

## ORIGINAL ARTICLE

### CIP2A expression in high grade prostatic intraepithelial neoplasia and prostate adenocarcinoma: a tissue microarray study

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#### Abstract

**Introduction:** CIP2A is an oncoprotein involved in the progression of several human malignancies. It has recently been described as a prognostic marker in many cancers. The present study aimed to investigate the immunohistochemical expression of CIP2A in benign prostatic hyperplasia (BPH), high grade prostatic intraepithelial neoplasia (HGPIN) and prostate cancer (PC), and to analyse the association with the clinicopathological parameters in PC cases to define its role in the development and progression of PC. **Materials and Methods:** Immunohistochemical staining for CIP2A was performed on the tissue microarray sections of 105 PC, 27 HGPIN and 27 BPH tissues. The CIP2A expression scores were compared with several clinicopathological parameters. **Results:** CIP2A was expressed in 96,2% of PC, 55,6% of HGPIN and 40,7% of BPH tissues. The expression of CIP2A in PC was significantly higher than in HGPIN ( $p<0.0001$ ) and BPH ( $p<0.0001$ ) cases. CIP2A expression score was significantly associated with Gleason score ( $p=0.032$ ) and lymphovascular invasion ( $p=0.039$ ). Nevertheless, there was no statistically significant association between the expression of CIP2A and perineural invasion, pT stage, metastasis and recurrence ( $p>0.05$ ). Multivariate analysis indicated that GS, lymphovascular invasion, distant metastasis were independent prognostic factors for PC patients but, CIP2A expression score was not found to be a prognostic factor. Additionally, there was no significant difference between the survival times of patients according to CIP2A expression ( $p=0.174$ ). **Conclusion:** According to our results, the expression of CIP2A protein is increased in PC and its expression may be involved in the development, differentiation, and aggressiveness of PC. However, further studies are needed to confirm our findings and to clarify the role of CIP2A in the development of PC.

**Keywords:** CIP2A; prostate cancer; high grade prostatic intraepithelial neoplasia

#### INTRODUCTION

Prostate cancer (PC) is the second most frequent malignancy and one of the main common causes of cancer-related deaths among men worldwide.<sup>1</sup> Surgical resection, androgen deprivation therapy, radiotherapy are established therapeutic methods; yet mortality and prognosis is still poor in managing advanced stages of PC.<sup>1</sup> Thus, investigation of mechanisms involved in prostate carcinogenesis remain crucial to optimise established therapy modalities and to identify new potential therapeutic targets.<sup>1</sup> Several studies investigated the aetiopathogenesis, prognosis, biological behaviour and therapy of PC but,

the underlying molecular pathways implicated in PC development are still poorly understood.

Cancerous inhibitor of protein phosphatase 2A (CIP2A), also referred as KIAA1524 or p90 tumour-associated antigen, is an oncoprotein that inhibits the degradation of c-Myc by inhibiting the protein phosphatase 2A (PP2A)-mediated dephosphorylation of Myc at serine 62.<sup>2</sup> Hence; stabilised c-Myc levels lead to uncontrolled activation of several oncogenic mechanisms and malignant transformation of human cells.<sup>3-6</sup> In addition to the c-Myc pathway, CIP2A interacts via PP2A inhibition with the signal pathways of PI3K/ Akt/ mTOR/ MAP/ ERK, which play a key

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role in cell proliferation and cell survival.<sup>7,8</sup> Thus, CIP2A plays a crucial role in tumour genesis and progression stimulating cell proliferation, cell renewal, escape from cell ageing and inhibition of apoptosis.<sup>8-10</sup>

Over the past years, numerous studies showed that CIP2A is overexpressed in various human malignancies including head and neck squamous cell carcinoma<sup>9</sup>, colon cancer<sup>11,12</sup>, oesophageal squamous cell carcinoma<sup>13</sup>, oesophageal adenocarcinoma<sup>14</sup>, gastric cancer<sup>15</sup>, renal cell carcinoma<sup>16</sup>, ovarian cancer<sup>17</sup>, breast cancer<sup>18</sup>, non-small cell lung cancer<sup>19</sup>, acute myeloid leukaemia<sup>20</sup>, and PC.<sup>21</sup> It is rarely expressed in non-malignant cells and its physiological function in the adult organism is unclear.<sup>2</sup> Besides, oncogenic factors like helicobacter pylori and papillomavirus 16 E7, have been claimed to upregulate the expression of CIP2A, which could be responsible for their oncogenic activities.<sup>22,23</sup> CIP2A expression has also been found to correlate with tumour aggressivity and poor prognosis in different cancer types.<sup>12,18,24-26</sup> but, the prognostic role of CIP2A in PC remains unknown.

As there are distinctive individual differences in the clinical course and biological behaviour of prostate adenocarcinoma, objective parameters concerning carcinogenesis, tumour behaviour, and biomarkers are needed. To the best of our knowledge, there are two articles in the literature that have investigated immunohistochemical expression of CIP2A in PC.<sup>21,27</sup> Besides, CIP2A expression in high grade prostatic intraepithelial neoplasia (HGPIN), the precursor lesion of PC, and association of CIP2A expression with overall survival, have not been investigated so far. In this study, we aimed to investigate the immunohistochemical expression of CIP2A in PC, HGPIN and BPH tissues in association with clinicopathological parameters to determine the role of CIP2A in the development, differentiation and progression of PC.

## MATERIALS AND METHODS

### *Patients and tissue samples*

This study included samples of 105 primary PC, 27 HGPIN and 27 BPH cases selected from routine archival material of our department of pathology. All of the BPH cases consisted of TUR materials and cases with HGPIN were selected from areas adjacent to tumour in radical prostatectomy materials. In addition to radical prostatectomy materials, TUR materials were included in PC cases to increase the rate

of high-grade tumours. Of these cases, 30 (28.6%) were TUR and 75 (71.4%) were radical prostatectomy materials. The hematoxylin-eosin (H&E) stained slides of the PC cases were re-evaluated histopathologically and their Gleason score (GS) was determined according to the WHO 2016 classification.<sup>28</sup> The presence or absence of vascular and perineural invasion was recorded in radical prostatectomy specimens. The pathological tumour stages (pT) of radical prostatectomy cases were performed according to the TNM system.<sup>1</sup> Data for the follow-up of patients such as metastasis or recurrence were obtained from patients' files and the hospital's electronic data system. None of the patients had received chemotherapy, hormonotherapy or radiotherapy before surgery. BPH tissues were used as a control group. The clinicopathological features of PC cases are summarised in Table 1.

Ethics committee approval was obtained for the study by report no. 90 on 03.05.2017 at the Süleyman Demirel University Medical Faculty Clinical Research Ethics Committee.

### *Immunohistochemistry*

Immunohistochemistry was performed on tissue microarray (TMA) slides of the cases. Briefly, suitable areas were marked on standard H&E stained slides. Two representative 2 mm diameter tissue cores were punched from formalin-fixed paraffin-embedded blocks of each case and inserted into a recipient paraffin block manually. A manual TMA Builder (Labvision, Thermo Fisher Scientific) instrument was used for the construction of these TMAs. The sections with a thickness of 4µm of TMA blocks were used for immunohistochemistry by streptavidin-biotin peroxidase technique for CIP2A. The sections were deparaffinised in xylene and dehydrated in descending dilutions of ethanol. Antigen retrieval was achieved by heat treatment at 98°C in citrate buffer (pH= 6.0) for 20 minutes. The immunostaining was performed using VENTANA BenchMark XT Autostainer (Fremont, CA, USA). Endogenous peroxidase activity was blocked by 20 minutes of incubation with 0.3% hydrogen peroxidase. Slides were tested with CIP2A (mouse monoclonal, Cat. No: SC-HL1925, Santa Cruz Biotechnology, San Diego, CA, 1/100 dilution, 60 minutes of incubation). Sections were tested with a streptavidin-biotin-peroxidase kit (UltraVision Large Volume Detection System Anti-Polyvalent, HRP, LabVision, USA), and after incubation, the reaction product was

**TABLE 1: Clinicopathological features of subjects with prostate cancer**

Clinicopathological features	n(%)
<b>Age (n=105)</b>	
≤60	17 (16.2%)
≥60	88 (83.8%)
<b>Gleason Score (n=105)</b>	
6	21 (20%)
7	38 (36.2%)
8	9 (8.6%)
9	29 (27.6%)
10	8 (7.6%)
<b>Lymphovascular Invasion (LV1) (n=75)</b>	
Positive	18 (24%)
Negative	57 (76%)
<b>Perineural Invasion (PNI) (n=75)</b>	
Positive	69 (92%)
Negative	6 (8%)
<b>Extraprostatic Invasion (EI) (n=75)</b>	
Positive	40 (53.3%)
Negative	35 (46.7%)
<b>pT Stage (n=75)</b>	
pT2a	3 (4%)
pT2b	6 (8%)
pT2c	26 (34.7%)
pT3a	16 (21.3%)
pT3b	24 (32%)
<b>Metastasis (n=101)</b>	
Positive	30 (29.7%)
Negative	71 (70.3%)
<b>Recurrence (n=86)</b>	
Positive	18 (20.9%)
Negative	68 (79.1%)

detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's Haematoxylin and mounted with DPX mountant (Code: RRSP29-E, Lot: 77719075591, Atom Scientific Ltd., United Kingdom). Kidney tissue containing cortical glomerular structures was used for CIP2A as a positive control.

*Evaluation of immunohistochemical staining*  
All immunohistochemical slides were

independently evaluated in a blinded fashion. Membranous and cytoplasmic staining was considered positive for CIP2A. Immunostaining was scored semiquantitatively based on the staining intensity as 0 (negative); +1 (weak) and +2 (strong).

#### *Statistical Analysis*

Statistical analysis was performed using SPSS 20.0 (IBM Inc, Chicago, IL, USA). The

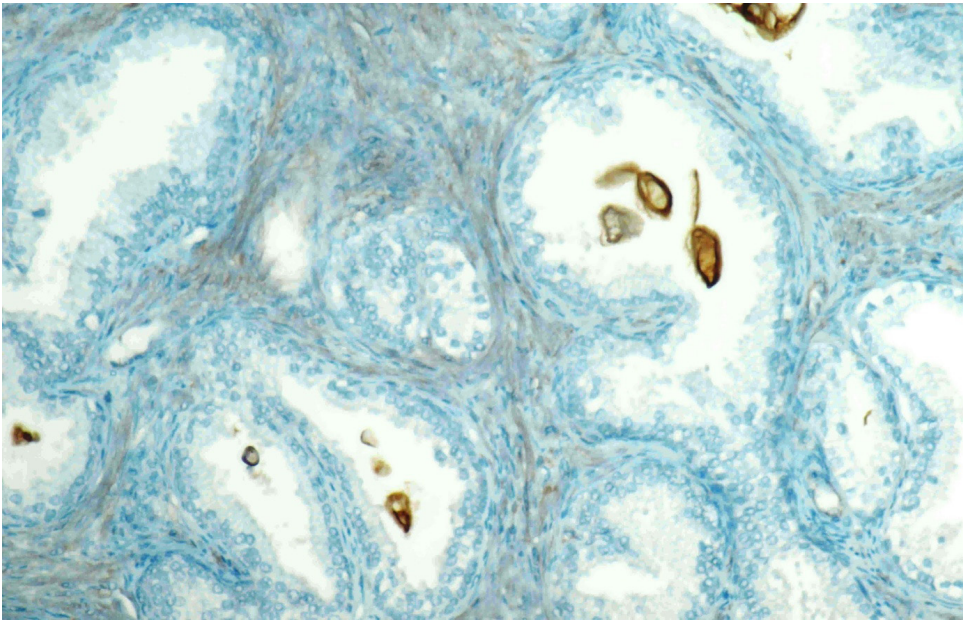


FIG. 1: Immunohistochemical staining of CIP2A in BPH; staining of stromal cells and corpora amylacea was observed but no expression was observed in the epithelial cells of the prostate (DAB x200)

comparison between the immunohistochemical staining of groups was tested by the Mann-Whitney U test and Spearman's rank correlation coefficient test. The relationship between CIP2A expression score and clinicopathological parameters was evaluated by Pearson's chi-square test. The relation between the survival and clinical factors was evaluated by the Monte Carlo Exact Chi-square test. The mean survival

times were calculated by Kaplan-Meier survival analysis, and the log-rank test was performed to determine the difference between CIP2A expression scores. The overall survival rate was plotted. The Cox regression model was established to determine the prognostic factors on overall survival. The  $p$ -values  $<0.05$  were considered as statistically significant.

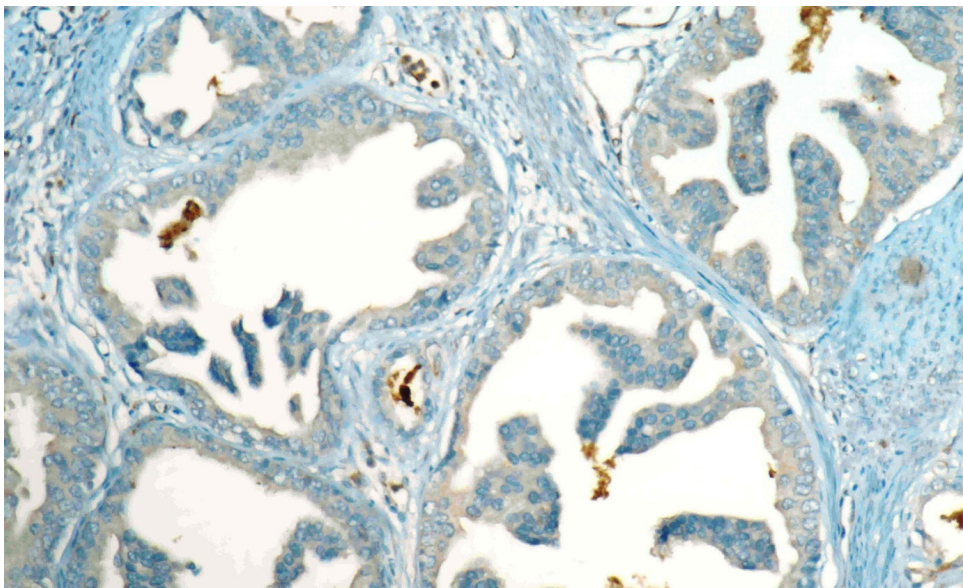


FIG. 2: Weak cytoplasmic CIP2A immunostaining in HGPIN (DAB x200)



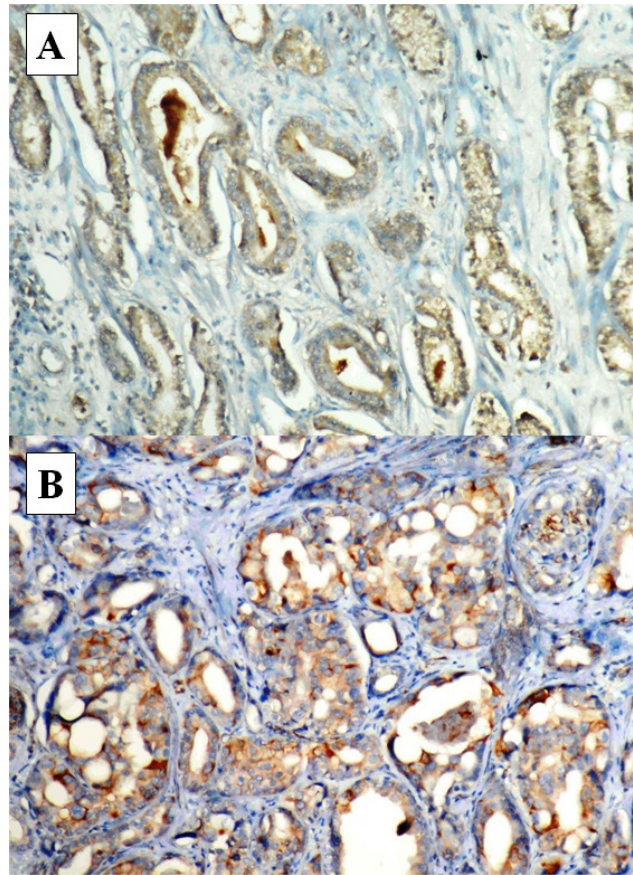


FIG. 3: Immunohistochemical staining intensity of CIP2A in A) low grade prostate cancer, and B) high grade prostate cancer (DAB×200)

## RESULTS

### *CIP2A expression in BPH, HGPIN and PC cases*

CIP2A showed cytoplasmic expression in BPH and HGPIN cases whereas, expression was cytoplasmic and rarely membranous in PC cases. Additionally, prostatic stroma was labelled focally in some cases. The staining intensity in PC tissues was remarkably denser than the other groups (Fig. 1-3). CIP2A expression was detected in 11 (40.7%) BPH, 15 (55.6%) HGPIN and 101 (96.2%) PC samples. Regarding expression scores; (Table 2) significant association was

found between CIP2A expression score and the groups ( $p < 0.0001$ ; Pearson's chi-square test). Besides, a positive correlation was detected between the two parameters ( $p < 0.0001$ ;  $r = 0.666$ ; Spearman's correlation test). As for the difference between the CIP2A expression score within the groups, statistically, significant associations were found between PC and HGPIN ( $p < 0.0001$ ), and also PC and BPH ( $p < 0.0001$ ); but there was not any significance between HGPIN and BPH ( $p = 0.140$ ; Mann-Whitney U test).

**TABLE 2: Distribution of CIP2A expression score in BPH, HGPIN and prostate cancer cases**

	CIP 2A Expression Score			p value
	0	1+	2+	
<b>BPH</b>	16 (59,3%)	11 (40,7%)	0	$p < 0.0001$
<b>HGPIN</b>	12 (44,4%)	11 (40,7%)	4 (14,8%)	
<b>PC</b>	4 (3,8%)	29 (27,6%)	72 (68,6%)	

### Correlation between CIP2A expression score and clinicopathological variables

As seen in Table 3, patients diagnosed with a higher grade GS (GS 8-10) ( $p=0.032$ ) and lymphovascular invasion ( $p=0.039$ ) exhibited increased CIP2A expression (Pearson's chi-square test). Considering other clinicopathological parameters of PC cases, no statistically significant correlation was found between CIP2A expression and the variables such as age, pT stage, perineural invasion, metastasis and recurrence in the PC group ( $p>0.05$ ).

### CIP2A expression and postoperative survival of PC patients

Overall survival (OS) was used for survival analysis. Death caused by PC was appointed as the endpoint of analysis. Overall survival was defined as the time interval between surgery and death. Univariate 5-year OS revealed that patients with high GS ( $GS \geq 8$ ), lymphovascular invasion, advanced pT stage (pT3a-3b), distant metastasis and recurrence had worse outcomes (Table 4). According to the Kaplan-Meier test, the mean overall survival time was 111.1 months for all patients, 118.5 months for patients with low CIP2A expression and 96.2 months for patients with high CIP2A expression. There was

no significant difference between the survival times of patients according to CIP2A expressions ( $p=0.174$ ) (Table 5). Kaplan-Meier curves of OS according to CIP2A expression were shown in Fig. 4. In addition, multivariate analysis indicated GS, lymphovascular invasion, distant metastasis were independent prognostic factors for PC patients (Table 6). CIP2A expression score, perineural invasion, pT stage and recurrence were not found to be prognostic factors.

## DISCUSSION

In this study, we demonstrated that CIP2A expression in PC tissues was significantly increased compared with benign prostate tissues, giving this marker a possible role in PC progression. This finding indicates that CIP2A was highly expressed in tumour tissues than nontumoural tissues, which is consistent with previous studies.<sup>13,15,18,19,25</sup> There are only a few studies investigating CIP2A expression in PC.<sup>21,27,29</sup> Shi *et al.*<sup>29</sup> found significantly increased p90 (CIP2A) autoantibodies in PC specimen comparing with BPH by ELISA and Western Blot methods. Also, Vaarala *et al.*<sup>21</sup> detected significantly increased CIP2A expression in PC as compared with BPH immunohistochemically.

**TABLE 3: Relationship between CIP2A expression score and clinicopathological parameters in prostate cancer**

		CIP2A Expression Score				p value
		Total	0 n (%)	1+ n (%)	2+ n (%)	
Gleason Score	6	21	1 (4.8)	5 (23.8)	15 (71.4)	p=0.032
	7	38	2 (5.3)	17 (44.7)	19 (50)	
	$\geq 8$	46	1 (2.2)	7 (15.2)	38 (82.6)	
pT Stage	pT2a-2c	35	0 (0)	12 (34.3)	23 (65.7)	p=0.251
	pT3a,3b	40	3 (7.5)	12 (30)	25 (62.5)	
LVI	positive	18	0 (0)	2 (11.1)	16 (88.9)	p=0.039
	negative	57	3 (5.3)	22 (38.6)	32 (56.1)	
PNI	positive	69	3 (4.3)	23 (33.3)	43 (62.3)	p=0.571
	negative	6	0 (0)	1 (16.7)	5 (83.3)	
Metastasis	positive	30	1 (3.3)	5 (16.7)	24 (80)	p=0.207
	negative	71	2 (2.8)	23 (32.4)	46 (64.8)	
Recurrence	positive	18	0 (0)	8 (44.4)	10 (55.6)	p=0.675
	negative	68	3 (4.4)	20 (29.4)	45 (66.2)	

**TABLE 4: Univariate 5-year overall survival of prostate cancer patients**

		Overall Survival	
CIP2A		n (%)	p
	Low expression	27 (32.9%)	0.728
	High expression	55 (67.1%)	
Gleason Score	GS 6 and 7	55 (67.1%)	<0.001*
	GS ≥ 8	27 (32.9%)	
LVI	negative	53 (79.1%)	0.003*
	positive	14 (20.9%)	
PNI	negative	6 (9.0%)	0.488
	positive	61 (91.0%)	
pT Stage	pT2a-2c	34 (50.7%)	0.029*
	pT3a,3b	33 (49.3%)	
Metastasis	negative	65 (79.3%)	<0.001*
	positive	17 (20.7%)	
Recurrence	negative	61 (81.3%)	0.042*
	positive	14 (18.7%)	

\*significant if p value is less than 0.05 level for Monte Carlo Exact Chi-Square test

**TABLE. 5: Survival time (months) of patients according to CIP2A expression**

	Mean survival time with 95% CI	
Low-CIP2A expression	118.5	99.3-137.7
High- CIP2A expression	96.2	84.5-108.1
Overall	111.1	97.3-124.9
Log-rank (mantel-Cox)	$X^2=1.852$	$P=0.174$

**TABLE 6: Cox regression analysis in predicting the overall survival of prostate cancer patients**

Overall survival		p	HR	95% CI
CIP2A				
Gleason Score	Low expression	0.317	0.730	0.394-1.352
	GS 6 & 7	0.023*	1.354	1.045-2.593
LVI	negative	0.036*	1.692	1.107-1.947
PNI	negative	0.469	0.692	0.256-1.873
pT Stage	pT2a-2c	0.343	0.753	0.419-1.353
Metastasis	negative	0.009*	2.551	1.243-5.716
Recurrence	negative	0.846	0.975	0.460-2.067

\*significant if p value is less than 0.05 for Cox Regression on mortality status

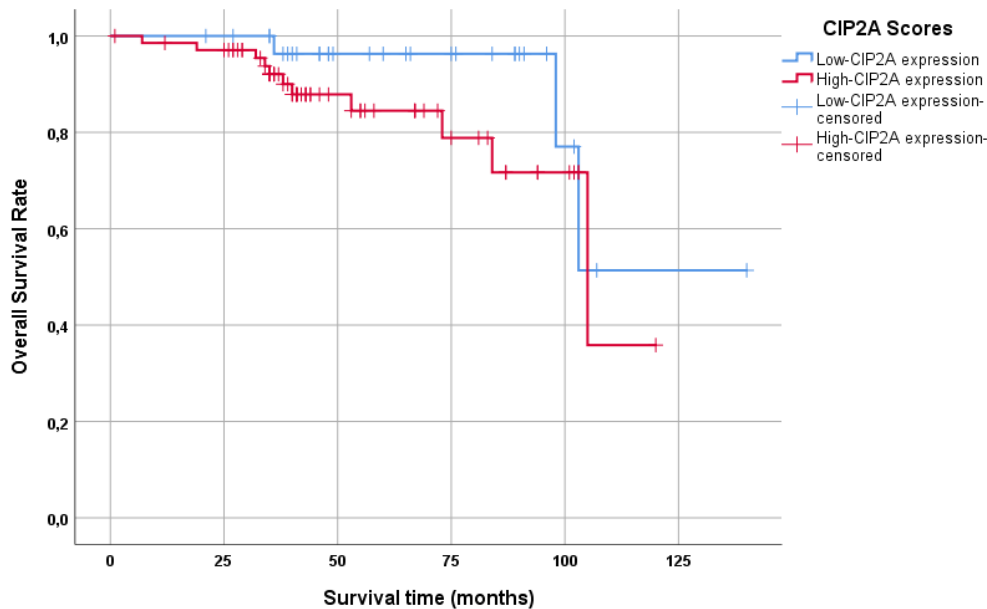


FIG. 4: Kaplan–Meier curves of OS according to CIP2A expression.

In the study of Khanna *et al.*<sup>27</sup> increased CIP2A mRNA levels were found in hormone-naïve primary PC and even more in castration-resistant PC compared to BPH by PCR method. Also, immunostaining showed higher protein levels in castration-resistant PC compared to hormone-naïve primary PC.<sup>27</sup>

In the present study we demonstrated for the first time the expression of CIP2A in HGPIN, the precursor lesion of PC. Additionally, we have demonstrated that CIP2A expression score was higher in PC tissues than in HGPIN tissues. Comparing HGPIN with BPH cases we found a tendency towards higher rates of strong staining in HGPIN cases but, the difference was not statistically significant which may be due to the limitation of a low number of cases. Although our findings suggest that CIP2A has no role in the early carcinogenesis of the prostate, it may play a role in PC development and the transformation of prostate epithelial cells into invasive malignant phenotype. Further investigations are needed especially in precursor lesions of different types of cancer to support our findings.

Previous reports have revealed that increased CIP2A expression was associated with poorly differentiated, high-risk tumours and tumour aggressivity in ovarian serous cancer<sup>25</sup>, breast cancer<sup>18</sup>, renal cell carcinoma<sup>16</sup>, non-small cell lung cancer<sup>30</sup>, oesophagogastric junction adenocarcinoma.<sup>31</sup>

In the study of Vaarala *et al.*<sup>21</sup> which is the

first study comparing CIP2A expression with prognostic parameters of PC, increased CIP2A expression was correlated with higher GS and pT stage. In concordance with the findings of Vaarala *et al.*<sup>21</sup>, we found a significant correlation between increased CIP2A expression score and higher GS. These findings suggest that CIP2A also plays a role in the differentiation and aggressive behaviour of PC. Unlike the study of Vaarala *et al.*<sup>21</sup>, we did not find any significant correlation between CIP2A expression score and pT stage of PC. This difference may be related to the case distribution of different pT stages.

CIP2A functions as oncogene and plays a multidirectional regulatory role in the signal pathways of Myc, E2F1, Plk1, DAPK1, which in turn play a role in the carcinogenesis of different types of cancer. It has emerged as a potential drug target for a range of different tumour types.<sup>8</sup> Natural compounds like Celastrol in non-small cell lung cancer, as well as Erlotinib derivatives in hepatocellular cancer, were therapeutically used aiming to inhibit CIP2A.<sup>2</sup> Khanna *et al.*<sup>27</sup> put forward that CIP2A could be a novel therapeutical target, especially in castration-resistant PC due to increased CIP2A expression levels in these type of cancer cells.

The function of CIP2A as a potential biomarker and its prognostic value has been investigated in different studies. Liu *et al.*<sup>32</sup> found anti-p90/CIP2A antibodies to be a useful serum biomarker in the screening and immunohistochemical diagnosis of early-stage breast cancer. In another



study, the prognostic role of CIP2A expression in serous ovarian cancer and its function as a novel biomarker for reduced survival have been proposed.<sup>25</sup> Similarly, Dong *et al.*<sup>19</sup> showed a correlation between increased CIP2A expression and poor prognosis in non-small cell lung cancer in their study. Conversely, in our study CIP2A expression did not associate with patient's prognosis even though there was a significant association between CIP2A expression and GS and lymphovascular invasion. The difference in sample size may be a possible explanation for this discrepancy.

Although immunohistochemistry can be practically applied in daily routine, an investigation of CIP2A by immunohistochemical methods alone is limiting our study. Nevertheless, immunohistochemistry assesses the expression of CIP2A protein, which is the functional gene product, while mRNA levels do not always correlate with protein levels. Also regarding the tissue microarray constructed in our study, although the 2-mm core was relatively large, our results may have been influenced due to the tissue heterogeneity.

In conclusion, detecting a correlation of CIP2A expression with important clinicopathological parameters such as GS and lymphovascular invasion underlines its probable role in differentiation and aggressiveness of PC. This is the first study investigating the relationship between CIP2A expression and survival in PC. However, our results are not consistent with those of other studies showing that high CIP2A expression is associated with poor prognosis in patients with various cancer types.

Drawing on our findings, further investigations are needed to concern the use of CIP2A protein as a biomarker and to determine its importance and role in differentiation, progression, prognosis and therapy of PC.

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**Conflict of interest:** The authors declare they have no conflict of interest.

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