^+$ or CD24$^+$ primary CRC cells relative to negative cells. (F) Real-time PCR analysis of miR-218-5p expression in primary CRC adherent spheres and cells. (G) Real-time PCR analysis of miR-218-5p expression in primary CRC adherent, spheres, and re-adherent cells. All results are presented as the mean ± SD, and statistical significance was assessed using a two-tailed Student's *t*-test. *P<0.05; ***P<0.001; ^{NS}P>0.05. PUM1, pumilio homologous protein 1; 3'-UTR, 3'-untranslated region; WT, wild-type; MUT, mutant; mRNA, messenger RNA; CT, cycle threshold; EpCAM, epithelial cell adhesion molecule; T-IC, tumor-initiating cell; CRC, colorectal cancer; PCR, polymerase chain reaction; SD, standard deviation.

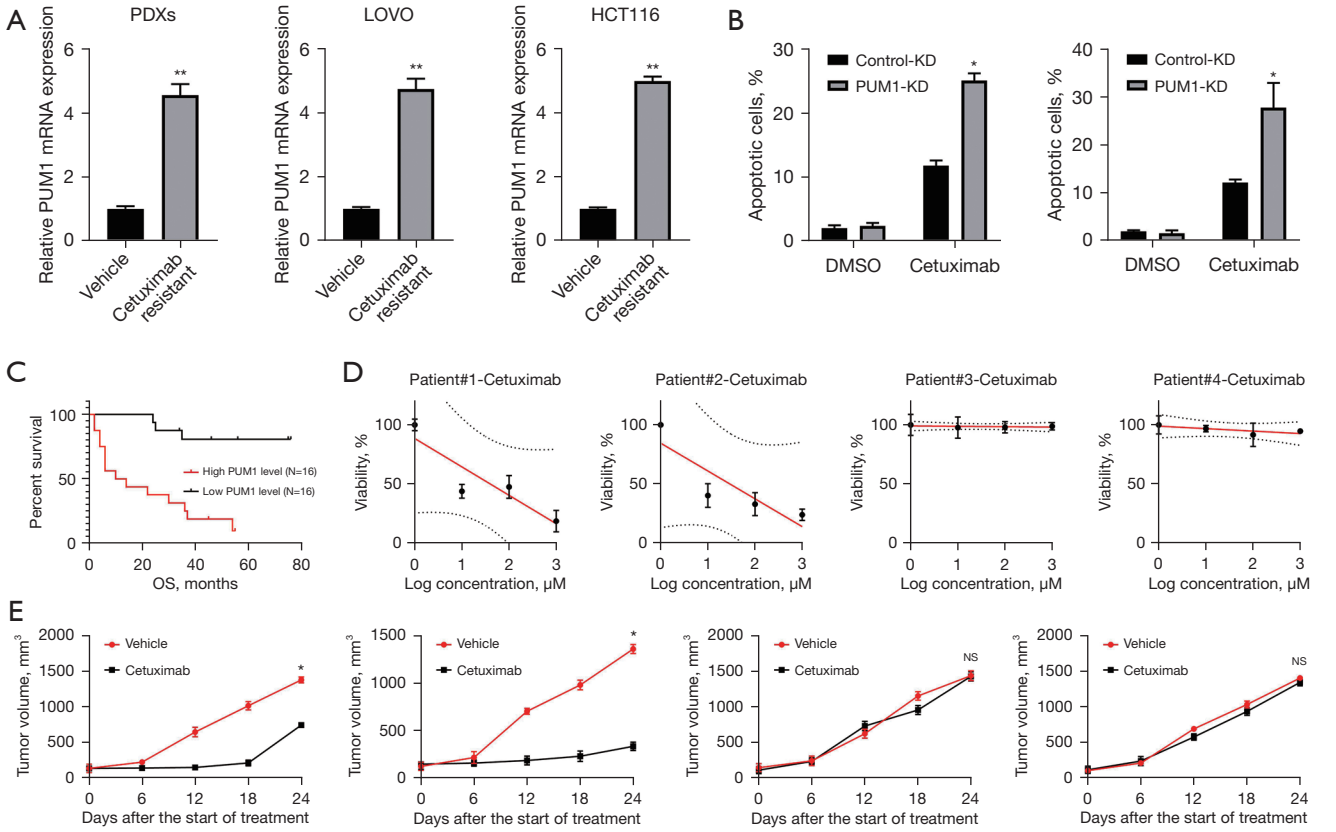


Figure 5 The PUM1 axis determines the cetuximab response in CRC. (A) Real-time PCR analysis of PUM1 expression in cetuximab-resistant CRC PDXs or cell lines. (B) Cetuximab (10 μM) was used to treat the Indicated CRC cells for 48 h and flow cytometry was employed to ascertain their apoptosis. (C) Kaplan-Meier analysis was carried out to evaluate the OS of patients between PUM1-low or PUM1-high groups. (D) Cetuximab was used to treat PDOs derived from the primary CRCs with high levels of PUM1 (Patient #1–2) or low PUM1 levels (Patient #3–4) for a period of 7 days and the corresponding cell survival curves were computed. (E) For 24 days, the vehicle or cetuximab (60 mg/kg body weight) was used to treat PDXs derived from the primary CRCs with high levels of PUM1 (Patient #1–2) or low levels of PUM1 (Patient #3–4), n=5 for each group. The growth of the xenograft was carefully examined. All results are presented as the mean ± SD, and statistical significance was assessed using a two-tailed Student's *t*-test. *P<0.05; **, P<0.01; ^{NS}P>0.05. PUM1, pumilio homologous protein 1; mRNA, messenger RNA; PDXs, patient-derived xenografts; KD, knock down; DMSO, dimethyl sulfoxide; OS, overall survival; CRC, colorectal cancer; PCR, polymerase chain reaction; PDOs, patient-derived organoids; SD, standard deviation.

to corresponding control samples (Figure S2A), and in CRC patients suffering from more advanced disease, miR-218-5p expression was notably low in comparison to the individuals with a less advanced stage of disease (Figure S2B). Furthermore, self-renewing spheroids and EpCAM$^+$ or CD24$^+$ primary CRC cells that were sorted showed decreased miR-218-5p expression (*Figure 4E,4F*). Notably, when the spheres were reattached, miR-218-5p levels returned to normal (*Figure 4G*). Together, the outcome demonstrates that by targeting PUM1, miR-218-5p restricted the expansion of T-ICs.

The PUM1 axis determines the cetuximab response in CRC

T-ICs are strongly linked to cancer's resistance to pharmacotherapy, according to mounting data (24,25). PUM1 expression was significantly elevated in cetuximab-resistant CRC PDXs and cell lines, as illustrated in *Figure 5A*. PUM1 overexpression in CRC cells decreased cetuximab sensitivity in CRC cells (*Figure 5B*). We continue to look at the role that PUM1 plays in patient response towards cetuximab based treatment. Compared to CRC patients having a high PUM1 expression, those with low

PUM1 expression had higher survival times after receiving cetuximab (*Figure 5C*). Next, we discovered that patient-derived organoids (PDOs) generated from tumors with high PUM1 levels were resistant to therapy with cetuximab (*Figure 5D*). Similar to primary CRCs, following cetuximab therapy, there was almost complete inhibition in the growth of PDXs derived from of high PUM1 carrying tumors (*Figure 5E*). These findings further show that PUM1 levels in tumors from patients may be a reliable indicator of cetuximab response.

Discussion

However, the incidence and death of CRC have not decreased despite improvements in research and treatment. T-ICs prevent the majority of CRC from being completely eliminated. The corresponding regulatory system for T-ICs, however, is poorly understood. In the current work, we discovered that PUM1 expression in T-ICs is downregulated due to miR-218-5p-mediated PUM1 messenger RNA (mRNA) degradation and that PUM1 inhibits T-ICs characteristics and tumorigenicity through the reduction of PI3K/AKT signaling. Our clinical studies further showed that PUM1 levels are related to patients' benefits from cetuximab.

Despite the fact that PUM1 was increased in CRC tissues and improved CRC metastasis, its function in T-ICs has not been explored as yet. The findings of this work demonstrated that PUM1 impaired the development of T-ICs in CRC, which promoted oncogenesis. In addition, we showed that spheroid-enriched T-ICs and $CD24^+$ or $EpCAM^+$ cells both had higher PUM1 levels. Functional studies revealed that PUM1 improved T-IC self-renewal and growth, promoting the onset and development of CRC. PUM1 is a viable therapeutic target since, taken together, our data show that it has a significant involvement in the spread of T-ICs.

In the progression of several malignancies, including CRC, the PI3K/AKT signaling pathway is stimulated (26,27). Our RNA-seq findings demonstrated that PUM1-mediated T-ICs growth needed inhibition of the PI3K/AKT signaling pathway. According to earlier research, tissue inhibitors of metalloproteinases (TIPMs) and matrix metalloproteinases (MMPs) had a role in PUM1-mediated tumor metastasis (16). On the other hand, we suggest that PUM1 controls T-ICs expansion through the AKT signaling pathway. These findings showed the critical function of the PUM1/PI3K/AKT axis in T-ICs as well as a unique mechanism for activating hepatic T-ICs. It is anticipated that targeting the PUM1/PI3K/AKT axis may turn out to be a cutting-edge strategy for the treatment of CRC, given the significant role played by the PUM1/PI3K/AKT axis in T-ICs.

Recent evidence demonstrates that T-ICs have dysregulated miRNA expression, which aids in the growth of T-ICs (9,25). It was thus demonstrated that PUM1 downregulation in T-ICs might result from the loss or increase of miRNAs that target this gene. The luciferase reporter test identifies, MiR-218-5p as an upstream PUM1 regulator that specifically targets PUM1. A marked downregulation of miR-218-5p in T-ICs has been demonstrated in the present study and has been shown to block the PUM1/PI3K/AKT axis, which is anticipated to be the cause of both carcinogenesis and self-renewal in T-ICs. The miR-218-5p/PUM1/PI3K/AKT axis has a critical function in T-ICs, according to these findings, which also offer a new method for the activation of liver T-ICs.

There are currently few clinical options for advanced CRC patients who acquire cetuximab resistance (28-32). Therefore, it is critical to look into the causes of cetuximab resistance and to find reliable biomarkers that can forecast how well CRC patients will respond to chemotherapy. In this investigation, it was discovered that CRC cells manifested more susceptibility to cetuximab-induced growth inhibition and death due to lower PUM1 expression. Low PUM1 levels were further shown to be related to improved response and survival amongst individuals who were treated with cetuximab in the cetuximab cohort, PDO, and PDX investigations. Therefore, before choosing an appropriate course of treatment, it is recommended to assess PUM1 expression in CRC tumors for the identification of patients who can potentially benefit from cetuximab therapy. There were some limited in current version: multiple methods for cell proliferation, migration, and invasion need to be used in future.

Conclusions

Collectively, our findings highlight the crucial role of the miR-218-5p/PUM1/PI3K/AKT regulatory circuit in regulating T-IC properties, suggesting potential therapeutic targets for CRC.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-6/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-6/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-6/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The present study obtained the approval of the Ethics Committee of Shanghai Fourth People's Hospital (No. 2022018-001), and informed consent was obtained from all participants. Animal experiments were performed under a project license (No. TJBH0122101) granted by the Ethics Committee of Shanghai Tongji University, in compliance with Shanghai Fourth People's Hospital guidelines for the care and use of animals.

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