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Original Article

RB1 and p53 are diagnostic markers for treatment-related neuroendocrine prostate cancer: a clinical and pathological analysis of 23 cases

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Abstract: The synergistic interplay between RB1 deletions and TP53 mutations drives androgen deprivation therapy (ADT) resistance and neuroendocrine transdifferentiation in advanced prostate cancer, culminating in treatment-related neuroendocrine prostate cancer (t-NEPC). This investigation systematically examines the clinicopathological characteristics and immunohistochemical phenotypes of t-NEPC to enhance diagnostic accuracy and prognostic understanding. We conducted a retrospective analysis of 23 t-NEPC cases diagnosed at the First Affiliated Hospital of Zhejiang University School of Medicine (2013-2024). We collected comprehensive clinical data, including patient demographics, treatment history, and serum biomarker profiles. Immunohistochemical evaluation was performed to determine expression patterns of prostate-associated antigens, neuroendocrine markers, and tumor suppressor proteins RB1/p53. The cohort demonstrated a mean age of 70 years at initial prostate cancer diagnosis, with t-NEPC emerging after a median ADT duration of 18 months. Biochemical profiles revealed a characteristic dissociation between suppressed prostate-specific antigen (PSA) levels and elevated neuroendocrine markers alongside other tumor-associated antigens, including carcinoembryonic antigen (CEA). The immunohistochemical signature of lineage transdifferentiation, indicated by the loss of androgen receptor (AR) and the expression of neuroendocrine markers, provides critical diagnostic clues for this aggressive variant. Molecular alterations were prevalent, with RB1 loss detected in 78.26% (18/23) and p53 abnormalities in 82.61% (19/23) cases. Notably, a histologically confirmed t-NEPC case with neuroendocrine marker negativity exhibited RB1/p53 co-alterations, molecularly aligning with most neuroendocrine-positive cases. These findings substantiate that combined RB1/p53 aberrations serve as robust diagnostic indicators for t-NEPC, particularly in tumors exhibiting small cell carcinoma morphology without neuroendocrine marker expression.

Keywords: Prostate cancer, neuroendocrine, p53, RB1, immunohistochemistry

Introduction

Prostate adenocarcinoma is androgen-dependent, and androgen deprivation therapy (ADT) is the cornerstone of treatment for metastatic and locally advanced prostate cancer, with most cases progressing to the more aggressive castration-resistant prostate cancer (CRPC) [1]. Treatment-related neuroendocrine prostate cancer (t-NEPC) is an important subset of CRPC, accounting for approximately 17-25% [2, 3], transformed from prostate adenocarcinomas following ADT, and is included as a separate subtype of prostate cancer in the 5th edition of the WHO Classification of Tumors of the Urological and Male Genital Organs in 2022 [4]. Compared to adenocarcinoma, t-NEPC exhibits greater aggressiveness, ceases PSA secretion, demonstrates resistance to ADT, and is associated with a markedly poor prognosis [5]. The most reliable method for diagnosing t-NEPC is through pathological examination, which typically necessitates a comprehensive evaluation incorporating histomorphology and immunohistochemical staining for neuroendocrine markers. However, these assessments are not always concordant, necessitating additional evidence to support the diagnosis. There is a lack of clinical awareness regarding the transition to t-NEPC following resistance to

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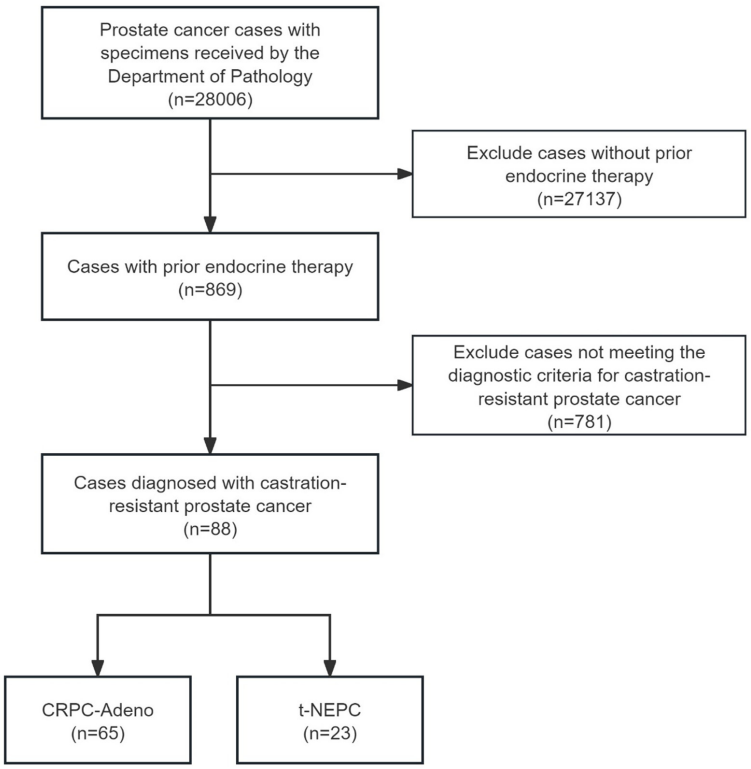

Figure 1. Flowchart of study design and case selection.

endocrine therapy in prostate cancer, and secondary biopsies are infrequently conducted. This oversight can lead to the underdiagnosis of t-NEPC.

Small cell carcinomas are predominantly observed in the lung, and extensive real-world cohort studies have demonstrated a remarkably high frequency of TP53 and RB1 oncogene inactivation in small-cell lung cancer (SCLC), which is recognized as a genomic hallmark of this malignancy [6-8]. It has been proposed that the concurrent inactivation of TP53 and RB1 in prostate adenocarcinoma similarly facilitates the acquisition of lineage plasticity and a stem cell-like phenotype in tumor cells, contributing to resistance against ADT and transformation into t-NEPC [9, 10]. The genomic landscape of metastatic castration-resistant prostate cancer (mCRPC) revealed an association between NE expression score, histologic NE features, and RB1/TP53 loss [11]. In this study, we examined 23 cases of t-NEPC exhibiting typical morphology and analyzed their clinicopathological characteristics, with a particular emphasis on the immunohistochemical phenotypes of t-NEPC, specifically the expression of p53 and RB1. This investigation aims to enhance the understanding of clinicians and pathologists regarding the spectrum of manifestations, diagnostic criteria, and prognostic implications of this disease, thereby reducing the incidence of missed diagnoses.

Material and methods

Case selection

Data were collected from patients treated for prostate cancer (PCa) at the First Hospital of Zhejiang University School of Medicine between 2009 and 2024, who met the criteria for CRPC and had undergone a secondary resection or biopsy of the lesion. All cases underwent blinded independent histopathological review by two pathologists, and 23 cases presenting with small-cell carcinoma morphology were classified as t-NEPC, while the remaining cases that retained conventional adenocarcinoma features were identified as castration-resistant prostate adenocarcinoma (CRPC-Adeno) (**Figure 1**). The morphology of small-cell carcinoma was characterized based on the established morphologic criteria for SCLC [12]. The cytomorphological score, ranging from 0 to 12, was determined by summing six cytomorphological parameters (**Table 1**), each scored as 0, 1, or 2, with lower scores indicating features more characteristic of small-cell carcinoma. A score of ≤5 was considered representative of small-cell carcinoma morphology. Cases with other significant illnesses that could impact survival assessment or with severely incomplete medical records were excluded from the study. Overall survival (OS) was defined as the period from the diagnosis of CRPC or t-NEPC to the date of death or last follow-up. The study received approval from the institutional ethics committee (IIT20240671B).

Collection of clinical and pathological characteristics

Data were collected from the medical records of 88 CRPC patients, covering patient age, sur-

Table 1. Morphological assessment

Parameters	Nuclear size	Nuclear molding	Chromatin pattern	Nucleoli	Cytoplasm	Length-to-width ratio
0	<3 lymphocytes	Abundant	Fine granules	Few	Scant	Around 2
1	Around 3	Some	Intermediate	Some	Some	Around 1.5
2	>3 lymphocytes	No	Coarse granules	Prominent	Prominent	Around 1

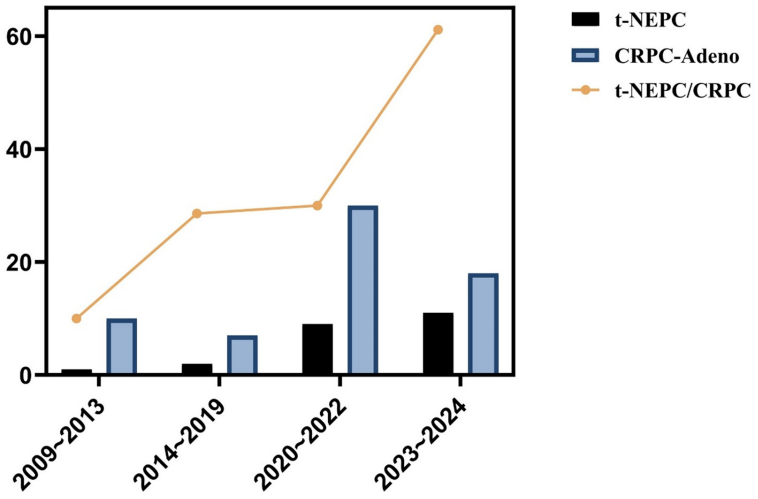

Figure 2. Temporal distribution of CRPC-Adeno and t-NEPC.

gical history, treatment regimens received, time to development of castration resistance, metastatic sites, and serum biomarker profiles. All specimens were routinely fixed in 10% formalin and embedded in paraffin. Tissue sections, with a thickness of four micrometers, were stained using hematoxylin and eosin (H&E) as well as immunohistochemical techniques. Immunohistochemical analysis was conducted on CRPC specimens to assess the expression of AR, prostate-associated markers (PSA, P504S, P501S, NKX3.1), neuroendocrine markers (Synaptophysin, Chromogranin A, CD56, INSM1), tumor suppressor proteins p53 and RB1, as well as POU2F3, a marker associated with a subtype of SCLC. At least 10% of tumor cells exhibiting positivity for at least one neuroendocrine marker or one prostate-associated marker were defined as positive for a neuroendocrine or prostate-associated marker. AR positivity was defined as nuclear staining in at least 10% of tumor cells. RB1 expression was categorized into two groups: loss (absence in at least 90% of nuclei), and wild-type. Similarly, p53 expression was classified into three categories: overexpression (strong nuclear staining in 80% or more of nuclei), loss (lack in 90% or more of nuclei), and wild-type.

Statistical analysis

For comparisons between t-NEPC and CRPC-Adeno cases, the Wilcoxon test was used for continuous variables, and the χ^2 test or Fisher's exact test for all other categorical variables. The data underwent statistical analysis using SPSS 27.0 software. Statistical significance was defined as $P< 0.05$. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test in a univariate analysis.

Results

Clinical information

In the cohort of t-NEPC cases, the initial instance was identified in 2013. Between 2009 and 2024, there was an observable increase in the proportion of t-NEPC cases relative to all CRPC cases (**Figure 2**). The age at which t-NEPC patients were initially diagnosed with prostate adenocarcinoma varied from 57 to 84 years, with both the mean and median ages calculated at 70 years. In marked contrast to CRPC-Adeno patients, the majority of t-NEPC patients (19/23) were diagnosed with PCa at an advanced stage, rendering them ineligible for curative surgical intervention. Radical prostatectomy was conducted in four cases, with postoperative pathological staging indicating a stage greater than T3, indicating tumor extension beyond the prostatic capsule and invasion into surrounding soft tissues or seminal vesicle glands.

Table 2. Comparative analysis of clinic characteristics between CRPC-Adeno and t-NEPC

Characteristics	CRPC-Adeno (N=65)	t-NEPC (N=23)	*P*-value
Age at PCa diagnosis (years)	67.63 (8.48)	70.01 (7.86)	0.214
Radical prostatectomy			
Yes	32 (49.23%)	4 (17.39%)	**0.008**
No	33 (50.77%)	19 (82.61%)	
Visceral metastasis			
Yes	24 (36.92%)	12 (52.17%)	0.201
No	41 (63.08%)	11 (47.83%)	
Bone metastasis			
Yes	43 (66.15%)	14 (60.87%)	0.648
No	22 (33.85%)	9 (39.13%)	
Time from PCa to CRPC (months)	45.00 (24.00, 67.00)	18.00 (12.00, 34.00)	**<0.001**
TPSA (ng/ml)			
At PCa diagnosis	30.88 (17.02, 71.80)	75.43 (19.45, 512.00)	0.063
At CRPC diagnosis	14.15 (2.37, 60.47)	0.16 (0.00, 4.57)	**<0.001**
NSE (ng/ml)	16.55 (12.50, 19.83)	58.90 (15.90, 208.90)	**0.007**
ProGRP (pg/ml)	53.70 (40.25, 61.05)	72.60 (37.90, 125.20)	0.613
CEA (ng/ml)	2.70 (1.70, 3.40)	20.75 (4.25, 108.60)	**<0.001**
CA199 (U/ml)	6.45 (4.05, 12.88)	21.90 (4.65, 145.95)	**0.016**
CA125 (U/ml)	14.65 (8.48, 24.50)	21.10 (11.25, 87.73)	0.123
LDH (U/L)	220.00 (180.50, 298.50)	244.00 (205.00, 395.00)	0.318

Bold values denote statistical significance at the P<0.05 level.

All CRPC patients had a documented history of endocrine therapy, which encompassed the use of oral androgen receptor inhibitors (such as bicalutamide and enzalutamide), androgen synthesis inhibitors (such as abiraterone), and subcutaneous gonadotropin-releasing hormone agonists (such as goserelin). Additionally, some patients received docetaxel chemotherapy and radiotherapy. Upon progression to CRPC, patients underwent re-excision or biopsy of the lesion due to a progressive exacerbation of dysuria, hematuria, and symptoms suggestive of distant metastasis, including bone pain. Compared to CRPC-Adeno patients, t-NEPC patients exhibited a significantly more rapid progression of the disease, with castration resistance developing at a median interval of 18 months (range: 6-84 months) following the initial diagnosis of prostate adenocarcinoma. Pathological specimens of t-NEPC were collected from various sites, including the prostate (9), bladder (5), liver (4), subcutaneous tissue (2), bone (1), mediastinum (1), and distant lymph nodes (1). In terms of serum biomarkers, t-NEPC patients showed lower total prostate-specific antigen (tPSA) levels and higher concentrations of neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9), as presented in **Table 2**.

Pathologic characteristics

The histomorphological and immunohistochemical characteristics exhibit significant variation among prostate adenocarcinoma, CRPC-Adeno, and t-NEPC, as illustrated in **Figure 3**. Prostate adenocarcinoma (Adeno-PCa) is characterized by distinct glandular structures, abundant cytoplasm, and prominent nucleoli, with expression of prostate-associated markers but an absence of neuroendocrine (NE) markers. At the initial diagnosis of prostate cancer, t-NEPC patients presented with high Gleason scores, with six cases scoring 9 and ten cases scoring 8. In CRPC-Adeno, independent glandular formations are infrequent, with tumors typically manifesting as sieve-like or diffuse patterns. The tumor cells are large and round, featuring prominent nucleoli and abundant cytoplasm, and express prostate-associated markers without NE marker expression. In contrast, t-NEPC is characterized by the absence of glandular structures, forming solid,

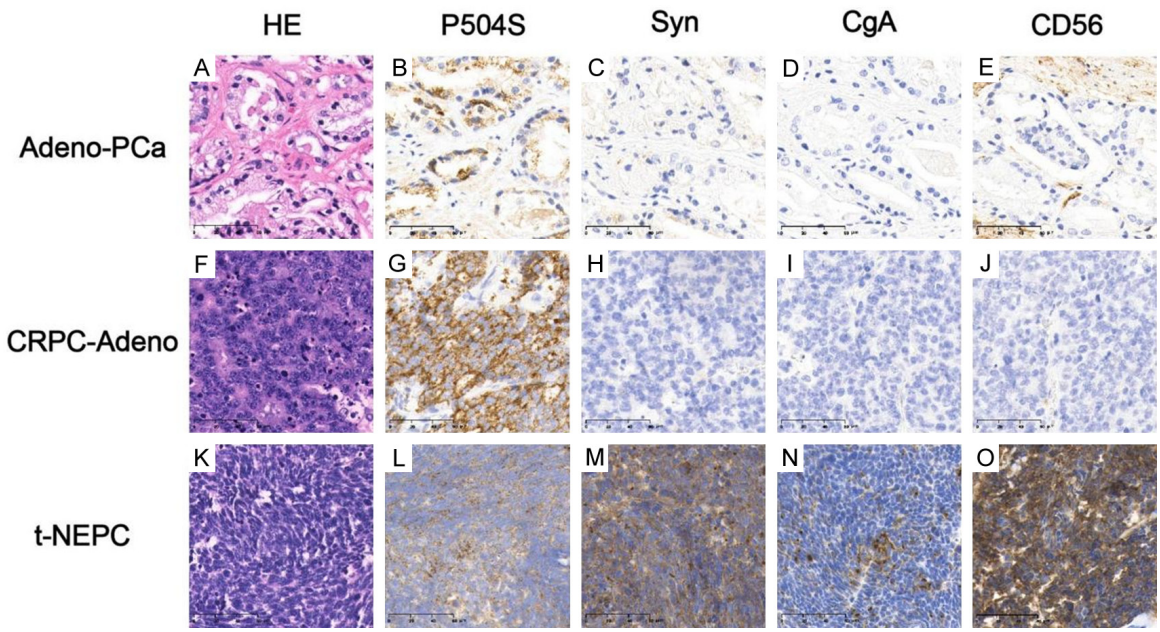

Figure 3. Representative HE, P504S, Syn, CgA, and CD56 staining of Adeno-PCa, CRPC-Adeno, and t-NEPC.

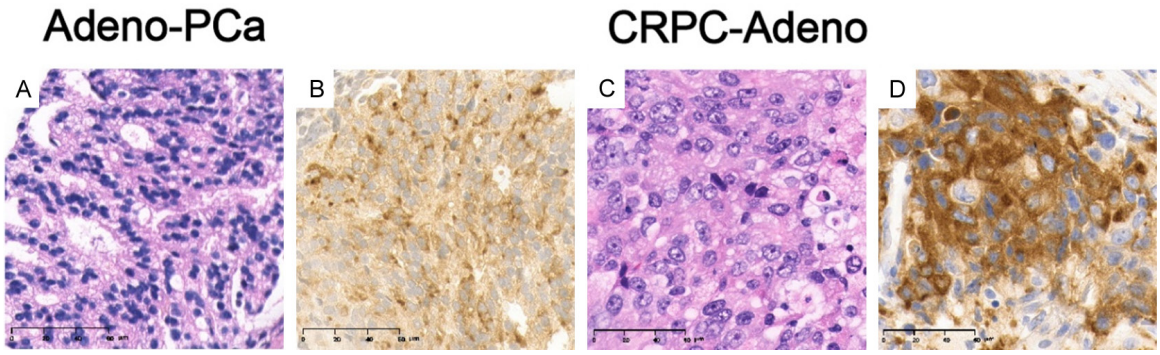

Figure 4. Positive staining of Syn in Adeno-PCa and CRPC-Adeno.

diffuse sheets of tumor cells. These cells are ovoid or short spindle-shaped, with minimal cytoplasm, dark nuclei, and fine granular chromatin. Necrosis, apoptosis, and karyorrhexis are frequently observed. Immunohistochemical analysis reveals the presence of NE markers such as synaptophysin (Syn), chromogranin A (CgA), CD56, and insulinoma-associated protein 1 (INSM1), while typically retaining prostate-associated markers such as P504S.

While some cases of CRPC-Adeno and Adeno-PCa also express neuroendocrine markers (**Figure 4**), the prevalence of neuroendocrine marker expression and AR loss is more pronounced in t-NEPC (**Table 3**). Among the 23 t-NEPC cases, 15 exhibited a neuroendocrine-positive (NE+)/AR-negative (AR-) phenotype, with 5 lacking prostate-associated markers; 7 cases displayed an NE+/AR+ phenotype (**Figure 5A-C**); and 1 case demonstrated an NE-/AR- phenotype (**Figure 5D-F**), also lacking prostate-associated markers.

We subsequently selected 30 cases from CRPC-Adeno characterized by lower morphological scores and minimal glandular structures for analysis through p53 and RB1 staining. These cases were then compared with t-NEPC. Abnormalities in RB1 and p53 were more frequently observed in t-NEPC than in CRPC-Adeno (**Table 4**). Specifically, 10 cases displayed p53 expression loss, indicative of nonsense mutations (**Figure 6I**), 9 cases showed p53 overex-

Table 3. Expression of NE markers and prostate-associated markers in CRPC-Adeno and t-NEPC

		CRPC-Adeno (N=65)	t-NEPC (N=23)	*P*-value
NE markers	Negative	49 (75.38%)	1 (4.35%)	<0.001
	Positive	16 (24.62%)	22 (95.75%)	
AR	Negative	4 (6.15%)	16 (69.57%)	<0.001
	Positive	61 (93.85%)	7 (30.43%)	
Prostate-associated markers	Negative	0 (0.00%)	8 (34.78%)	<0.001
	Positive	65 (100.00%)	15 (65.22%)	

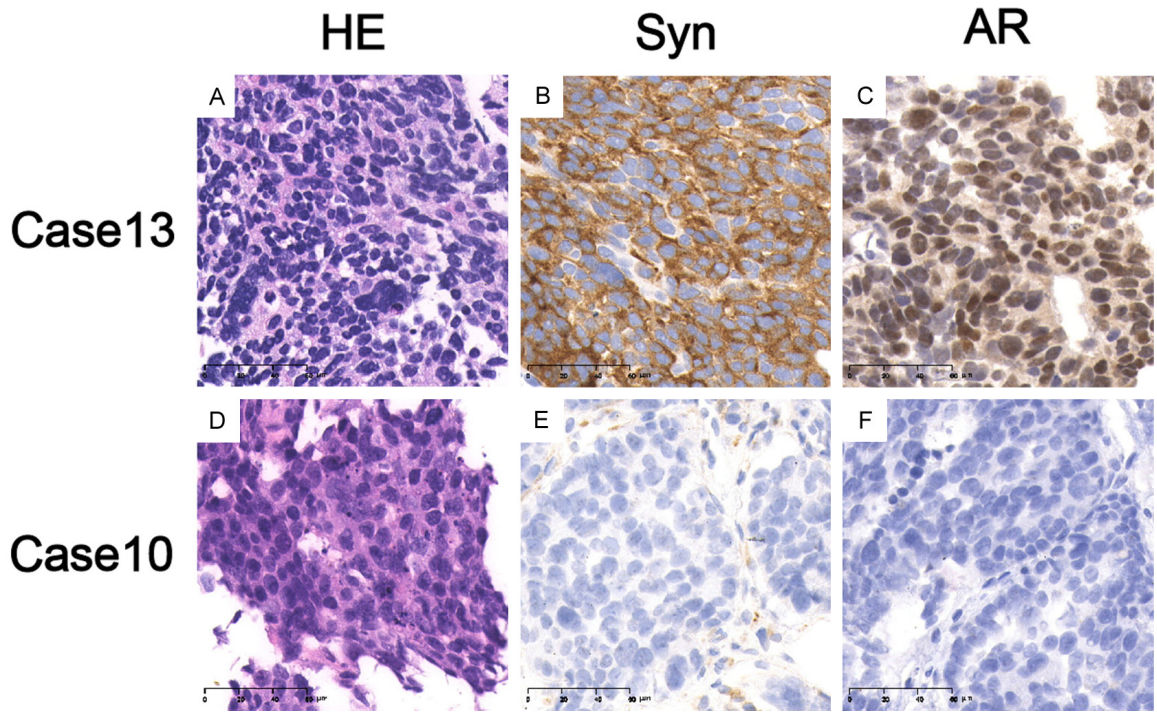

Figure 5. Syn and AR staining in t-NEPC.

pression, indicative of missense mutations (**Figure 6F**), and 4 cases retained wild-type p53 expression (**Figure 6C**). Furthermore, 18 cases demonstrated RB1 expression loss (**Figure 6E**, **6H**), while 5 cases retained wild-type RB1 expression (**Figure 6B**). None of the cases expressed POU2F3. The immunohistochemical profiles of 23 cases of t-NEPC are presented in **Table 5**.

Follow-up results

After the diagnosis was confirmed by pathology, 10 t-NEPC patients were treated with platinum-based chemotherapy, supplemented with radiotherapy and endocrine therapy, 10 underwent novel endocrine therapy only, and 3 received conservative symptomatic treatment. In the cohort of 65 CRPC-Adeno patients, 41 individuals received a combination of novel endocrine therapy and either radiotherapy or chemotherapy. The chemotherapy regimen predominantly involved docetaxel. Additionally, 21 patients were administered novel endocrine therapy alone, while 3 patients underwent conservative treatment (**Table 6**). Among 23 patients monitored over an average follow-up period of 9 months (range: 2-34), 17 patients died from multi-organ tumor metastasis, with a median overall survival (OS) of 4 months (95% CI: 3.2-4.8). Notably, CRPC-Adeno patients exhibited a median OS of 9 months (95% CI: 6.7-11.3). In contrast, t-NEPC patients demonstrated significantly shorter survival durations than CRPC-Adeno patients, with a 2-year survival rate of less than 10% (**Figure 7**).

Table 4. Expression of p53 and RB1 in CRPC-Adeno and t-NEPC

		CRPC-Adeno (N=30)	t-NEPC (N=23)	*P*-value
p53	Wild-type	13 (43.33%)	4 (17.39%)	0.005
	Loss/overexpression	17 (56.67%)	19 (82.61%)	
RB1	Wild-type	24 (80.00%)	5 (21.74%)	<0.001
	Loss	6 (20.00%)	18 (78.26%)	
p53 and RB1	Not both abnormal	25 (83.33%)	6 (26.08%)	<0.001
	Both abnormal	5 (16.67%)	17 (73.92%)	

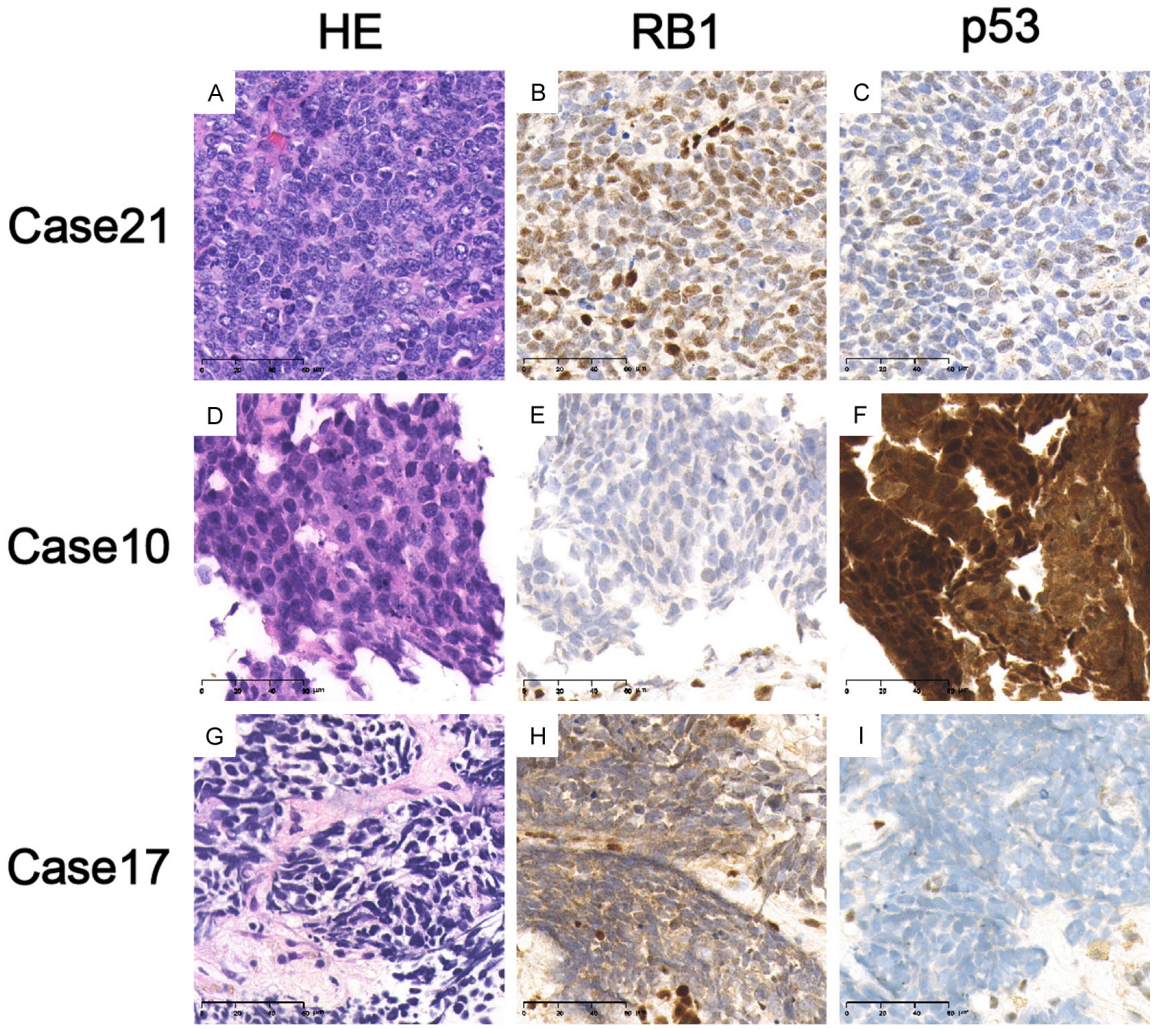

Figure 6. RB1 and p53 staining in t-NEPC.

When stratifying t-NEPC patients by immunohistochemical phenotypes, those who were AR-positive exhibited a higher median OS of 8 months (95% CI: 2.0-14.0) compared to AR-negative patients, who had a median OS of 4 months (95% CI: 3.1-4.9); however, this difference was not statistically significant. Similarly, patients with wild-type expression of RB1 and/or p53 demonstrated a higher median OS of 5 months (95% CI: 2.9-7.1) compared to those with aberrant expression of both RB1 and p53, who had a median OS of 4 months (95% CI: 3.0-5.0); again, this difference did not reach statistical significance (**Figure 8**).

Table 5. IHC results of 23 t-NEPC cases

Case	Prostate-associated marker	NE marker	AR	p53	RB1	POU2F3
1	+	+	-	Loss	Loss	-
2	+	+	-	Loss	Loss	-
3	+	+	+	Loss	Loss	-
4	+	+	-	Wild-type	Wild-type	-
5	+	+	+	Wild-type	Wild-type	-
6	+	+	+	Loss	Wild-type	-
7	+	+	-	Overexpression	Loss	-
8	+	+	-	Loss	Loss	-
9	+	+	+	Wild-type	Loss	-
10	-	-	-	Overexpression	Loss	-
11	+	+	-	Loss	Loss	-
12	+	+	+	Overexpression	Loss	-
13	+	+	+	Loss	Loss	-
14	+	+	-	Loss	Loss	-
15	-	+	-	Loss	Loss	-
16	+	+	-	Overexpression	Loss	-
17	-	+	-	Loss	Loss	-
18	-	+	-	Overexpression	Loss	-
19	-	+	-	Overexpression	Loss	-
20	+	+	+	Overexpression	Loss	-
21	-	+	-	Wild-type	Wild-type	-
22	+	+	-	Overexpression	Loss	-
23	+	+	-	Overexpression	Wild-type	-

Table 6. Treatment of CRPC-Adeno and t-NEPC patients

Treatment	CRPC-Adeno (N=65)	t-NEPC (N=23)
Novel endocrine therapy only	41	10
Radiotherapy/Chemotherapy	21	10
Conservative symptomatic treatment	3	3

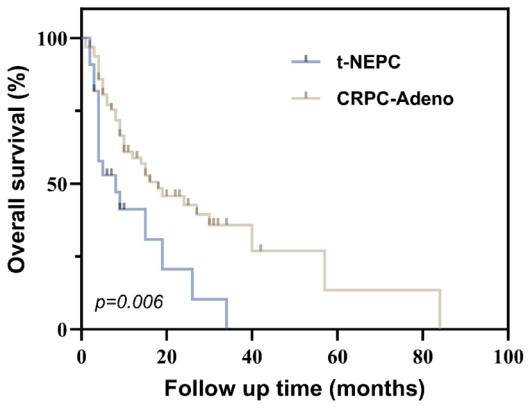

Figure 7. Overall survival of CRPC-Adeno and t-NEPC patients.

Discussion

The histopathological profile of prostate adenocarcinoma exhibits considerable morphological and immunohistochemical phenotypic alterations following endocrine therapy. A significant development is the emergence of t-NEPC, a clinically aggressive variant characterized by neuroendocrine differentiation that arises as an adaptive resistance mechanism to prolonged endocrine therapy. This transdifferentiation process exemplifies tumor lineage plasticity driven by epigenetic reprogramming and the selective pressure exerted by androgen receptor-targeted agents [3, 13]. Our findings indicate that the incidence of t-NEPC has increased over the past decade, potentially linked to the widespread clinical adoption of next-generation androgen receptor signaling pathway inhibitors. From 2009 to 2024, the First Hospital of Zhejiang University School of Medicine conducted over 28,000 pathological examinations on prostate cancer specimens. In contrast, only 88 cases (0.31%) of secondary pathological examination for CRPC were documented, highlighting a potential deficiency in clinical vigilance regarding t-NEPC. Our cohort of CRPC patients' median overall survival is shorter than that reported in certain other studies. This discrepancy may be attributable to selection bias, as only a subset of CRPC patients with more severe conditions underwent secondary biopsy or lesion resection [3, 13].

Following the initial diagnosis of prostate adenocarcinoma, t-NEPC patients exhibit a more rapid disease progression, develop resistance to ADT more quickly, and experience shorter progression-free intervals compared to CRPC-

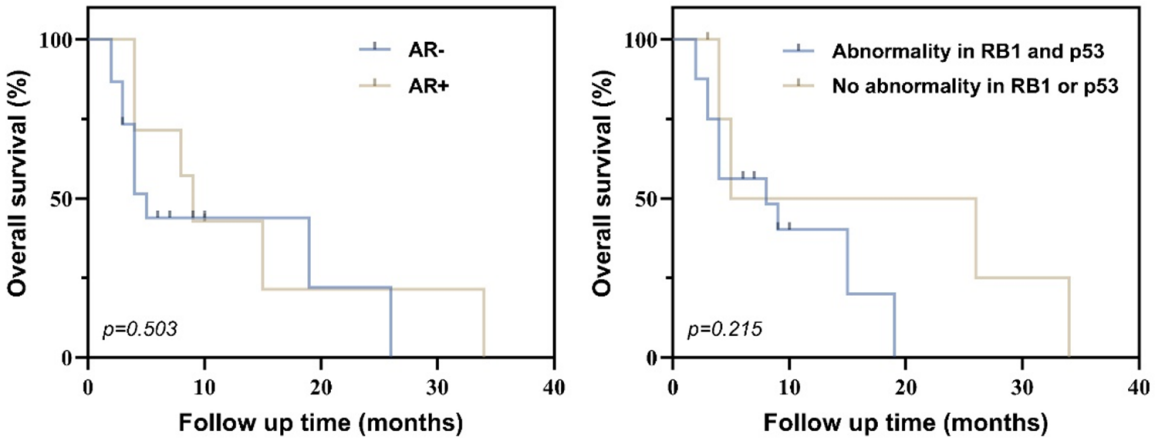

Figure 8. Overall survival of t-NEPC patients in different subgroups.

Adeno patients. t-NEPC patients demonstrated lower tPSA levels while exhibiting elevated levels of NSE, CEA, and CA199. NSE, a biomarker found in neural and neuroendocrine tissues, is extensively used as an indicator for neuroendocrine malignancies, particularly SCLC. NSE concentration is strongly correlated with tumor burden and facilitates early diagnosis [16], while its overexpression enhances the migration and invasion capabilities of SCLC cells [17]. CA199 and CEA are widely used tumor markers integral to the diagnosis and monitoring of various cancers, however, their sensitivity and specificity are generally lower than that of PSA in typical prostate cancers. In t-NEPC cases, PSA levels may not be sufficiently elevated to indicate disease progression. Nonetheless, CA199 and CEA can still provide valuable diagnostic insights in this context. CA199, similar to other markers like PSA, can be utilized in combination to enhance the diagnostic accuracy of prostate cancer [18], as well as to more effectively assess the condition and prognosis of the disease. Elevated CA199 levels may signify disease progression or recurrence [19, 20]. CEA has been linked to tumor progression and prognosis in neuroendocrine-differentiated prostate cancers [21]. Elevated CEA expression may correlate with the dedifferentiation of prostate cancer cells, a condition typically associated with a more aggressive tumor phenotype [22]. Consequently, monitoring fluctuations in biomarkers such as CA199 and CEA can furnish clinicians with critical reference data, facilitating timely modifications to diagnostic and therapeutic strategies. In cases where prostate adenocarcinoma progresses to the CRPC stage, and imaging reveals significant tumor advancement despite low PSA levels that are disproportionate to the tumor burden, along with elevated non-specific tumor markers (such as CEA, CA199) and serum neuroendocrine markers (such as NSE and ProGRP), the possibility of t-NEPC should be considered [23, 24]. It is advisable to conduct a re-biopsy of rapidly progressing lesions.

The inclusion criteria for t-NEPC in this study were determined based on morphological characteristics. Morphology continues to be the gold standard for diagnosing t-NEPC, which typically manifests as small cell carcinoma characterized by scant cytoplasmic tumor cells, elevated nucleoplasmic ratios, and inconspicuous nucleoli. In comparison to small cell carcinomas in other organs, t-NEPC exhibits a broader spectrum of morphological features, including more open chromatin, small nucleoli, and the presence of tumor giant cells, which can complicate its differentiation from adenocarcinomas with a Gleason score of 5+5. Nevertheless, morphological interpretation is inherently subjective and prone to significant inter-observer variability. Therefore, immunohistochemical staining for neuroendocrine markers is recommended to rule out t-NEPC in patients with CRPC who do not exhibit typical adenocarcinoma morphology upon repeat biopsy following the failure of endocrine therapy.

Immunohistochemistry (IHC) serves as a crucial technique for identifying the origin of tumors and assessing the presence or absence of neu-

roendocrine differentiation. Our study observed focal expression of neuroendocrine markers in the initial prostate adenocarcinoma of certain t-NEPC patients. Furthermore, in cases of CRPC that did not fulfill the histological criteria for small cell carcinoma, the tumors exhibited characteristics of more poorly differentiated adenocarcinomas, with a higher prevalence of neuroendocrine marker expression. The immunohistochemical phenotypes of t-NEPC align with that of small-cell neuroendocrine cancer found in other anatomical locations, characterized by the expression of neuroendocrine markers such as Syn, CgA, CD56, and INSM1. Tumors classified as t-NEPC frequently exhibit reduced or absent expression of prostate-associated markers, complicating the determination of tumor origin. Consequently, it is imperative to exclude other potential tumor sources by integrating medical history and imaging examinations. A limited number of neuroendocrine cells are dispersed within normal prostate glands [25]. Additionally, focal expression of neuroendocrine markers is observed in morphologically typical ductal or vesicular adenocarcinomas of the prostate, and this expression is positively correlated with the Gleason grade of prostate adenocarcinoma [26]. The prognostic significance of focal neuroendocrine differentiation in conventional prostate adenocarcinoma remains contentious. However, the majority of contemporary studies indicate that it does not have a direct correlation with either disease-specific or overall patient survival. Consequently, in the absence of morphological evidence of neuroendocrine differentiation, routine immunohistochemical staining for neuroendocrine markers is not recommended during the clinical diagnosis of prostate adenocarcinoma.

In contrast to primary small cell carcinoma of the prostate, t-NEPC exhibits varying levels of AR nuclear expression, indicating that the AR pathway remains transcriptionally active under epigenetic regulation at this stage [3]. In this study, t-NEPC cases demonstrated immunohistochemical phenotypes of NE+/AR-, NE+/AR+, and NE-/AR-. Conversely, CRPC-Adeno predominantly exhibited the NE-/AR+ immunohistochemical phenotype, with the most common NE+/AR- phenotype in t-NEPC not being observed. Furthermore, we identified NE+/AR+ and NE-/AR- immunohistochemical phenotypes in CRPC-Adeno, wherein AR-negative cases still expressed at least one prostate-associated biomarker. These immunohistochemical phenotypes may represent distinct stages in the transdifferentiation process from prostate adenocarcinoma to neuroendocrine carcinoma. In the clinicopathological diagnosis of prostate cancer, a distinct subtype known as amphitrite prostate carcinoma (AMPC) is characterized by bidirectional differentiation, exhibiting both adenocarcinoma and neuroendocrine features within the same cell population. This is evidenced by the presence of amphophilic cytoplasm and tissue morphology resembling high-grade adenocarcinoma, with frequent mitotic figures. Immunohistochemical analysis reveals the concurrent expression of prostate-associated and neuroendocrine markers. AMPC exemplifies the plasticity inherent in the prostate cancer lineage and predominantly arises following endocrine therapy, although it can also occur as a primary condition [27]. Notably, this subtype has yet to be explicitly defined in the current World Health Organization classification of prostate tumors.

The molecular characteristics of t-NEPC are typically characterized by a reduction or inactivation of the AR signal transduction pathway, deletion of the RB1 gene, and mutation of the TP53 gene. The loss of Rb1 protein is nearly universal in prostatic small-cell neuroendocrine carcinoma, suggesting its potential utility as a biomarker for t-NEPC [28]. Our study demonstrates that abnormal expression of p53 and RB1 is significantly more prevalent in t-NEPC compared to CRPC-Adeno with a Gleason score of 5+5. Immunohistochemical evaluation of these markers provides a practical method for distinguishing t-NEPC from CRPC-Adeno with a Gleason score of 5+5, with the latter potentially harboring AMPC components. Given the limitations of traditional neuroendocrine markers and morphological evaluation alone, immunohistochemical analysis of RB1 and p53 is instrumental in diagnosing t-NEPC, particularly when the tumor displays small cell carcinoma morphology with inadequate expression of neuroendocrine markers. However, aberrant expression of RB1 and p53 is neither necessary nor sufficient for the diagnosis of t-NEPC. Single-cell transcriptome sequencing (scRNA-seq) data analysis from Chinese prostate cancer patients revealed the absence of RB1 dele-

tion or TP53 mutation in certain t-NEPC patients [29]. This suggests that adenocarcinoma cells may transform t-NEPC via alternative molecular pathways.

With the growing body of research on the molecular pathological classification of prostate cancer, it has become imperative to develop a molecular classification for t-NEPC. Neuroendocrine cancers are regulated by various transcription factors, and the molecular classification of small cell lung cancer (SCLC) based on transcription factors such as ASCL1, NEUROD1, POU2F3, and YAP1 provides a foundation for precision-targeted therapies in lung cancer patients [30]. Given the similarities between t-NEPC and small-cell lung cancer, establishing a molecular classification for t-NEPC and identifying its disease drivers are essential for advancing precision-targeted therapies for t-NEPC. POU2F3, a lineage-defining transcription factor, plays a crucial role in the differentiation of tuft cells, which are epithelial chemosensory cells found in numerous organs, including the respiratory tract [31]. RNA-seq data from a clinical cohort indicated that POU2F3 is expressed in a subset of patients with CRPC and t-NEPC. The expression of POU2F3 appeared to be mutually exclusive with ASCL1 and inversely correlated with RB1 expression. POU2F3 is present in clinical cases of CRPC and t-NEPC and may serve as a biomarker for the transition from CRPC to t-NEPC [32]. In 13.41% (33/246) of SCLC cases, POU2F3 exhibited strong nuclear staining, with negative or minimal labeling for neuroendocrine markers. Compared with POU2F3-negative SCLC, SCLC-P harbored fewer TP53 and RB1 mutations [33]. Nonetheless, POU2F3 expression was absent in all 23 cases of t-NEPC within this cohort. This absence may be attributable to the infrequent occurrence of cases exhibiting wild-type expression of p53 and RB1 or lacking neuroendocrine marker expression in our cohort. Consequently, these findings imply that the sensitivity of POU2F3 as a biomarker for t-NEPC might be constrained. Further research is necessary to elucidate the heterogeneity of POU2F3 expression in CRPC and t-NEPC populations.

Currently, the management of t-NEPC predominantly depends on platinum-based chemotherapy regimens, which are associated with limited efficacy and a notably poor prognosis. However, small-molecule inhibitors targeting potential therapeutic targets such as MYCN-AURKA [34], KIT [35], and DDL3 [36] are undergoing research and clinical trials. These advancements offer new possibilities and hope for the development of individualized and precise treatment strategies for t-NEPC.

The limitations of this study are that it was limited to IHC testing and there may be differences at the genetic level. The small cohort size and the fact that this was a retrospective study limit the generalisability of the results. Nonetheless, our findings provide new insights into the diagnosis of t-NEPC and add to our understanding of the disease process, thus contributing to improved management of t-NEPC.

Conclusions

This study analyzed the immunohistochemical findings in a cohort of 23 Chinese patients diagnosed with t-NEPC, with a particular focus on the proteins RB1 and p53. CRPC Patients with a brief recurrence period, low serum tPSA levels, and elevated NSE, CEA, and CA199 levels should remain vigilant regarding the potential transformation to t-NEPC. The immunohistochemical evaluation of RB1 and p53 is very helpful in some difficult cases. The accumulation of additional cases is essential to advance the molecular classification of t-NEPC.

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Disclosure of conflict of interest

None.

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