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Original Research

N6-methyladenosine Methyltransferase METTL3 Enhances PTGER2 Expression to Increase Ovarian Cancer Stemness and Chemoresistance

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Abstract

Background: Ovarian cancer is the second leading cause of gynecologic cancer-associated deaths. Cancer stemness and chemoresistance are responsible for ovarian cancer metastasis and the poor prognosis of patients. In this study, we determined the function of N^6-methyladenine (m^6A) RNA methylation and prostaglandin E receptor 2 (PTGER2) in ovarian cancer progression. **Methods**: The m^6A RNA methylation-associated PTGER2 in ovarian cancer was identified using bioinformatics analysis. The role of PTGER2 in ovarian cancer was elucidated in cell lines and clinical samples with cellular and molecular experiments. **Results**: In this investigation, bioinformatics analysis based on a public cancer database was used to elucidate the impact of m^6A modification on the prognosis of patients with ovarian cancer. Moreover, PTGER2 was identified as a potential oncogene associated with the distant metastasis of ovarian cancer and poor patient prognosis. Interestingly, PTGER2 expression was experimentally shown to be enhanced by N^6-adenosine-methyltransferase 70 kDa subunit (METTL3)-mediated m^6A modification. In addition, PTGER2 enhanced cancer stem cell self-renewal properties, the epithelial-mesenchymal transition, and DNA damage repair, thus potentiating cell stemness, therapy resistance to carboplatin, proliferation, and metastasis of ovarian cancer. Importantly, PTGER2 expression in clinical samples was associated with distant metastasis, predicted poor patient prognosis, and independently served as a prognostic predictor in ovarian cancer. **Conclusions**: Our work defines PTGER2 as an oncogene and reveals that PTGER2 is a prognostic predictor and novel therapeutic target for the management of ovarian cancer.

Keywords: N^6-methyladenosine; PTGER2; ovarian cancer; stemness; chemoresistance

1. Introduction

Ovarian cancer is characterized by a high prevalence and high mortality rate and ranks as the second leading factor causing gynecologic cancer-related deaths in women worldwide [1]. Platinum-based chemotherapy is still the main treatment for advanced ovarian cancer [2]. Although ovarian cancer mortality has declined in recent years [3], ovarian cancer stemness, chemoresistance, and metastasis are common reasons for treatment failure and poor patient prognosis [4]. Therefore, identifying novel targets to overcome these tumorigenic properties is now an urgent priority for this disease.

N^6-methyladenine (m^6A) RNA methylation is the most abundant RNA modification type in non-coding RNAs and mRNAs and can regulate downstream effectors to affect ovarian cancer progression [5]. The m^6A RNA methylation process is mediated by many proteins including methyltransferases, demethylases, and m^6A readers [6]. Previous studies have suggested that m^6A regulator-mediated methylation modification patterns can affect cancer progression [7,8]. Copy number variation (CNV) gains are associated with an increase in gene expression, whereas CNV deletions are correlated with a decrease in gene expression. Moreover, advanced-stage cancers harbor more CNV events of m^6A regulators [9], and patients with CNV gain of N6-adenosine-methyltransferase 70 kDa subunit (METTL3) have a poor prognosis [10]. METTL3 functions as a methyltransferase and plays an oncogenic role in ovarian cancer [11,12]. However, the dysregulation of downstream effectors of these m^6A regulators remains largely undetermined.

Prostaglandin E2 receptor (subtype EP2), namely PTGER2, belongs to the G protein-coupled receptor superfamily. The activation of PTGER2 results in the increased activity of cAMP-dependent signaling [13]. Previous studies have revealed that PTGER2 acts as an oncogene in colorectal cancer [14] and prostate cancer [15] and serves as a tumor suppressor in neuroblastoma [16]. However, the biological role and clinical significance of PTGER2 and the mechanism of PTGER2 dysregulation in ovarian cancer remain unclear.

Here, we elucidated the role of m^6A modification in ovarian cancer and identified specific genes modulated by

m^6A regulators using a public cancer database. In particular, we focused on the mechanism by which METTL3-mediated m^6A modification of PTGER2 mRNA results in its upregulation, and explored the impact of these aberrant interactions on ovarian cancer cell stemness, chemoresistance, proliferation, and metastasis. Our work further suggests the potential application of PTGER2 as a reliable prognostic predictor and a novel therapeutic target for ovarian cancer treatment by using ovarian cancer cell lines and clinical samples.

2. Materials and Methods

2.1 Bioinformatics Analysis

Bioinformatics analysis was performed according to our previous study [17]. The genomic data, mRNA expression profiles, and clinical data of The Cancer Genome Atlas (TCGA) ovarian serous cystadenocarcinoma (OV) database were downloaded for conducting differential analyses, survival analyses, correlation analyses, unsupervised clustering analyses, gene set variation analysis (GSVA), and gene set enrichment analysis (GSEA). The gene set representing platinum drug resistance was constructed using data obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database as previously reported [18]. Based on the best cut-off value obtained from survival analysis, samples were assigned as low and high expression groups.

2.2 Cell Cultures

Ovarian cancer cell lines OV90 and SKOV3 with short tandem repeat verification were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) harboring 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2. It was confirmed that the cell lines did not have mycoplasma contamination.

2.3 Cell Transfections

Single hairpin RNA (shRNA) negative control and shRNA targeting PTGER2 (sh-PTGER2) were constructed and inserted into lentiviral particles by GeneChem Corporation (Shanghai, China). Small interfering RNA (siRNA) against METTL3 (si-METTL3) was synthesized by RiboBio Corporation (Guangzhou, China). Polybrene and Lipofectamine 3000 were used for lentivirus particle transfection and siRNA transduction, respectively. After transfection or transduction for 48–72 h, ovarian cancer cells were subjected to further investigation.

2.4 RT-PCR and Quantitative PCR

Total RNAs of ovarian cancer cells were used for reverse transcription with a kit purchased from TaKaRa Corporation (Dalian, China). RT-PCR and quantitative PCR (qPCR) were conducted with the generated cDNAs and specific primers. Bio-Rad GelDoc XR+ (Hercules, CA, USA) was applied to capture images for RT-PCR, and the $2^{-\Delta\Delta Ct}$ method was used to determine the relative expression of mRNA for qPCR.

2.5 Western Blot Analysis

Total proteins of ovarian cancer cells in lysis buffer were quantified by the BCA method as previously described in our investigation [19]. Then the separated and transferred proteins were incubated with antibodies against the following proteins: METTL3, PTGER2, Myc, cyclin D1 (CCND1), vimentin, and β-actin. The chemiluminescence method was adopted for protein detection, and the Bio-Rad GelDoc XR+ was applied to record images.

2.6 Methylated RNA Immunoprecipitation

The Methylated RNA Immunoprecipitation (MeRIP) m^6A Kit purchased from RiboBio Corporation (Guangzhou, China) was used to conduct MeRIP as previously described in our investigation [8]. Briefly, total RNAs of ovarian cancer cells were used to generate fragments that were pulled down with magnetic beads harboring the m^6A antibody. Enriched RNA was purified and used for RT-PCR and qPCR.

2.7 Luciferase Reporter Assay

PmirGLO luciferase reporter vectors (Promega Co., Madison, WI, USA) containing wild-type or mutant 3'-untranslated region (UTR) of PTGER2 were transfected into ovarian cancer cells. After transfection for 48 h, the luciferase activity in cells was measured to evaluate the effects of METTL3 on PTGER2 transcriptional levels on the BioTek Luminometer Synergy H1 (Winooski, VT, USA) using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

2.8 Tumorsphere Formation Assay

Ovarian cancer cells seeded on 6-well ultra-low-attachment plates were incubated in serum-free DMEM/F12 (20 ng/mL epidermal growth factor, 20 ng/mL fibroblast growth factor, and 2% B27). The images of tumorspheres were captured, and the number of tumorspheres was counted after a 14-day culture. Three continuous generations of tumorspheres number were calculated for analysis.

2.9 Immunofluorescence

Ovarian cancer cells seeded on coverslips were treated with paraformaldehyde (4%) for fixation, incubated with Triton X-100 (0.2%) for permeabilization, and subjected to incubation with antibodies against the following proteins: cluster of differentiation 44 (CD44), CD133, and gamma-H2A histone family member X (γH2AX). DAPI was used to co-stain the cells, and the images of stained cells were captured with a fluorescence confocal microscope.

2.10 Colony Formation Assay

To detect cell proliferation and chemoresistance, ovarian cancer cells were subjected to treatment with carboplatin for 6 h at the indicated concentrations and then seeded in wells for 10 days. The generated colonies were subjected to fixation, staining, and recording for analysis.

2.11 Transwell Assays

The migration and invasion ability of ovarian cancer cells were detected by transwell assays. For the migration assay, serum-free cells were seeded in the upper chamber of a transwell without Matrigel coating. For the invasion assay, serum-free cells were plated in the upper chamber of a transwell with Matrigel coating. DMEM containing 10% FBS was added to the lower chambers of a transwell were for both the migration and invasion assays. The migrated and invaded cells were subjected to fixation, staining, and recording for analysis.

2.12 Patient Tissues

One hundred and fifty-eight ovarian cancer specimens described in our previous study [19] were collected for analysis. All specimens had a pathological diagnosis. Patient written consent and ethics approval from the Ethics Committee of the hospital were obtained (K2020-036-01). All procedures strictly adhered to the policies approved by the Institutional Ethics Committee and Declaration of Helsinki.

2.13 Immunohistochemistry

Immunohistochemistry (IHC) and staining evaluation were carried out as previously described [19]. Paraffin-fixed sections from ovarian cancer specimens were deparaffinized and rehydrated before antigen retrieval in citrate buffer. Then the sections were subjected to the eradication of endogenous peroxidase activity and blocking of non-specific antigens. After incubation with anti-PTGER2 antibody, the sections were treated with Diaminobenzidine substrate and subjected to the evaluation of staining intensities. For statistical analysis, a score of 7–12 was assigned as high expression, and a score of 0–6 was considered low expression.

2.14 Statistical Analyses

SPSS 22.0 (IBM SPSS Inc., Chicago, IL, USA) or RStudio 9.0 was applied for analysis of the data expressed as the mean $\pm$ standard deviation from three independent experiments. The Wilcoxon rank-sum test and Student's two-tailed *t*-test were used to test the statistical significance between two groups. One-way analysis of variance was applied to reveal the statistical significance among multiple groups. Spearman's rank correlation test and Chi-square test were applied for the correlation analyses. Survival analysis was performed by plotting Kaplan-Meier survival curves with log-rank tests. Univariate and multivariate Cox regression models were applied to identify the correlation between PTGER2 expression and the overall survival of patients with ovarian cancer. Statistical significance was achieved with $p < 0.05$.

3. Results

3.1 Bioinformatics Analyses Indicate the Important Role of m^6A RNA Methylation in Ovarian Cancer Progression

To explore the role of m^6A regulators in ovarian cancer progression, we adopted 9 m^6A writers (including Cbl Proto-Oncogene Like 1 [CBLL1], METTL3/14/16, RNA-binding motif protein 15/15B [RBM15/15B], Vir-like m^6A methyltransferase associated, WT1-associated protein [WTAP], and zinc finger CCCH-type containing 13 [ZC3H13]), 2 m^6A erasers (AlkB homolog 5, RNA demethylase [ALKBH5] and fat mass and obesity-associated protein), and 15 m^6A readers (including eukaryotic translation initiation factor 3 subunit A, ELAV like RNA-binding protein 1 [ELAVL1], fragile X messenger ribonucleoprotein 1 [FMR1], heterogeneous nuclear ribonucleoprotein A2/B1 [hnRNPA2B1], hnRNPC, insulin-like growth factor 2 mRNA-binding protein 1/2/3 [IGF2BP1/2/3], leucine rich pentatricopeptide repeat containing, YTH domain-containing protein 1/2 [YTHDC1/], YTHDF1/2/3 and RNA-binding motif protein X-linked), which are dysregulated in human cancers. Since the deletion or amplification of gene copy number affects the corresponding gene expression and is regarded as the trigger of cancer development [8], we applied bioinformatics analyses based on TCGA OV database to identify CNVs in these 26 m^6A regulators and found that 23 m^6A regulators contained CNVs in ovarian cancer (**Supplementary Fig. 1**). Then the 23 CNV-carrying genes in TCGA database were used to continue our analysis of the clinical significance of these m^6A regulators in ovarian cancer. Gene interaction network analysis suggested that the expression of ALKBH5, CBLL1, ELAVL1, FMR1, HNRNPA2B1, HNRNPC, IGF2BP2, METTL14, METTL16, METTL3, YTHDC2, YTHDF2, and WTAP was correlated with the overall survival of patients with ovarian cancer and indicated a strong correlation among the expression of 23 m^6A regulators (**Supplementary Fig. 2A**), suggesting these dysregulated m^6A regulators as contributors to ovarian cancer development and progression.

To further examine how the 23 m^6A regulators exert their clinical significance in ovarian cancer, we performed unsupervised clustering and identified two distinct patterns of m^6A modification in TCGA OV patients according to the expression of 23 m^6A regulators (**Supplementary Fig. 2B**). Survival analyses showed the superior prognosis of patients with ovarian cancer in cluster A patterns than in cluster B patterns (**Supplementary Fig. 2C**). Differently enriched Gene Ontology (GO) and KEGG terms between these two clusters were elucidated by conducting GSVA. The analysis verified that cluster B was involved in RNA processing (**Supplementary Fig. 2D**) and cancer-

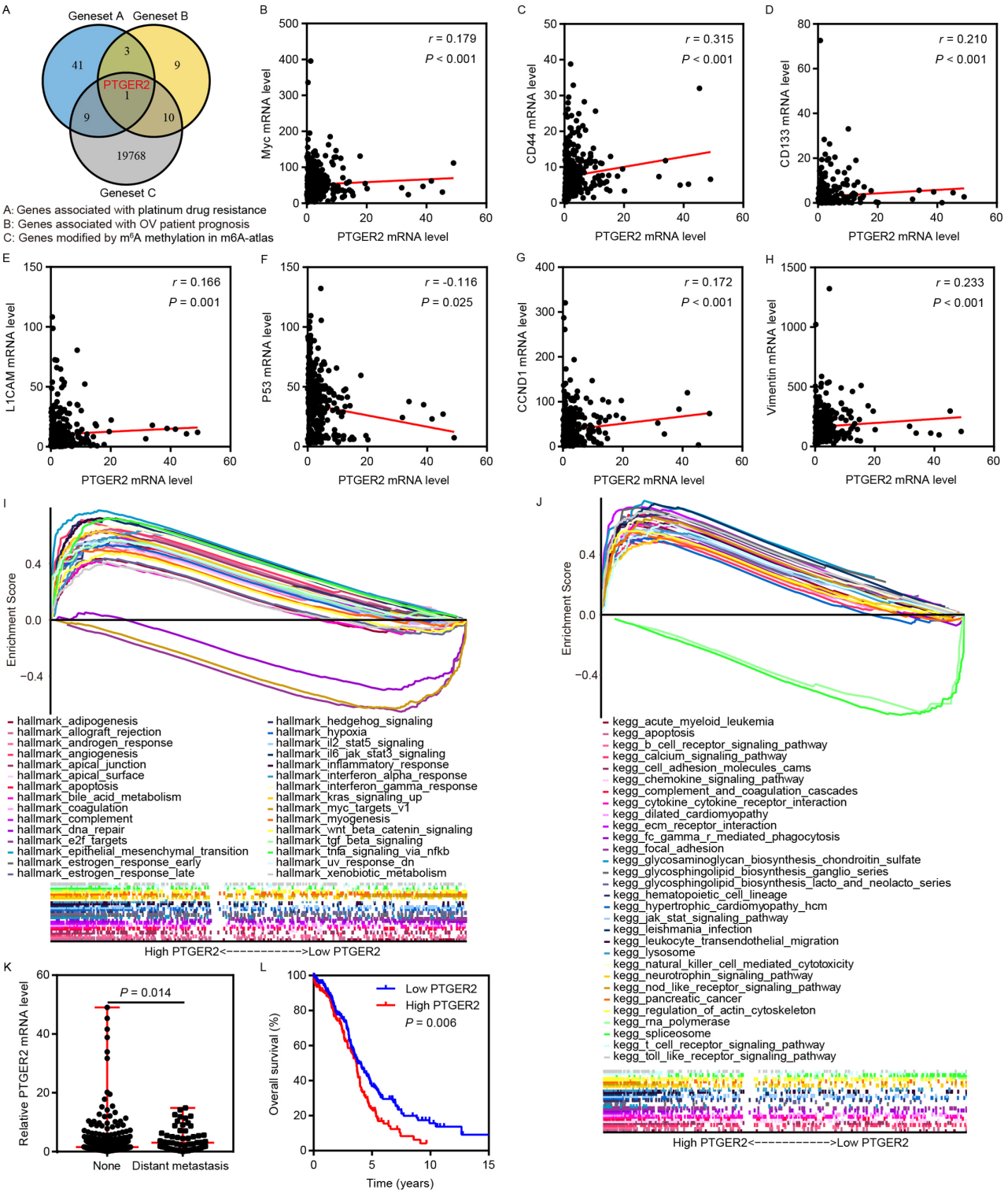

Fig. 1. PTGER2 expression serves as an m^{6}A RNA methylation-mediated oncogene in ovarian cancer as per The Cancer Genome Atlas (TCGA) database. (A) Venn diagram showing genes correlated with chemoresistance, m^{6}A modification, and patient prognosis in ovarian cancer. (B–H) The associations among Myc, CD44, CD133, L1CAM, p53, CCND1, vimentin, and PTGER2 expression. (I,J) Gene set enrichment analysis (GSEA) of PTGER2 showing enrichment of dysregulated pathways and processes in Hallmark (Top 30) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Top 30). (K) Comparison of PTGER2 expression in ovarian cancer patients with or without distant metastasis. (L) The Kaplan-Meier curve delineating the overall survival of patients with ovarian cancer based on PTGER2 expression.

associated signaling pathways (**Supplementary Fig. 2E**), such as the Notch and Wnt pathways, as well as DNA replication, cell cycle, base excision repair, nucleotide excision repair, homologous recombination, non-homologous end joining, and mismatch repair compared to cluster A patterns, further indicating the critical role of m^6A modification in ovarian cancer progression.

3.2 PTGER2 Expression is Associated with Aberrant m^6A RNA Methylation and Functions as a Tumorigenic Gene in Ovarian Cancer

To identify specific genes that could be affected by m^6A RNA methylation in ovarian cancer, we carried out differential expression analyses to compare the identified cluster A with cluster B and elucidated 492 differentially expressed genes (fold change $\geq$2.0, $p < 0.05$). Then we integrated and analyzed the differential expression data with another database to identify specific genes participating in ovarian cancer progression. First, we calculated the index of platinum drug resistance by GSVA in TCGA OV patients and performed correlation analyses to elucidate genes associated with chemoresistance (Geneset A). Furthermore, we conducted survival analyses to reveal genes correlated with the prognosis of TCGA OV patients ($|r| \geq 2.0$, $p < 0.05$) (Geneset B). In addition, we extracted genes potentially modified by m^6A RNA methylation as suggested by the m^6A-Atlas database with experimental evidence (Geneset C). Interestingly, the intersection of these three genesets only identified PTGER2 as an overlapping gene (Fig. 1A).

Subsequent correlation analyses revealed a positive correlation between PTGER2 expression and the expression of Myc, CD44, CD133, L1 cell adhesion molecule (CAM), CCND1, and vimentin, which serve as stemness, proliferation, and epithelial-mesenchymal transition (EMT)-associated markers, and showed a negative association between PTGER2 expression and expression of the DNA damage repair-associated marker p53 (Fig. 1B–H). Moreover, GSEA confirmed that PTGER2 positively participated in oncogenic processes and pathways such as DNA repair, E2F targets, EMT, Myc targets, Wnt/β-catenin signaling, CAMs, focal adhesion, and the extracellular matrix receptor interaction (Fig. 1I,J). Importantly, PTGER2 expression was upregulated in ovarian cancer patients with distant metastasis compared to those without distant metastasis (Fig. 1K), and high PTGER2 expression predicted poor patient prognosis in TCGA (Fig. 1L). These findings collectively suggest PTGER2 as a potential oncogene in ovarian cancer.

3.3 METTL3-Mediated m^6A Modification Elevates PTGER2 Expression

Based on the findings of the above-mentioned bioinformatics analyses, we further investigated whether PTGER2 expression is modulated by m^6A modification. PTGER2 was potentially modified by METTL3, METTL14, and IGF2BP1 in the m6A2Target database (http://m6a2target.canceromics.org/). Since our above survival analysis suggests METTL3 as a risk factor for the prognosis of ovarian cancer patients and PTGER2 serves as a potential oncogene, we explored the impact of METTL3 on PTGER2 and found that silencing METTL3 downregulated the mRNA and protein levels of PTGER (Fig. 2A–C). Moreover, m^6A individual-nucleotide-resolution cross-linking and immunoprecipitation and m^6A MeRIP-sequencing data collected in the RMVar database (http://rmvar.renlab.org) revealed three potential m^6A modification sites in the coding sequence (CDS) and 3′ UTR of PTGER mRNA (Fig. 2D). Considering the location relationship of these three sites, we synthesized two pairs of primers covering site 1, and sites 2 and 3, respectively. Furthermore, m^6A MeRIP analyses validated that PTGER mRNA was immunoprecipitated by anti-m^6A antibody and could be detected by qPCR with the primers covering sites 2 and 3, but not site 1 (**Supplementary Fig. 3**). Moreover, the decrease in m^6A modification of PTGER mRNA was detected in METTL3-silenced ovarian cancer cells (Fig. 2E,F). To further elucidate the site responsible for m^6A modification of PTGER mRNA, we constructed plasmids harboring mutant sites 2 or site 3 (Fig. 2G). Luciferase reporter assays further indicated that METTL3 knockdown can inhibit the transcription of wild-type PTGER, whereas mutation at site 2 but not at site 3 abolished this suppression (Fig. 2H). These findings demonstrate that METTL3-mediated m^6A RNA methylation contributes to controlling PTGER2 expression in ovarian cancer.

3.4 PTGER2 Knockdown Inhibits Ovarian Cancer Stemness, Chemoresistance, Proliferation, and Metastasis

According to the results obtained from the above bioinformatics analyses, we determined the impact of PTGER2 on ovarian cancer cell stemness, chemoresistance, proliferation, and metastasis. First, shRNA targeting PTGER2 (sh-PTGER2) was used for PTGER2 silencing in ovarian cells. Then tumorsphere formation and immunofluorescence assays showed that PTGER2 knockdown suppressed the stemness of ovarian cancer cells and impaired the expression of stem cell markers CD44 and CD133 in ovarian cancer cells (Fig. 3A–C). Colony formation assays indicated that PTGER2 depletion inhibited the carboplatin resistance and proliferation of ovarian cancer cells (Fig. 3D,E), and transwell assays measured the inhibition of migration and invasion by PTGER2 knockdown in ovarian cancer cells (Fig. 3F,G). Furthermore, immunofluorescence assays detected the upregulation of γH2AX by PTGER2 depletion in ovarian cancer cells treated with carboplatin, indicating enhanced DNA damage (Fig. 3H). Moreover, the expression of cell stemness, proliferation, and EMT-associated proteins including Myc, CCND1, and vimentin were impaired in PTGER2-silenced ovarian cancer cells (Fig. 3I). These findings demonstrate that PTGER2 func-

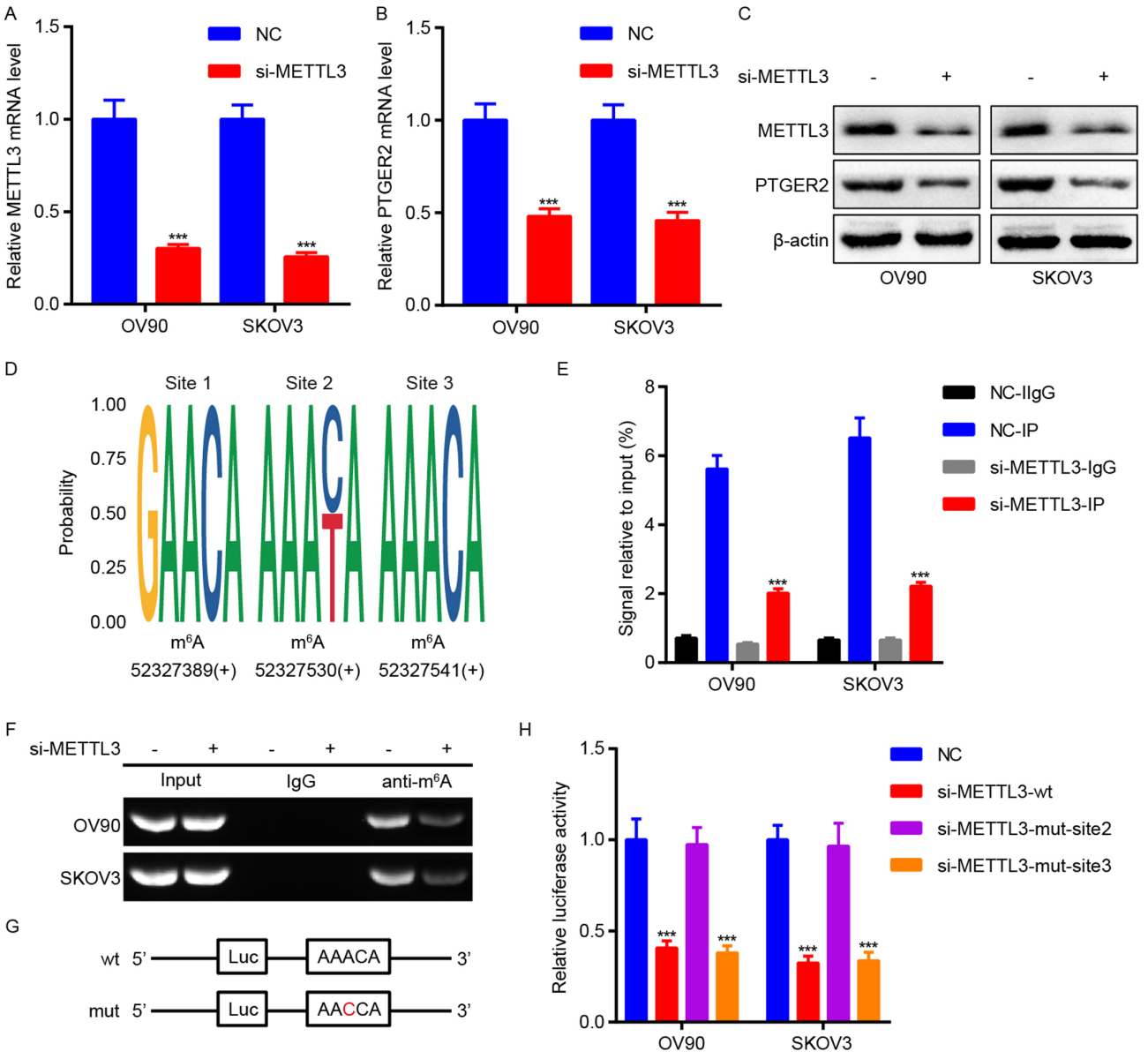

Fig. 2. METTL3 is responsible for m^{6}A modification of PTGER2 mRNA. (A) quantitative PCR (qPCR) analysis determining the efficiency of METTL3 knockdown in OV90 and SKOV3 cells. (B) qPCR analysis showing the effect of METTL3 knockdown on PTGER2 mRNA expression in OV90 and SKOV3 cells. (C) Western blot analysis suggesting the impact of METTL3 knockdown on PTGER2 protein expression in OV90 and SKOV3 cells. (D) Bioinformatics analysis identifying m^{6}A modification sites within the CDS and 3'UTR regions of PTGER2 mRNA. (E,F) m^{6}A MeRIP assays measuring the influence of METTL3 knockdown on m^{6}A modification of PTGER2 mRNA in OV90 and SKOV3 cells. The enriched RNAs were further subjected to qPCR (E) and RT-PCR (F). (G) The sequences of wild-type and mutant m^{6}A modification sites. (H) Luciferase reporter assays revealing the impact of METTL3 depletion on the post-transcriptional inhibition of PTGER2 in OV90 and SKOV3 cells. $^{***}p < 0.001$. NC, negative control.

tions as an oncogene by stimulating cell stemness, chemoresistance, proliferation, and metastasis.

3.5 PTGER2 Expression is Associated with the CLinical and Pathological Characteristics of Patients with Ovarian Cancer

To gain further knowledge of the vital role of PTGER2 in the progression of ovarian cancer, we performed IHC in 158 ovarian cancer tissues. IHC showed the different levels of PTGER2 protein expression in the detected ovarian cancer samples (Fig. 4A), and PTGER2 protein expression was upregulated in ovarian cancer samples with metastasis compared to those without metastasis ($p = 0.021$) (Fig. 4B, **Supplementary Table 1**). However, PTGER2 protein expression had no significant correlation with other clinical and pathological characteristics. Survival analysis was performed and revealed that high PTGER2 protein expression conferred poor overall survival for patients with ovarian

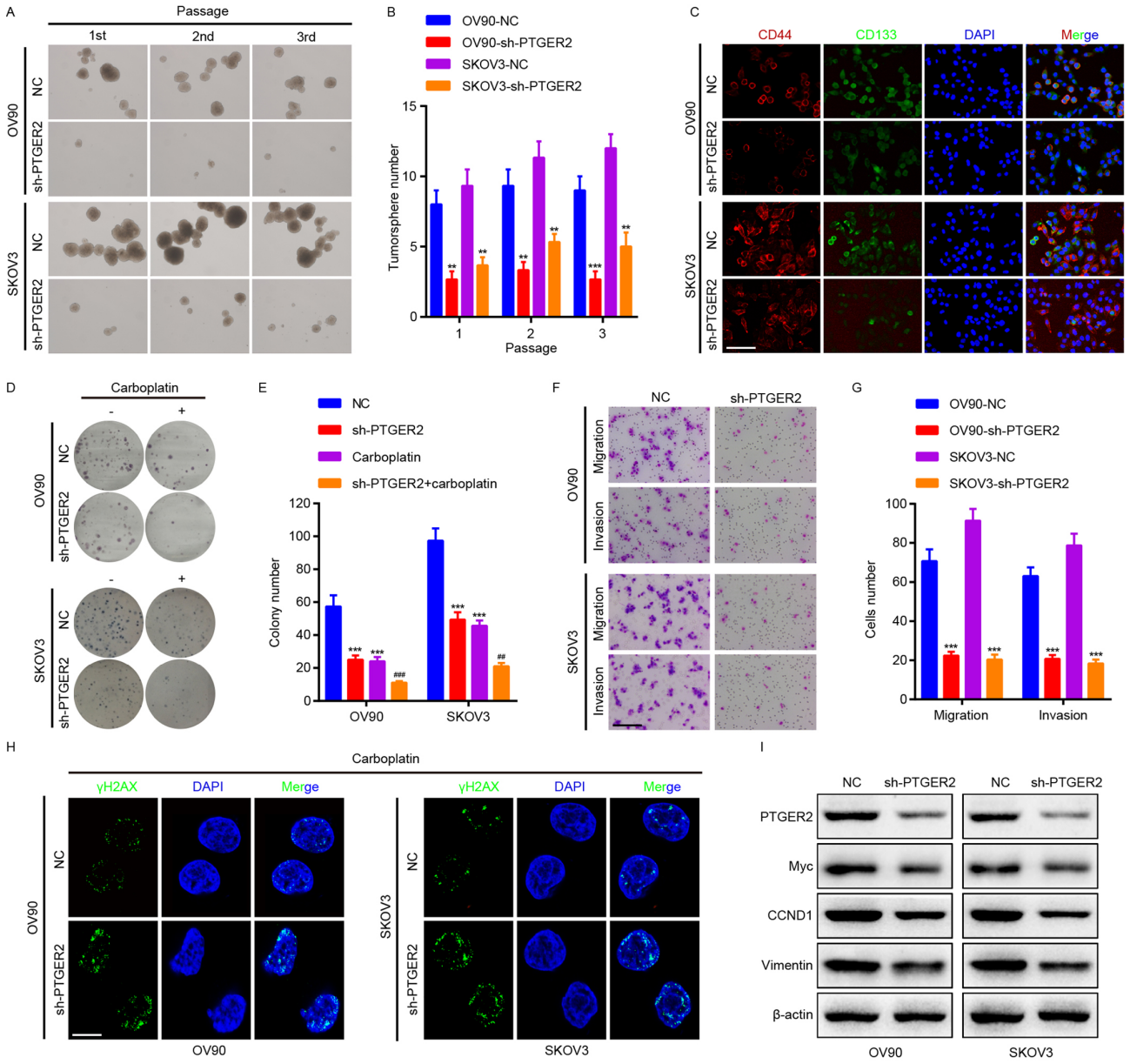

Fig. 3. PTGER2 increases ovarian cancer stemness, chemoresistance, proliferation, and metastasis. (A–G) Tumorsphere formation (A,B), immunofluorescence (scale bar: 12.5 μm) (C), Clonogenic (D,E), transwell (Scale bar: 50 μm) (F,G) assays were adopted for detecting the stemness, chemoresistance, proliferation, and metastasis of PTGER2-depleted OV90 and SKOV3 cells and the controls. (H) Immunofluorescence analysis (scale bar: 8 μm) was applied to measure γH2AX expression in PTGER2-depleted OV90 cultured with carboplatin, PTGER2-depleted SKOV3 cells cultured with carboplatin, and the controls. (I) Western blot analysis showing the expression of proteins correlated with stemness and chemoresistance (Myc), proliferation (CCND1), and metastasis (vimentin) in PTGER2-silenced OV90 and SKOV3 cells and the controls. $^{**}p < 0.01$, $^{***}p < 0.001$ (compared with the control group). $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ (compared with the carboplatin group). NC, negative control.

cancer ($p = 0.009$, log-rank test) (Fig. 4C). Univariate analyses were subsequently conducted and elucidated the positive associations between high PTGER2 protein expression, old age, advanced T classification, advanced N classification, distant metastasis, and poor overall survival of patients with ovarian cancer (Fig. 4D). Multivariate analyses were performed and revealed that PTGER2 protein expression (HR = 1.095, 95% confidence interval [CI] 1.061–1.180, $p = 0.027$), T classification, and lymph node metastasis served as independent and unfavorable prognostic factors for patients with ovarian cancer (Fig. 4E).

4. Discussion

Developing new biomarkers for cancer diagnosis, treatment, and prognosis evaluation are the key issues in cancer research [20,21]. Stemness and chemoresistance

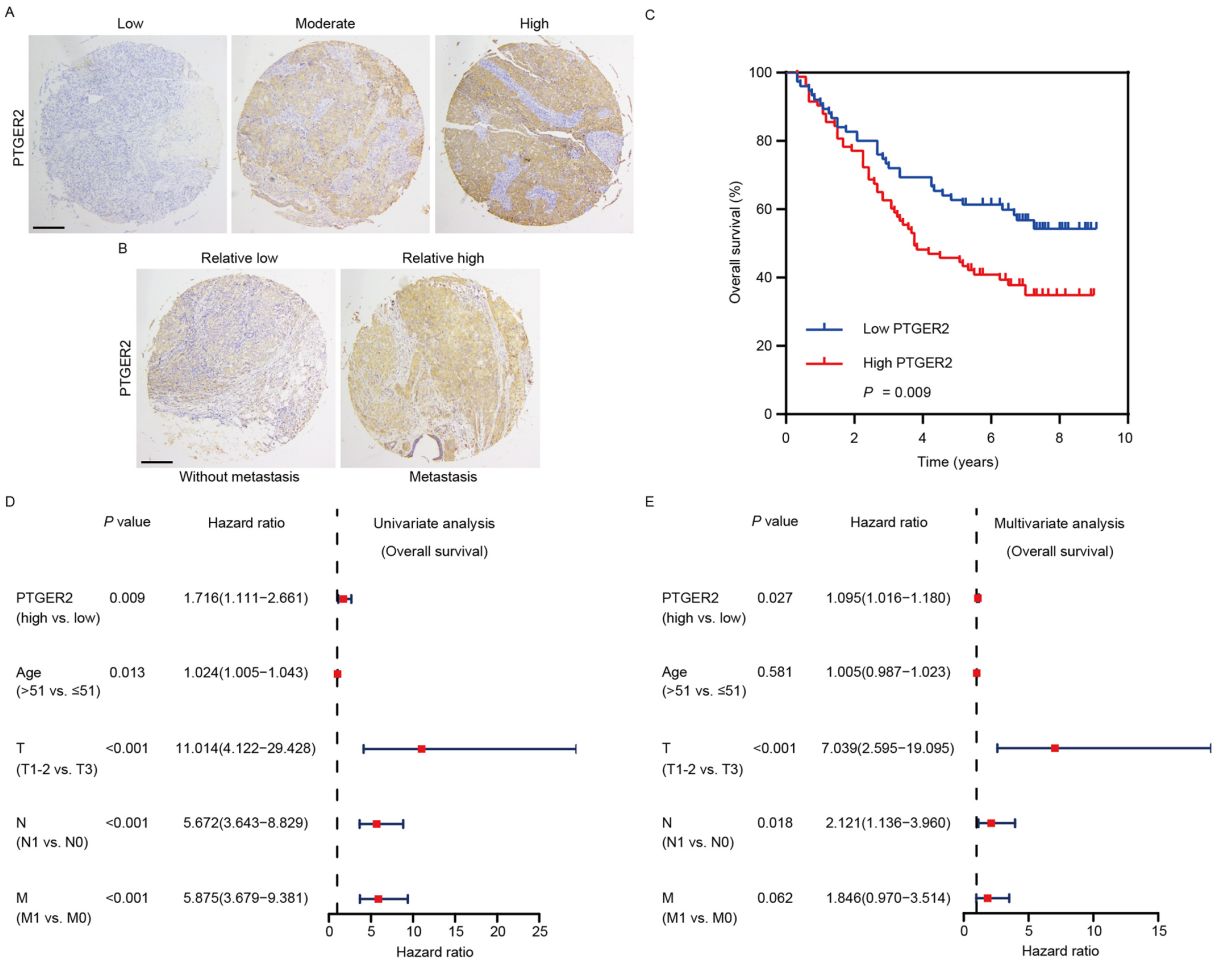

Fig. 4. PTGER2 expression predicts the prognosis of ovarian cancer patients. (A) Images representing the differential PTGER2 expression in ovarian cancer tissues (scale bar: 125 μm). (B) Images representing the differential PTGER2 expression between ovarian cancer patients with or without distant metastasis (scale bar: 125 μm). (C) Kaplan-Meier curve displaying the overall survival of patients with ovarian cancer based on PTGER2 protein levels. (D) Univariate analysis was used to correlate PTGER2 protein levels, clinicopathological parameters, and overall survival of patients with ovarian cancer. (E) Multivariate analysis was conducted to correlate PTGER2 protein levels, clinicopathological parameters, and overall survival of patients with ovarian cancer.

are responsible for proliferation and metastasis and are regarded as contributors to poor prognosis of patients with ovarian cancer [19]. m^{6}A modification plays a key role in cancer development and progression by regulating many biological and pathological processes including cell stemness, drug resistance, proliferation, and metastasis [22]. In this investigation, notable links between m^{6}A modification and ovarian cancer progression were identified using bioinformatics analyses. In addition, our study suggested that METTL3-mediated m^{6}A RNA methylation modulated the expression of PTGER2, which subsequently promoted ovarian cancer stemness, chemoresistance, proliferation, and metastasis by affecting the expression of associated key regulators. Importantly, high PTGER2 expression predicted the poor overall survival of patients with ovarian cancer.

Distinct m^{6}A modification patterns can affect the prognosis of cancer patients [7]. In this work, we conducted bioinformatics analyses and established two m^{6}A modification patterns according to 23 CNV-harboring m^{6}A regulators. The expression of 23 m^{6}A regulators was affected by DNA CNV, suggesting the significance of their gene expression in cancer progression [23]. The aberrant expression of these m^{6}A regulators together with their prognostic role in ovarian cancer further revealed the importance of m^{6}A modification in cancer progression. Based on the vital value of m^{6}A modification in ovarian cancer, we further identified PTGER2 as an oncogene potentially modified by m^{6}A modification in a subsequent investigation.

Previous studies have shown that METTL3 promotes ovarian cancer proliferation, migration, and invasion [24, 25]. In addition, METTL3 is an inducer of cancer stem cell self-renewal and chemoresistance in human cancers [26,27]. In this work, the regulatory impact of m^{6}A RNA methylation on the expression of PTGER2, which served as a promoter of ovarian cancer stemness, chemoresistance,

proliferation, and metastasis, was experimentally verified by the observation that METTL3 enhanced PTGER2 expression through m^{6}A modification, further suggesting the oncogenic role of METTL3 in ovarian cancer. Comprehensive analysis has suggested that METTL3 mainly regulates m^{6}A RNA methylation in the CDS region, stop codon, and 3' UTR of its targets [28]. In this investigation, we also found that METTL3 modulated m^{6}A RNA methylation of PTGER2 in the 3' UTR region, thus enhancing PTGER2 expression. However, some experts and scholars have proposed that m^{6}A-sensitive PCR and MeRIP mapping of specific mRNA do not provide definitive evidence of m^{6}A. Therefore, additional studies are needed to confirm the m^{6}A RNA methylation of PTGER2 mRNA.

PTGER2 plays dual roles as an oncogene or a tumor suppressor in human cancers [13,15,16]. PTGER2 facilitates cancer stemness, chemoresistance, proliferation, and metastasis [14,29]. In the present work, we also revealed the role of PTGER2 in promoting ovarian cancer stemness, chemoresistance, proliferation, and metastasis. Cancer stem cell self-renewal properties contribute to regulating the EMT process and DNA damage repair [30]. The facilitation of cancer chemoresistance can be regulated by several mechanisms, including modulation of cancer stemness, DNA damage repair, and the EMT [31]. In our investigation, we further discovered that PTGER2 increased cancer stem cell self-renewal properties, the EMT, and DNA damage repair to enhance cell stemness, resistance to carboplatin, proliferation, and metastasis, thus potentiating ovarian cancer progression. Moreover, bioinformatics analysis of TCGA ovarian cancer samples and immunohistochemical assays in our collected clinical samples confirmed that PTGER2 functioned as an oncogene and was correlated with ovarian cancer distant metastasis. Importantly, PTGER2 expression was identified as an independent and unfavorable factor in patient tissues, further supporting the conclusions drawn from ovarian cancer cells.

5. Conclusions

Overall, our study provides a new regulatory axis consisting of METTL3 and PTGER2 in the modulation of ovarian cancer progression. METTL3-mediated m^{6}A RNA methylation of PTGER2 is responsible for the oncogenic role of PTGER2 in ovarian cancer. This work delineates the role of m^{6}A RNA methylation in a specific network of signal transduction and highlights the foundation for the clinical translation of PTGER2 both as a prognostic predictor and as a novel target for the management of ovarian cancer.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

YL and BX conceived and designed the present study. YL wrote the manuscript. BX checked and revised the manuscript. Both authors contributed to the article and approved the submitted version. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Patient written consent and ethics approval from the Ethics Committee of the hospital were obtained (K2020-036-01).

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2809199.

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