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Centrosome Protein 78 Is Overexpressed in Muscle-Invasive Bladder Cancer and Is Associated with Tumor Molecular Subtypes and Mutation Signatures

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1,2 **Xiaoli Huang***
ABCDEF 3 **Yang Yan***
BD 2 **Rong Wei**
CD 2 **Hu Liu**
BD 2 **Xingchen Zhu**
ACFG 2 **Dexi Bi**
AEG 1,2 **Qing Wei**
AE 3 **Xudong Yao**

1 Department of Pathology, Shanghai Clinical College, Anhui Medical University, Hefei, Anhui, P.R. China
2 Department of Pathology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, P.R. China
3 Department of Urology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, P.R. China

Corresponding Authors:

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* Xiaoli Huang and Yang Yan contributed equally to this work

Qing Wei, e-mail: weiqing1971@126.com, Xudong Yao, e-mail: yaouxudong67@sina.com

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Background:

Centrosome aberrations have long been linked to tumorigenesis. Centrosome protein 78 (CEP78) is a centrosome component that is required to regulate the cell cycle, but its role in bladder cancer has not been elucidated.

Material/Methods:

Real-time quantitative polymerase chain reaction and immunohistochemistry were used to examine the expression of CEP78 in bladder cancer tissues and adjacent non-cancer tissues.

Results:

Analysis of the RNA-Seq data from the TCGA (The Cancer Genome Atlas) MIBC cohort (n=408) revealed that CEP78 was overexpressed in tumor tissues, which was confirmed with fresh-frozen and formalin-fixed paraffin-embedded specimens collected from 28 and 33 MIBC patients, respectively, in the present study. The clinicopathological relevance of CEP78 was further investigated. High CEP78 expression was found to be correlated with non-papillary histological type, luminal, basal-squamous and neuronal molecular subtypes, TP53 mutation, RB1 mutation, wild-type FGFR3, PPARG fusion and amplification, high total number of single-nucleotide variants, and high neoantigen load, but it was not associated with tumor stages or overall survival.

Conclusions:





The results of this study suggest that CEP78 plays in a role in promoting the development of MIBC and could be a novel diagnostic and therapeutic target.

MeSH Keywords:

Cell Cycle • Centrosome • Mutation • Urinary Bladder Neoplasms

Full-text PDF:

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Background

Bladder cancer is the most common urinary track malignancy worldwide, ranking 9th in morbidity and 13th in mortality among all types of cancer [1]. About 75% of newly diagnosed cases are non-muscle-invasive bladder cancer (NMIBC), while 25% are muscle-invasive bladder cancer (MIBC) [2]. MIBC is a lethal disease with much lower 5-year overall survival rates than NMIBC [3]. Although understanding of bladder cancer has improved in recent years [4], novel biomarkers for diagnosis and treatment remain elusive.

Centrosome aberrations have long been proposed to be associated with tumorigenesis [5]. The centrosome is the main microtubule-organizing center in most eukaryotic cells and is essential for cell division [5]. Studies have shown that dysregulation of the centrosome is involved in various types of cancer [5]. Centrosome-associated family proteins (CEPs) are active components that participate in the functions of the centrosome. Aberrant expression of CEPs may lead to abnormalities in centrosome function, which in turn results in chromosomal instability [5,6]. Of note, CEP55 has been reported to be expressed at high levels in prostate cancer [7], epithelial ovarian cancer [8], colon cancer [9], oral squamous cell carcinoma [10], and bladder cancer [11], but CEP63 is expressed at low levels in bladder cancer [12]. CEP78 also has been recognized as a component of the centrosome that regulates G2/M transition of mitotic cell cycle [6] and is associated with prostate cancer [13], differentiated thyroid carcinoma [14], and colorectal cancer [15], but its role in bladder cancer is unknown.

In the present study we explored the clinical significance of CEP78 in MIBC. We assessed the expression of CEP78 in MIBC tissues and examined its association with clinical and pathological features.

Material and Methods

Ethics

This study was approved by the Ethics Committee of Shanghai Tenth People's Hospital (2019K113) and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

The Cancer Genome Atlas dataset

The clinical and pathological information and RNA-Seq data of the MIBC patients in The Cancer Genome Atlas (TCGA MIBC cohort) were retrieved from the GDC Data Portal (<https://portal.gdc.cancer.gov>). For the RNA-Seq data, log₂-transformed upper-quartile-normalized FPKM were used for comparison.

Patients and tissue samples

Tissue samples were collected from 2 independent cohorts of primary MIBC patients who received radical cystectomy at Shanghai Tenth People's Hospital between January 2016 and June 2019. Fresh-frozen tumor tissues and adjacent normal tissues were collected from 28 patients (fresh-frozen cohort). The fresh tissue specimens were immediately snap-frozen in liquid nitrogen and stored at -80°C. Clinical formalin-fixed paraffin-embedded (FFPE) specimens were collected from 33 patients (FFPE cohort).

RNA extraction and quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from human tissues or cultured cells as previously described [16]. qRT-PCR was performed with the SYBR Green PCR Kit (Takara Biotechnology) on an ABI Prism 7500 Sequence Detection System (Applied Biosystems). The primer sequences were 5'-TGGCAGGGAGCAGATCAC-3' (forward) and 5'-AAGCCAGCCATACAGTCAAGA-3' (reverse) for *CEP78*; and 5'-ACAGTCAGCCGCATCTTCTT-3' (forward) and 5'-GACAAGCTTCCCGTTCTCAG-3' (reverse) for *GAPDH*. The qRT-PCR were set as 95°C for 15 s, 40 cycles of 95°C for 5 s, and 60°C for 35 s, and 4°C for 10 min. Experiments were performed in triplicate.

Immunohistochemistry (IHC)

The FFPE specimens were cut into 3-µm sections, placed on polylysine-coated slides, deparaffinized, and stained on a BenchMark ULTRA automated immunohistochemical staining system (Roche) with the CEP78 antibody (1: 200; NBP2-48920, Novus). The percentage of stained cells (0 for 0%, 1 for 1–10%, 2 for 11–25%, 3 for 26–50%, 4 for 51–75%, and 5 for 76–100%) and staining intensity (0 for negative, 1 for weak, 2 for moderate, and 3 for strong staining) were scored [17] independently by 2 pathologists. For each sample, the means of the 2 statistical scores were multiplied to obtain an IHC score.

Statistical analysis

Data were analyzed with GraphPad Prism (version 5) or SPSS (version 19). Wilcoxon signed rank test, Mann-Whitney test, or Kruskal-Wallis test followed by Dunn's multiple comparisons test was used for the comparison of quantitative data, as applicable. The chi-square test or Fisher's exact test were used for the comparison of categorical data. Kaplan-Meier method was used for survival analysis. Spearman rank correlation test was used for correlation analysis. A two-tailed $p < 0.05$ was considered statistically significant.

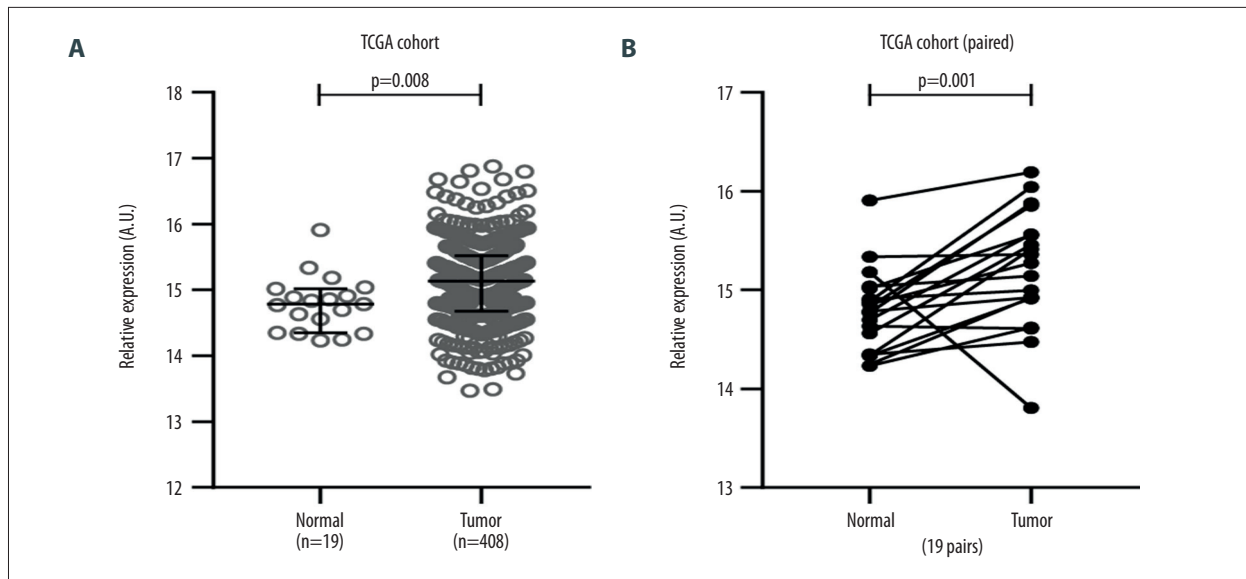


Figure 1. Upregulation of *CEP78* mRNA expression in MIBC revealed in the TCGA cohort. **(A)** Comparison of the *CEP78* mRNA levels between all tumor and available normal tissues from 408 patients in the TCGA MIBC cohort with Mann-Whitney test. **(B)** Paired comparison of the *CEP78* mRNA levels between the tumor and matched normal tissues available in 19 patients in the TCGA MIBC cohort with Wilcoxon signed rank test. Medians and interquartile ranges are shown.

Results

Analysis of the TCGA cohort revealed upregulation of *CEP78* mRNA expression in MIBC

Analysis of the RNA-Seq data of the TCGA MIBC cohort (n=408) was performed. We conducted unpaired comparison between normal and tumor tissues with all available data (19 normal vs. 408 tumor tissues, Mann-Whitney test) as well as paired comparison with matched data available in 19 cases (Wilcoxon signed rank test). Both showed that, compared to normal tissues, the mRNA expression of *CEP78* was upregulated in tumor tissues (Figure 1A, 1B, $p=0.008$ and $p=0.001$, respectively).

Validation in clinical samples confirmed *CEP78* is overexpressed in MIBC

Validation in clinical specimens collected by this study was carried out. We examined the expression of *CEP78* in paired fresh-frozen tumor tissues and adjacent normal tissues collected from 28 MIBC patients (fresh-frozen cohort) with qRT-PCR, and obtained similar results, showing that the tumor tissues had high *CEP78* expression (Figure 2A, $p=0.013$). Further, IHC staining of *CEP78* in an independent cohort of paired FFPE samples from 33 MIBC patients (FFPE cohort) showed that tumor tissues had higher IHC scores than the normal ones ($p<0.001$), confirming that *CEP78* was overexpressed in MIBC (Figure 2B, 2C).

CEP78 expression is associated with molecular pathological features in MIBC

In addition, we evaluated the clinicopathological relevance of *CEP78* in the TCGA cohort (Figure 3). The *CEP78* expression was found to be significantly associated with molecular subtype, papillary/non-papillary histological subtype, and certain genetic alterations, whereas it showed weak association with sex, age, tumor stage, lymphovascular invasion, squamous pathology, lymph node status, or overall survival (Figure 3A–3C).

MIBC has previously been categorized into 5 molecular subtypes according to transcriptome profiles [4]. Among them, we found that high *CEP78* expression correlated to luminal, basal-squamous, and neuronal subtypes (Figure 3A, 3D).

Moreover, high *CEP78* expression also was found to be correlated with *TP53* mutation, *RB1* mutation, wild-type *FGFR3* and *PPARG* fusion and amplification (Figure 3A). The *CEP78* expression also displayed positive correlations with total number of single-nucleotide variants (SNVs) (Spearman $\rho=0.2471$, $p<0.001$) and neoantigen load (Spearman $\rho=0.2466$, $p<0.001$) (Figures 3E, 3F), but we did not observe a correlation between *CEP78* expression and APOBEC mutation load, *FGFR3* fusion, or *FGFR3* amplification (Figure 3A).

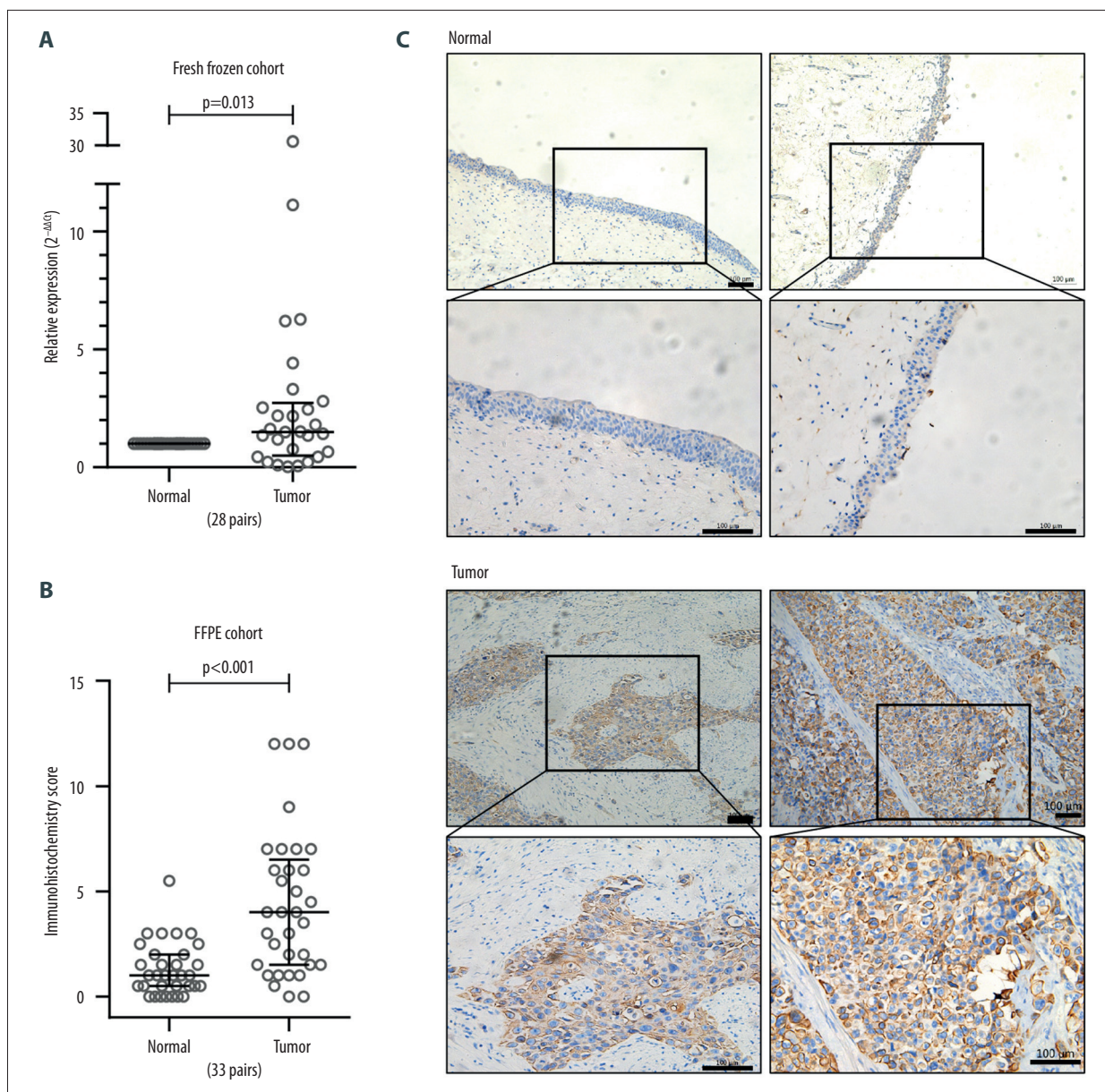


Figure 2. CEP78 overexpression confirmed in clinical samples collected by this study. **(A)** Comparison of the relative *CEP78* expression in fresh-frozen tumor and adjacent normal tissues from 28 MIBC patients with Mann-Whitney test. **(B)** IHC staining scores of CEP78 in FFPE tumor and adjacent normal tissues from 33 MIBC patients compared with Mann-Whitney test or Wilcoxon signed rank test. The Mann-Whitney test p value is shown in the figure; while the Wilcoxon signed rank test p value also was <0.001. **(C)** Representative IHC staining images of the tumor and matched adjacent normal tissues. Images for one pair are placed in one row. All images were separately taken. Scale bar, 100 μ m. Medians and interquartile ranges are shown for **(A)** and **(B)**.

Discussion

This study examined the expression of CEP78 in MIBC tumor tissues. We obtained consistent results from the TCGA and 2 in-house MIBC cohorts, showing that CEP78 was highly expressed in tumor tissues at both mRNA and protein levels, and we revealed its association with the molecular subtypes and mutation signatures.

The centrosome is the main microtubule-organizing center for human cell proliferation, while the replication of the centrosome is controlled by centrosome proteins (CEPs) [5,6]. Recent studies have suggested that centrosome aberrations contribute to tumorigenesis in many ways [5]. CEP78 has been reported to regulate G2/M transition of the mitotic cell cycle [6], and thus regulates cell proliferation. Therefore, overexpression of CEP78 may play a role in promoting the development of MIBC.

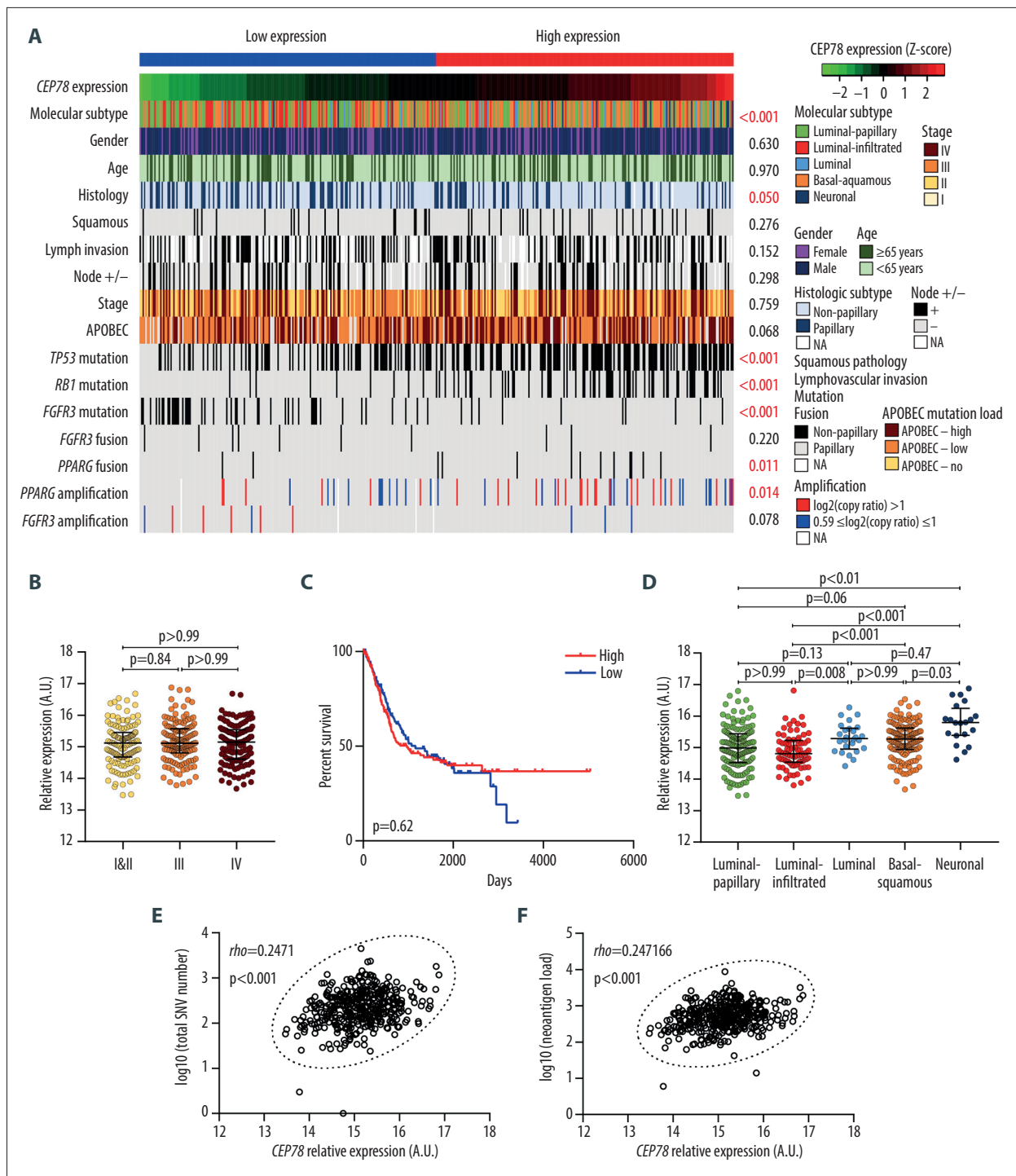


Figure 3. Clinicopathological relevance of CEP78 analyzed in the TCGA cohort. **(A)** Association between the expression level (high or low) of CEP78 and the studied clinicopathological features. Each column indicates a case in the heatmap. Chi-square test or Fisher's exact test p values are shown by the right side of the heatmap and those < 0.05 are highlighted in red. **(B)** CEP78 expression in different tumor stages (Kruskal-Wallis test followed by Dunn's multiple comparisons test). **(C)** CEP78 was not associated with overall survival (log-rank test). **(D)** CEP78 expression in different molecular subtypes (Kruskal-Wallis test followed by Dunn's multiple comparisons test). **(E)** Correlation between CEP78 expression and total number of single-nucleotide variants (SNVs) (Spearman rank correlation test). **(F)** Correlation between CEP78 expression and neoantigen load (Spearman rank correlation test). For **(B)** and **(D)**, medians and interquartile ranges are shown.

Interestingly, we also found that *CEP78* expression was associated with many key pathological features in MIBC, especially the genetic alterations of *TP53*, *RB1*, *FGFR3*, and *PPARG* and total SNV number, which may be involved in the chromosomal instability caused by centrosome dysregulation. MIBC recently has been classified into 5 molecular subtypes based on multiplatform analysis, including luminal-papillary, luminal-infiltrated, luminal, basal-squamous, and neuronal subtypes, which have not just prognostic value, but also show great potential in personalized therapy [4]. Subtyping with multi-omics data would be expensive and resource-consuming in clinical practice; thus, several representative molecular and histological features have been proposed for subtype stratification [4]. Our study found that *CEP78* expression also had a significant correlation with these molecular subtypes, indicating that *CEP78* could be useful as a subtyping maker. Moreover, the genetic alterations of *TP53*, *RB1*, *FGFR3*, and *PPARG* and total SNV number also are hallmarks of different molecular subtypes [4]. The association between *CEP78* expression and these specific mutation signatures might help to understand the mechanisms governing different subtypes. It has been reported that in MIBC, the APOBEC-a and -b signatures accounted for 67% of all SNVs and that some mutations not belonging to APOBEC-signature mutagenesis might be also APOBEC-mediated [4], but here we did not find a significant correlation between *CEP78* expression and APOBEC mutation load ($p=0.068$), which might suggest that the correlation ($\rho=0.2471$) between *CEP78* expression

and total SNV number was mainly influenced by non-APOBEC-mediated mutations. The mutagenesis in MIBC undoubtedly involves complex mechanisms and multiple factors, and the *CEP78* expression showed only a weak association with total SNV number. Therefore, further investigations are needed to define the underlying molecular mechanism.

Overall, this study showed elevated expression of *CEP78* in patients with MIBC. Our results shed light on the role of centrosome abnormalities, particularly the aberrant expression of *CEP78*, in the tumorigenesis of bladder cancer. We envision that *CEP78* could be a potential diagnostic biomarker and therapeutic target for MIBC.

Conclusions

This study investigated the expression of *CEP78* at the mRNA and protein levels in MIBC and paracancerous tissues, and conducted clinical and pathological analyses in the TCGA database, to promote understanding of the pathological process of MIBC and provide a new potential treatment target for MIBC.

Conflict of interests

None.

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