Expression analysis and potential validity of PRMT3, IGF2BP1 in adrenocortical carcinoma and their correlation with FOXM1

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Background
PRMT3 and IGF2BP1 overexpression has been detected in several malignancies and has been recognized by their oncogenic role. No studies have handled the expression of PRMT3 and IGF2BP1 in adrenocortical carcinoma (ACC) and their association with outcome. This work aimed at exploring the significance of PRMT3 and IGF2BP1 expression in ACC and their correlation with FOXM1 expression.

Patients and methods
Immunohistochemistry accessed quantitative expression of PRMT3, IGF2BP1, and FOXM1 proteins in 60 cases of ACC and 20 cases of adrenocortical adenoma. IGF2BP1 mRNA and PRMT3 mRNA were also evaluated using a quantitative real-time PCR.

Results
PRMT3, IGF2BP1, and FOXM1 expression were statistically highly significant in ACC compared to adrenocortical adenoma (P<0.001). PRMT3 high expression showed a significant correlation with ENSAT stage (P<0.001), distant metastasis (P<0.001), recurrence (P<0.001), Weiss score, and Ki-67 index (P<0.05). IGF2BP1 high expression showed a significant correlation with ENSAT stage (P<0.001), Weiss score (P<0.001), recurrence (P<0.001), distant metastasis (P<0.05), and Ki-67 index (P<0.05). PRMT3 and IGF2BP1 showed a high significant correlation with FOXM1 (P<0.001).

Conclusion
PRMT3, IGF2BP1, and FOXM1 are upregulated in ACC. PRMT3, IGF2BP1, and FOXM1 contribute to aggressive phenotypes of ACC and might serve as poor potential prognostic factors.

Keywords:
adrenocortical carcinoma, FOXM1, IGF2BP1, PRMT3

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Introduction

Adrenocortical carcinoma (ACC) is considered the most common primary malignant disease in the adrenal gland. It is the second-most frequent cancer of an endocrine origin behind anaplastic thyroid carcinoma (Lam, 2021). Due to the aggressive nature and recurrence tendency, the prognosis is generally poor. The overall 5-year survival rate is approximately under 35%. Currently, curative treatment is limited to complete surgical resection in addition to Mitotane, the only adrenolytic drug therapy. Therefore, the establishment of new prognostic factors and innovative therapeutic strategies is mandatory in ACC (Basile *et al.*, 2021).

Posttranslational modifications (PTMs) of proteins are essential for the maintenance of basic cellular functions. PTMs are mediated by a number of enzymatic processes, such as phosphorylation, methylation, ubiquitination, and acetylation. Among these PTMs, arginine methylation has been accentuated as a fundamental regulatory mechanism (Ramazi and Zahiri, 2021).

The process of methylation of arginine residues in proteins by protein arginine methyltransferases (PRMTs) is an vital modification modulating miscellaneous cell processes such as signal transduction, gene transcription, DNA repair, and messenger RNA processing (Hartley and Lu, 2020). The family of PRMTs is considered the main "writer" of arginine methylation. A developing range defined the significance of PRMTs in multiple diseases, particularly in the progression of cancers (Al-Hamashi *et al.*, 2020).

The PRMT3 is documented as type-I PRMT. Its C-terminal region comprises a catalytic domain with methyltransferase activity (Min *et al.*, 2019). PRMT3 has been shown to be involved in the management of several pathological processes. PRMT3 overexpression was detected in various malignancies such as hepatocellular

carcinoma and colorectal carcinoma, and is associated with poor clinical outcomes (Wang *et al.*, 2019).

Insulin-like growth factor 2 mRNA-binding (IGF2BP1) is a oncofetal protein that is upregulated in many cancers. IGF2BP1 promotes the expression of oncogenes like MYC and LIN28B by impairing the decay of their mRNA (Haase *et al.*, 2021). IGF2BP1 functions as a posttranscriptional regulator of some essential mRNAs, noncoding RNAs, or miRNAs, which are critical for control of cancer cell proliferation, invasion, and chemoresistance. IGF2BP1 is associated with metastasis and poor survival in several types of cancers (Biegel *et al.*, 2021).

Forkhead Box M1 (FOXM1) is considered a member of the Forkhead box family. FOXM1 is mainly responsible for growth and maturation during embryogenesis as well as homeostasis and repair of adult tissues in normal cells (Huang *et al.*, 2019). Meanwhile, it acts as an oncogene that affects cell migration, invasion, epithelial–mesenchymal transition, recruitment of tumor-associated macrophages, angiogenesis, stem cell renewal, DNA damage repair, cellular senescence, and drug resistance (Chen *et al.*, 2023).

The expression and prognostic potential of PRMT3 and IGF2BP1 have not yet been studied in ACC. This study aimed to assess PRMT3 and IGF2BP1 expression in ACC by quantitative real-time PCR and immunohistochemistry and to correlate both markers with different clinicopathological factors besides their correlation with the classic oncogene FOXM1.

Patients and methods

Patients

This is a retrospective study carried out on 60 cases of ACC and 20 cases of adrenocortical adenoma. The material included archival formalin-fixed, paraffin-embedded blocks collected from the Pathology Department, Faculty of Medicine, Benha University, processed in the period from 2011 to 2017 clinicopathological data as age, sex, tumor size, functional status, Weiss score, Ki-67 proliferation index, distant metastasis, ENSAT stage, recurrence, and 5 years overall survival were extracted from the pathology reports and medical records after approval by the ethical committee at Faculty of Medicine, Benha University (code number: RC 12-3-2024).

Quantitative real-time RT-reassessment of IGF2BP1 and PRMT3 mRNA

Total RNA was extracted from carcinoma and adenoma tissues using the RNeasy extraction kit (Qiagen, California, USA) according to the manufacturer's protocol. The RNA concentration was measured using a nanodrop spectrophotometer (Biowave II, Berlin, Germany). Then, RNA was reverse transcribed into cDNA using the iScriptc DNA Synthesis Kit (Bio-Rad, Houston, USA). Quantitative PCR was performed using the Ready Mix PCR Reaction Mix kit (iScriptTM One-Step RT-PCR Kit with SYBR Green; Bio-Rad, California, USA). Thermal cycling conditions were: 10 min at 50°C, 5 min at 95°C then 40 cycles, 10 s at 95°C 30 s at 55°C, and 1 min at 55°C using Rotorgene real-time PCR system and the related software for analysis and interpretation (Qiagen, Seoul, South Korea). β-actin was used as a reference gene for internal control. The PCR primer sequences are shown in Table 1. Data were analyzed using the comparative Ct ($2^{-\Delta\Delta CT}$) method.

Histopathological study

Four-micrometer thick sections were stained by conventional hematoxylin and eosin. Each slide was examined by two specialists for:

(1) Confirmation of the previous diagnosis according to the 2022 WHO Classification of adrenal cortical tumors.
(2) Assessment of the histologic type of ACC (conventional, myxoid, and oncocytic).
(3) Assessment of the Weiss score (Mondal *et al.*, 2013). The nine histological parameters evaluated in this system are high nuclear grades, mitotic rate more than 5/50 high power fields, atypical mitotic figures, clear tumor cell cytoplasm (<25% tumor cells), diffuse architecture (>33% of tumor), necrosis, venous invasion, sinusoidal invasion, and capsular invasion. The Weiss score (4–7) is considered low, while the score 6–9 is considered high.
(4) Assessment of tumor stage according to the European Network for the Study of Adrenal Tumors (ENSAT) (Fassnacht *et al.*, 2010).

Immunohistochemical study

For immunohistochemical (IHC) staining, 10% formalin-fixed, paraffin-embedded, 4-μm tissue sections were prepared and immunostained for the

Table 1 The PCR primer sequences in the study cases

Gene	Forward primer	Reverse primer
PRMT3	5′-TCCTTTGGGGTCCA GCCTTG-3′	5′-ATGCCTGTGGAGTT GGGGCT-3′
IGF2BP1	5′TGCAGCTAGGGATGT GAATCTTC-3′	5′GGAGCCCAGTCCATC AGAACT3′
β–actin	5′GTGACATCCACACC CAGAGG-3′	5′ ACAGGATGTCAAAAC TGCCC-3′

primary antibodies. PRMT3 rabbit monoclonal antibody (1: 100, EPR13279; Abcam, USA). IGF2BP1 rabbit monoclonal antibody (1: 100, Ab82968; Abcam, Cambridge, UK). FOXM1 rabbit monoclonal antibody (1: 100, EPR17379; Abcam). DAP was utilized as a chromogen. IHC staining was performed using a detection kit (Thermoscientific, USA) according to the manufacturer's data. Human cervix carcinoma tissue, human testis tissue, and human colon tissue were used as positive control for PRMT3, IGF2BP1, and FOXM1, respectively. Negative control for all markers was achieved by omitting the primary antibody.

Immunohistochemical assessment

The staining of sections was blindly assessed and scored by two independent pathologists.

(1) PRMT3: PRMT3 expression was cytoplasmic. The staining intensity was graded as 0 (negative), 1 (weak), 2 (medium), and 3 (strong). The percentage of positive cells was scored from 0 to 4 (0, 1–25, 26–50, 51–75, 76–100%). The total score was between 0 and 12 by multiplying these two scores. The classification of each sample as low (0–3) staining or high (4–12) staining was determined (Lei *et al.*, 2022).
(2) IGF2BP1: IGF2BP1 expression was cytoplasmic. The percentage and intensity of the stained cells were taken into consideration while determining the final score. Grades for staining intensity were 0 (no staining), 1 (weak staining), 2, and 3 (strong staining). The percentages were assessed as 1 (≤25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The final scores were calculated by multiplying these two scores. For statistical purposes, a score less than or equal to 6 was regarded as low expression, and more than 6 as high expression (Kuai *et al.*, 2021).
(3) *FOXM1: FOXM1* expression was unclear. IHC evaluation depends on a semiquantitative scoring system calculated by multiplying the intensity by the percentage of stained cells. Grades for staining intensity were (negative as 0, mild as 1, moderate as 2, and strong as 3) percentage of stained cells graded as 0: absent, 1: 1–25%, 2: 26–50%, 3: 51–75%, and 4: 76–100%. Scores of multiplication were graded as follows: –: 0, +: 1–3, ++: 4–8, and +++: 9–12. For statistical analysis, the – and 1+ scores were considered the low-expression group, and the 2+ and 3+ cases were considered the high-expression group (Soliman *et al.*, 2019).
(4) The Ki-67 index retrieved from pathology records was also categorized into low (<20%) and high (≥20%).

Statistical analysis

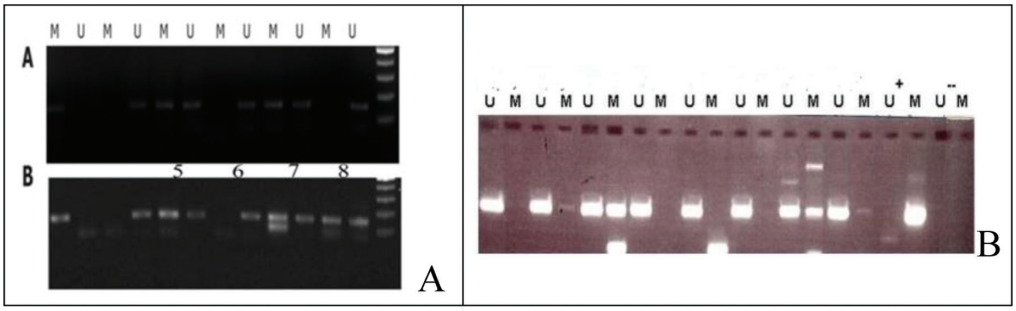
The collected data were tabulated and analyzed using SPSS, version 16 software (SPSS Inc., Chicago, Illinois, USA). Categorical data were presented as numbers and percentages, using χ^2) or Fisher's exact test for their analysis. Continuous data were expressed as mean±SD, median, IQR, and range. Data were tested for normality using the Shapiro–Wilks test, assuming normality at P value more than 0.05. Differences between the two groups were tested using the Student t test for normally distributed variables or Mann–Whitney U (Z_{MWU}) test for nonparametric ones. Differences among three groups were tested by the Kruskal–Wallis test. Spearman's correlation coefficient (rho) was used to assess correlations among nonparametric variables. Receiver operating characteristic curve analysis was used to detect cutoff values for the studied markers with optimum sensitivity and specificity in the early diagnosis of the cancerous group. The accepted level of significance in this work was stated at 0.05 (P<0.05 was considered significant).

Results

RT-PCR results

The mRNA level of PRMT3 and IGF2BP1 was measured by qRT-PCR in 60 cases of ACC and 20 cases of adrenocortical adenoma.

Figure 1



(a) Semiquantitative RT-PCR showing PRMT3 mRNA expression in adrenocortical carcinoma. (b) Semiquantitative RT-PCR showing IGF2BP1 mRNA expression in adrenocortical carcinoma.

The PRMT3 mRNA expression level was detected in 45 (75%) of 60 of ACC compared with three (15%) of 20 of adrenocortical adenoma with high significant difference (P <0.001) (Fig. 1a).

The IGF2BP1 mRNA expression level was detected in 42 (70%) of 60 of ACC compared with two (10%) of 20 adrenocortical adenoma with a high significant difference (P <0.001) (Fig. 1b).

Clinicopathological results

The present retrospective study included 60 cases of ACC and 20 cases of adrenocortical adenoma. Twenty-five (36.7%) ACC cases had distant metastasis, which was confirmed at the initial diagnosis (synchronous metastasis) in 10 cases. The clinicopathological data of ACC cases were summarized in Table 2.

Immunohistochemical results

PRMT3, IGF2BP1, and FOXM1 expression in study cases

By immunohistochemistry, the levels of PRMT3, IGF2BP1, and FOXM1 were significantly higher in ACC than in adrenocortical adenoma (P<0.001), as shown in Table 3.

Receiver operating characteristic curve for the validity of the studied markers in diagnosis of adrenocortical carcinoma

Regarding sensitivity, PRMT3 is more sensitive than IGF2BP1 and FOXM1 in the diagnosis of ACC. However, FOXM1 is more specific than PRMT3 and IGF2BP1, as shown in Table 4.

Correlation PRMT3 with clinicopathological variables in adrenocortical carcinoma cases

PRMT3 high expression showed a high significant correlation with ENSAT stage, distant metastasis, and recurrence (P<0.001). PRMT3 high expression showed a significant correlation with Weiss score and Ki-67 index (P<0.05). There were no statistical differences between PRMT3 expression and age, sex, tumor size, histologic type, and functioning tumors, as detailed in Table 5, Fig. 2.

Correlation of IGF2BP1 with clinicopathological variables in adrenocortical carcinoma cases

IGF2BP1 high expression showed a high significant correlation with ENSAT stage, Weiss score, and recurrence (P<0.001). IGF2BP1 high expression showed a significant correlation with distant metastasis and Ki-67 index (P<0.05).

There were no statistical differences between IGF2BP1 expression and age, sex, histologic type, tumor size, and functional status, as detailed in Table 6 and Fig. 2.

Table 2 Clinicopathological data of the adrenocortical carcinoma study cases

Study groups	*n* (%)
Age	
Mean±SD (range)	48.56±17.83 (5–71)
Sex	
Male	27 (45)
Female	33 (55)
Histologic type	
Conventional	44 (73.4)
Oncocytic	8 (13.3)
Myxoid	8 (13.3)
Functional status	
Functioning	33 (46.7)
Nonfunctioning	27 (53.3)
Tumor size	
<5 cm	31 (51.7)
≥5	29 (48.3)
Ki-67 proliferation index	
Low (<20%))	27 (45)
High	33 (55)
Weiss score	
Low (1–4)	21 (35)
High (5–9)	39 (65)
ENSAT stage	
Stage I	8 (13.3)
Stage II	15 (25)
Stage III	22 (36.7)
Stage IV	15 (25)
Distant metastasis	
Positive	25 (36.7)
Negative	35 (63.3)
Recurrence	
Present	33 (38.3)
Absent	27 (61.7)
Outcome within 5 years	
Alive	28 (46.7)
Dead of disease	32 (53.3)
Total	60 (100)

Correlation of FOXM1 with clinicopathological variables in adrenocortical carcinoma cases

FOXM1 high expression showed a high significant correlation with ENSAT stage, recurrence, and distant metastasis (P<0.001), as detailed in Tables 6 and 7, Fig. 2.

Correlation between PRMT3, IGF2BP1 and FOXM1 the studied adrenocortical carcinoma cases

A positive high statistical significant correlation between PRMT3 and IGF2BP1 (rho=0.933) (P<0.001). A positive high statistically significant correlation between PRMT3 and FOXM1(rho=0.826) (P<0.001). A positive high statistically significant correlation between IGF2BP1 and FOXM1 (rho=0.759) (P<0.001).

Kaplan–Meier survival analysis

Kaplan–Meier survival analysis showed that the 5-year overall survival rate in the group of patients with a low

Table 3 PRMT3, IGF2BP1, and FOXM1 immunostaining in studied cases

Markers	Adrenocortical adenoma (*N*=20) [*n* (%)]		Adrenocortical carcinoma (*N*=60) [*n* (%)]		Test of significance	*P*
PRMT3						
Low	16	80	23	38.3	28.2	<0.001 (HS)**
High	4	20	37	61.7		
IGF2BP1						
Low	15	75	25	41.7	33.9	<0.001 (HS)**
High	5	25	35	58.3		
FOXM1						
Low	17	85	24	40	30.8	<0.001 (HS)**
High	3	15	36	60		

**Correlation is highly significant at the 0.01 level (two-tailed).

Table 4 Receiver operating characteristic curve results

Marker	Sensitivity %	Specificity %	PPV%	NPV%	Accuracy%	AUC	*P* value
PRMT3	85.4	86.8	83.3	85	86.1	0.896	<0.001 (HS)
IGF2BP1	80.5	82.9	89.2	82.2	85.4	0.882	<0.001 (HS)
FOXM1	82.7	90.2	90.5	915	91.5	0.915	<0.001 (HS)

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

expression level of PRMT3, IGF2BP1, and FOXM1 was significantly longer than that for patients with a high level of PRMT3, IGF2BP1, and FOXM1 immunoreactivity (*P*<0.001) (Fig. 3).

Discussion

ACC is an endocrine neoplasm characterized by an extremely aggressive disease and poor outcomes. ACC has a 5-year survival rate of less than 15% and accounts for 0.2% of all cancer-related deaths. Surgical resection is considered the first-line treatment for ACC; however, almost 50% of patients develop recurrence or metastasis (Miele *et al.*, 2020) (Figs 4–7).

The factors involved in ACC progression and metastasis remain unclear. Identifying the progression elements and prognostic factors of ACC remains a research focus and a challenge (Paragliola *et al.*, 2020). The relevant roles and mechanisms of PRMT3 and IGF2BP1 in ACC progression have not yet been studied. The current research is the first to analyze PRMT3 and IGF2BP1 expression and their prognostic value in ACC.

The present study aims to detect IHC expression of PRMT3, IGF2BP1, and FOXM1 and gene expression of PRMT3, IGF2BP1 in ACC and adenoma and their relevance to the various clinicopathological features of ACC. Besides, the study revealed the correlation between PRMT3, IGF2BP1, and FOXM1.

Involvement of PRMT3 in the development of different diseases, particularly in cancer progression, has obtained interest. Accumulating evidence has indicated the oncogenic role of PRMT3 through gene regulation in cancer cells in many types of tumors (Xu and Richard, 2021). However, the role of PRMT3 in ACC remains unidentified.

The current study demonstrated PRMT3 expression at the mRNA level using the qRT-PCR technique and at the protein level using IHC. PRMT3 expression was statistically highly significant in ACC compared to adrenocortical adenoma (*P*<0.001). The PRMT3 mRNA expression level was detected in 75% of ACC compared with 15% of adrenocortical adenoma with a high significant difference (*P* <0.001). This suggests that PRMT3 might act as an oncoprotein and may have a role in the carcinogenesis of ACC.

In addition, the present work showed that the overexpression of PRMT3 was significantly correlated with ENSAT stage (*P*<0.001), distant metastasis (*P*<0.001), recurrence (*P*<0.001), Weiss score (*P*<0.05), and Ki-67 index (*P*<0.05) in ACC. The Kaplan–Meier curve demonstrated that high PRMT3 expression was significantly correlated with poor survival. All this gives an impression of the potential role of PRMT3 in the aggressiveness and progression of ACC.

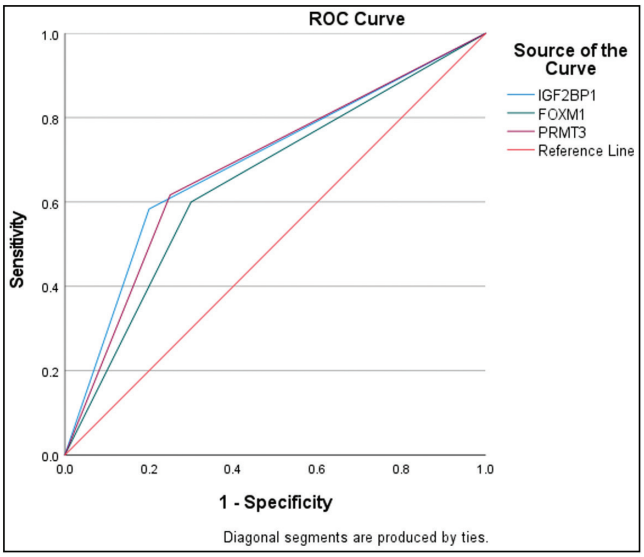
There are many studies that match our results but in other types of cancer. Zhang *et al.* (2021) published that PRMT3 was highly expressed in colorectal cancer, and the expression level was correlated with the overall survival of patients. Moreover, analysis showed that PRTM3 modulates the HIF1/VEGFA signaling pathway by stabilizing HIF1α.

Table 5 Correlation PRMT3 with clinicopathological variables in adrenocortical carcinoma cases

Variables	*N*	PRMT3 [*n* (%)]		*P* value
		Low	High	
Age (years) (mean±SD)	60	53.4±13.6	53.6±12.7	0.68 (NS)
Sex				
Male	27	9 (33.3)	18 (66.7)	0.480 (NS)
Female	33	14 (42.4)	19 (57.6)	
Histologic type				
Conventional	44	18 (40.9)	26 (59.1)	0.106 (NS)
Oncocytic	8	3 (37.5)	5 (62.5)	
Myxoid	8	2 (25.0)	6 (75.0)	
Tumor size				
<5 cm	31	13 (41.9)	18 (58.1)	0.561 (NS)
≥5	29	10 (34.5)	19 (65.5)	
Functional status				
Functioning	33	15 (45.5)	18 (54.5)	0.216 (NS)
Nonfunctioning	27	8 (29.6)	19 (70.4)	
Ki-67 proliferation index				
Low	27	15 (55.6)	12 (44.4)	<0.05 (S)
High	33	8 (24.2)	25 (75.8)	
Weiss score				
Low	21	12 (57.1)	9 (42.9)	<0.05 (S)
High	39	11 (28.2)	28 (71.8)	
ENSAT stage				
Stage I	8	7 (87.5)	1 (12.5)	<0.001 (HS)
Stage II	15	11 (73.3)	4 (26.7)	
Stage III	22	4 (18.2)	18 (81.8)	
Stage IV	15	1 (6.7)	14 (93.3)	
Distant metastasis				
Positive	25	4 (16)	21 (84)	<0.001 (HS)
Negative	35	19 (54.3)	16 (45.7)	
Recurrence				
Present	27	2 (7.4)	25 (92.6)	<0.001 (HS)
Absent	33	21 (63.6)	12 (36.4)	
Total	60	23 (38.3)	37 (61.7)	

HS, highly significant; NS, nonsignificant; S, significant.

Figure 2



(a) Moderate PRMT3 cytoplasmic expression in adrenocortical carcinoma (immunohistochemical ×400). (b) High strong PRMT3 cytoplasmic expression in adrenocortical carcinoma (immunohistochemical ×400).

Lei *et al.* (2022) explained that upregulation of PRMT3 is significant in HCC, and high expression correlates with poor prognosis. Furthermore, PRMT3 overexpression improves the glycolysis in hepatocellular carcinoma cells, thus promoting tumor cell proliferation by enhancing arginine methylation of lactate dehydrogenase A and metabolic reprogramming.

Liao *et al.* (2022) demonstrated that PRMT3 is highly enriched in glioblastoma multiform and that it promotes the progression of glioblastoma multiforme through enhancing HIF1A and metabolic glycolysis signaling. Hu *et al.* (2021) detected that PRMT3 stabilizes C-MYC in colorectal carcinoma and that PRMT3 function in tumorigenesis was dependent on C-MYC.

In addition, PRMT3 overexpression has been approved to be established in prostate cancer (Grypari *et al.*, 2023) and breast cancer (Zhi *et al.*, 2023) and is linked to poor clinical outcomes. It was discovered

Table 6 Correlation of IGF2BP1 with clinicopathological variables in adrenocortical carcinoma

Variables	*N*	IGF2BP1 [*n* (%)]		*P* value
		Low	High	
Age (years) (mean±SD)	60	54.4±12.7	54.8±18.2	0.540 (NS)
Sex				
Male	27	11 (40.7)	16 (59.3)	0.897 (NS)
Female	33	14 (42.4)	19 (57.6)	
Histologic type				
Conventional	44	20 (45.5)	24 (54.5)	0.277 (NS)
Oncocytic	8	3 (37.5)	5 (62.5)	
Myxoid	8	2 (25.0)	6 (75.0)	
Tumor size				
<5 cm	31	14 (45.2)	17 (54.8)	0.578 (NS)
≥5	29	11 (37.9)	18 (62.1)	
Functional status				
Functioning	33	17 (51.5)	16 (48.5)	0.090 (NS)
Nonfunctioning	27	8 (29.6)	19 (70.4)	
Ki-67 proliferation index				
Low	27	16 (59.3)	11 (40.7)	<0.05 (S)
High	33	9 (27.3)	24 (72.7)	
Weiss score				
Low	21	14 (66.7)	7 (33.3)	<0.001 (HS)
High	39	11 (28.2)	28 (71.8)	
ENSAT stage				
Stage I	8	6 (75)	2 (25)	<0.001 (HS)
Stage II	15	10 (66.7)	5 (33.3)	
Stage III	22	8 (36.4)	14 (63.6)	
Stage IV	15	2 (13.3)	13 (86.7)	
Distant metastasis				
Positive	25	7 (28)	18 (72)	<0.05 (S)
Negative	35	20 (57.1)	15 (42.9)	
Recurrence				
Present	27	5 (18.5)	22 (81.5)	<0.001 (HS)
Absent	33	20 (60.6)	13 (39.4)	
Total	60	25 (41.7)	35 (58.3)	

HS, highly significant; NS, nonsignificant; S, significant.

Table 7 Correlation of FOXM1 with clinicopathological variables in adrenocortical carcinoma cases

Variables	*N*	FOXM1 [*n* (%)]		*P* value
		Low	High	
Age (years) (mean±SD)	60	52.4±13.7	52.8±19.2	0.940 (NS)
Sex				
Male	27	9 (33.3)	18 (66.7)	0.349 (NS)
Female	33	15 (45.5)	18 (54.5)	
Histologic type				
Conventional	44	19 (54.5)	25 (56.8)	0.344 (NS)
Oncocytic	8	3 (37.5)	5 (62.5)	
Myxoid	8	2 (25.0)	6 (75.0)	
Tumor size				
<5 cm	31	12 (38.7)	19 (61.3)	0.836 (NS)
≥5	29	12 (41.4)	17 (58.6)	
Functional status				
Functioning	33	16 (48.5)	17 (51.5)	0.143 (NS)
Nonfunctioning	27	8 (29.6)	19 (70.4)	
Ki-67 proliferation index				
Low	27	15 (55.6)	12 (44.4)	<0.05 (S)
High	33	9 (27.3)	24 (72.7)	
Weiss score				
Low	21	10 (47.6)	11 (52.4)	0.385 (NS)
High	39	14 (35.9)	25 (64.1)	
ENSAT stage				
Stage I	8	5 (62.5)	3 (37.5)	<0.001 (HS)
Stage II	15	10 (66.7)	5 (33.3)	
Stage III	22	8 (36.4)	14 (63.6)	
Stage IV	15	1 (6.7)	14 (93.3)	
Distant metastasis				
Positive	25	3 (12.0)	22 (88.0)	<0.001 (HS)
Negative	35	21 (60.0)	14 (40.0)	
Recurrence				
Present	27	3 (11.1)	24 (88.9)	<0.001 (HS)
Absent	33	21 (63.6)	12 (36.4)	
Total	60	24 (40.0)	36 (60.0)	

that PRMT3 regulates tumors' chemoresistance. Gemcitabine-resistant pancreatic cancer cells had an elevated expression of PRMT3, according to Hsu *et al.* (2018).

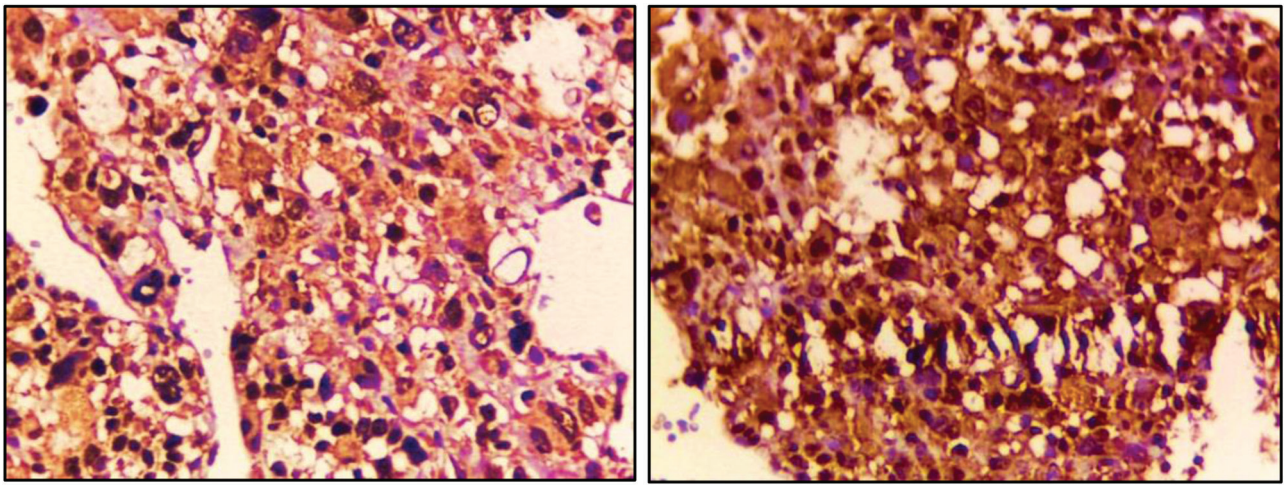
In brief, the promoting role of PRMT3 in cancer can be elucidated by many functions. Accumulating evidence has indicated the oncogenic role of PRMT3 through gene regulation in cancer cells. In addition, all recent studies point to the potential role of PRMT3 in metabolic reprogramming, enhanced glycolysis, chemoresistance, and immune evasion. Understanding of PRMT3-mediated tumorigenesis is still an area of research.

IGF2BP1 is considered an RNA-binding protein acting as a posttranscriptional modulator that promotes the expression of pro-oncogenic cancer-related mRNA targets. IGF2BP1 overexpression is significantly associated with poor prognosis in a variety of cancers (Dhamdhere *et al.*, 2023). However, no scientific studies have investigated the role of IGF2BP1 in ACC.

The current study demonstrated IGF2BP1 expression at mRNA level using qRT-PCR technique and protein level using IHC was statistically highly significant in ACC compared to adrenocortical adenoma (P<0.001). The IGF2BP1 mRNA expression level was detected in 70% of ACC compared with 10% of adrenocortical adenoma with a high significant difference (P <0.001). This may give an impression about the oncogenic role of IGF2BP1 in the carcinogenesis of ACC.

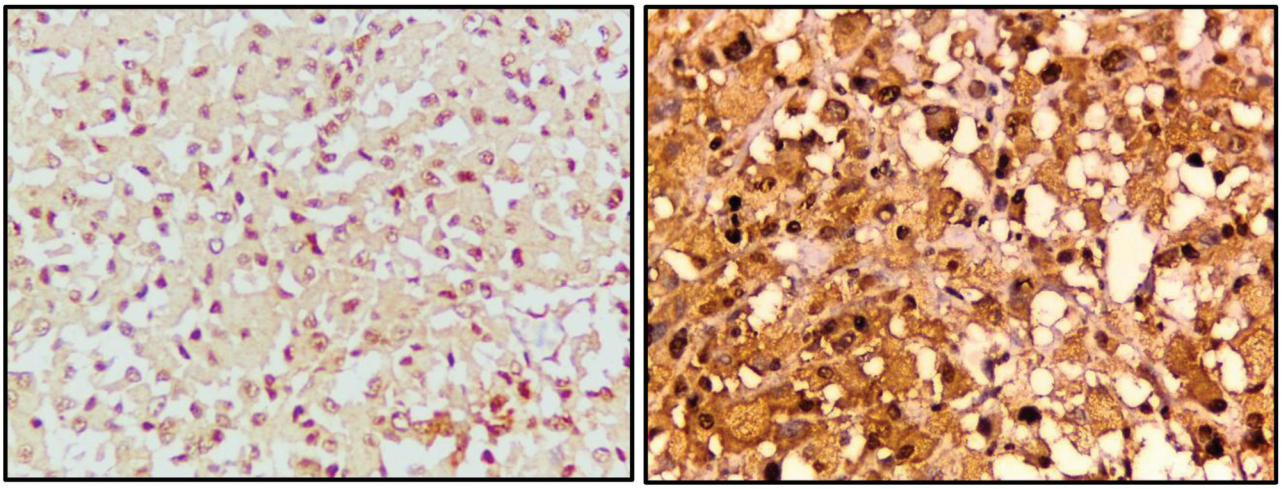
Furthermore, the present work pointed to that the overexpression of IGF2BP1 was significantly correlated with ENSAT stage (P<0.001), recurrence (P<0.001), Weiss score (P<0.001), distant metastasis (P<0.05), and Ki-67 index (P<0.05) in ACC. The Kaplan–Meier curve demonstrated that high IGF2BP1 expression was correlated significantly with poor survival. This

Figure 3



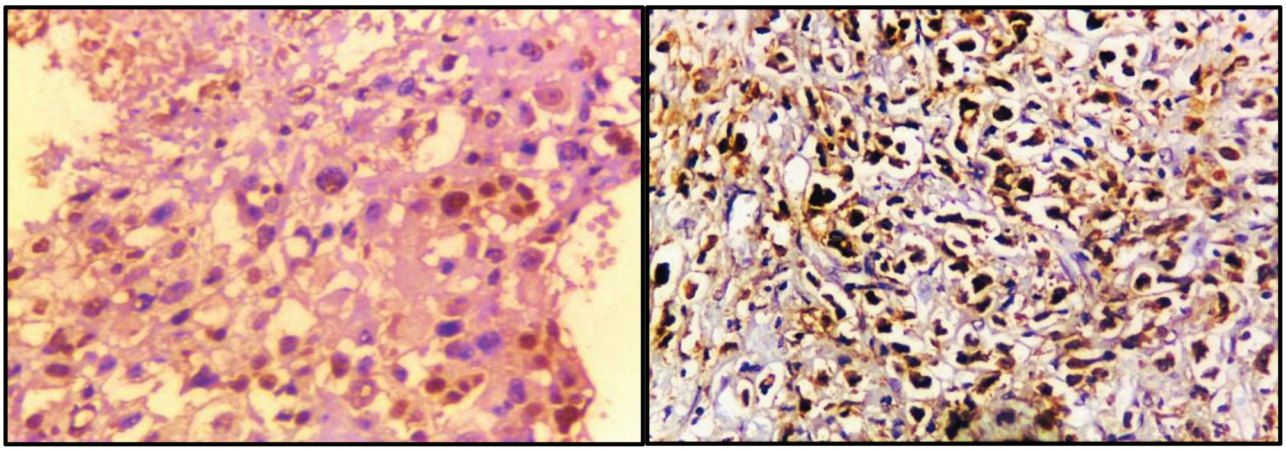
The receiver operating characteristics curve for the validity of PRMT3, IGF2BP1, and FOXM1 to predict early diagnosis of adrenocortical carcinoma.

Figure 4



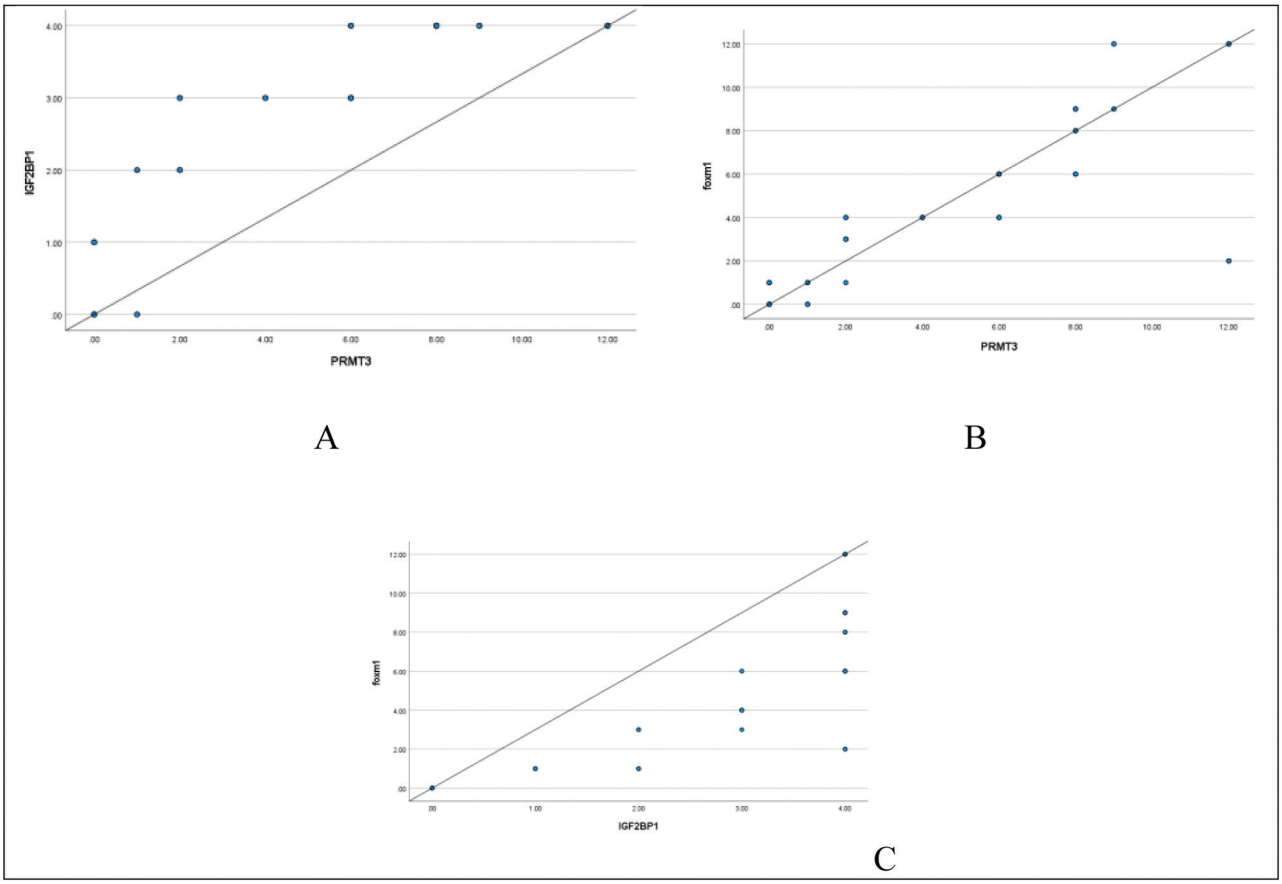
(a) Low IGF2BP1 cytoplasmic expression in adrenocortical carcinoma (immunohistochemical ×400). (b) High IGF2BP1 cytoplasmic expression in adrenocortical carcinoma (immunohistochemical ×400).

Figure 5



(a) Low FOXM1 nuclear expression in adrenocortical carcinoma (immunohistochemical ×200). (b) High FOXM1 nuclear expression in adrenocortical carcinoma (immunohistochemical ×200).

Figure 6



(a) Scatter graph showing a significant positive correlation between PRMT3 and IGF2BP1 scores. (b) Scatter graph showing a significant positive correlation between PRMT3 and FOXM1 scores. (c) Scatter graph showing a significant positive correlation between IGF2BP1 and FOXM1 scores.

means that IGF2BP1 expression is associated with poor prognostic features, suggesting its potential role as an independent prognostic factor in ACC.

Our results match those of other studies performed on different types of cancer. Chen *et al.* (2021) reported that IGF2BP1 was upregulated in colorectal carcinoma and was correlated with advanced-stage, liver metastasis, chemoresistance, and poor survival.

Many studies within the literature confirm the essential role of IGF2BP1 in promoting distinct cancer pathways, including KRAS-driven signaling in lung adenocarcinoma (Rosenfeld *et al.*, 2019) as well as MYC/MYCN-driven gene expression in liver cancer (Huang *et al.*, 2018). Moreover, Müller *et al.* (2020) demonstrated that IGF2BP1 promotes the E2F-driven gene responsible for the progression of the cancer cell cycle.

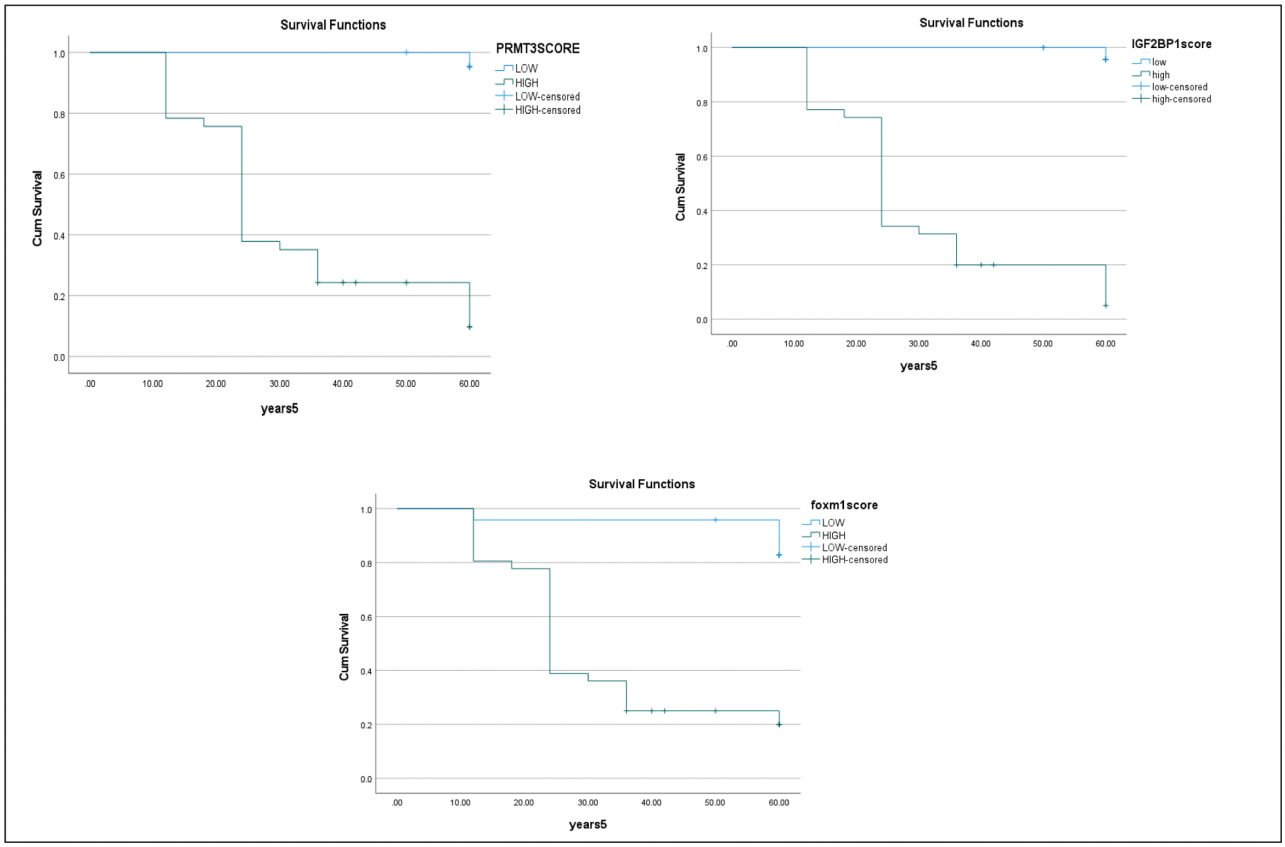
Luo and Lin (2022) stated that IGF2BP1 is overexpressed in gastric carcinoma and serves as a predictor of poor outcomes. IGF2BP1 directly interacted with c-MYC mRNA in an m6A-dependent manner. Furthermore, IGF2BP1 promoted aerobic glycolysis and cancer cell migration of gastric carcinoma cells.

Shi *et al.* (2023a, 2023b) pointed out that overexpression of the IGF2BP1 was clinically correlated with metastasis in breast cancer patients. Moreover, IGF2BP1 promoted distant metastasis *in vitro* and *in vivo*. Zhang *et al.* (2020) declared that IGF2BP1 was overexpressed in NSCLC cells, and IGF2BP1 knockdown inhibited cancer cell proliferation, migration, and invasion.

IGF2BP1 has an essential role in embryogenesis, tumorigenesis, and chemoresistance via serving as a posttranscriptional regulator by expression of some essential mRNA targets required for tumor cell growth and proliferation invasion as well as chemotherapy resistance resulting in poor overall survival and metastasis in various types of cancers (Hagemann *et al.*, 2023).

The current study revealed a positive correlation between PRMT3 overexpression and IGF2BP1 overexpression

Figure 7



Kaplan–Meier survival curves of adrenocortical carcinoma patients with low and high expression of PRMT3, IGF2BP1, and FOXM1protein; follow-up period=60 months.

in ACC (P<0.001). Our results may be explained by the study of Shi *et al.* (2023a, 2023b), who declared that IGF2BP1 is considered a key substrate of PRMT3 and that IGF2BP1 methylation is mandatory for PRMT3-mediated chemoresistance in hepatocellular carcinoma. We believe that more research is required to explain the unknown interactions between PRMT3 and IGF2BP1 in carcinogenesis in ACC and other cancers.

To the best of our knowledge, this may be the first study that reported high IGF2BP1 and PRMT3 expression in ACC, in addition to their correlation with poor prognostic factors and poor overall survival in ACC.

FOXM1 is a member of the Forkhead transcription factor family. FOXM1 is a protooncogene transcription factor that strongly contributes to cell cycle progression (Sher *et al.*, 2022).

In the current study, FOXM1 high expression was detected in 65.0% of ACC and 33.3% of adrenocortical adenoma. The difference in FOXM1 expression was statistically highly significant. This may give more indication of the oncogenic role of FOXM1 in ACC.

Moreover, our study revealed that FOXM1 immunoexpression was significantly associated with ENSAT stage (P <0.001), recurrence (P <0.001), distant metastasis (P <0.001), and Ki-67 index (P<0.05).

In fact, limited studies handled FOXM1 expression in ACC. Liang *et al.* (2019) have declared that FOXM1 overexpression was obvious in ACC compared with adrenocortical adenoma and that overexpression of FOXM1 was associated with poor prognostic factors and immune evasion. This is matching our results. Our study might provide further evidence of FOXM1 being a biomarker of aggressiveness and poor prognosis in ACC.

In many previous studies, FOXM1's high expression has been associated with aggressiveness and poor prognostic factors in different types of malignancies.

Liu *et al.* (2021) pointed out that FOXM1 is enriched in ovarian cancer and that FOXM1 promotes critical oncogenic phenotypes in ovarian cancer, as cell proliferation, invasion and metastasis, chemotherapy resistance, cancer stem cell properties, genomic instability, and altered cellular metabolism.

Rather *et al.* (2023) found that FOXM1 was upregulated in cancer colon tissues and that elevated FOXM1 was strongly correlated with larger tumor size, lymph node status, lymphovascular invasion, perineural invasion, lymph node metastasis, late stage, localization, and recurrence.

Madhi *et al.* (2022) stated that FOXM1 has a dual function in controlling lung cancer cell proliferation and immune evasion. FOXM1 knockdown decreased PD-L1 expression, promoted cell death, and downregulated some proliferation-associated proteins, such as cyclin D1, cyclin E1, and MYC.

Concerning the relation between PRMT3 and FOXM1 in the present study, PRMT3 high expression showed a significant correlation with FOXM1 (P<0.001). This may be the first study documenting the relation between PRMT3 and FOXM1 In cancer. This raises the question of the possibility of arginine methylation mediated by PRMT3 in the upregulation of FOXM1. Further studies are required to explain the underlying direct molecular mechanism of FOXM1 regulation by PRMT3.

Zhang *et al.* (2021) found that PRMT3 stabilizes HIF1. In addition, Tang *et al.* (2019) declared that the increase in FOXM1 expression was due to the transcriptional regulation of the FOXM1 promoter by HIF-1α. This may explain the relationship between PRMT3 and FOXM1 through PRMT3–HIF-1α–FOXM1 pathway.

Concerning the relation between IGF2BP1 and FOXM1 in the present study, IGF2BP1's high expression showed a significant correlation with FOXM1 (P<0.001). Similar results had been documented by Huang *et al.* (2019), who declared that IGF2BP1 expression was positively associated with FOXM1 expression in lung adenocarcinoma.

On brief, PRMT3 and IGF2BP1 may be a functional upstream regulator of classical oncogene FOXM1, which further established their critical role in the initiation and development of ACC.

Conclusion

(1) This may be the first study to report high PRMT3 and IGF2BP1 expression in ACC.
(2) High levels of PRMT3, IGF2BP1, and FOXM1 are significantly associated with poor prognostic factors and poor survival rates in ACC.
(3) PRMT3, IGF2BP1, and FOXM1 expression appears to be a novel and independent prognostic marker in ACC, and their identification may detect eligible candidates for targeted therapy.
(4) PRMT3 and IGF2BP1 may be a functional upstream regulator of classical oncogene FOXM1 in ACC.

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Conflicts of interest
There is no conflict of interest.

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