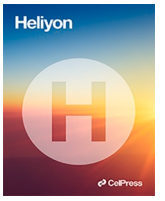
Heliyon 10 (2024) e34864

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Changes in immunophenotypes after neoadjuvant endocrine therapy for prostate cancer and their clinical significance

Mei Li [a,b,1], Kun Zhong [c,1], Guifang He [a], Yu Yin [a,c,*]

[a] Department of Pathology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, 230022, PR China
[b] Department of Pathology, NO.2 People' s Hospital of Fuyang City, Fuyang, Anhui, 236015, PR China
[c] Department of Pathology, School of Basic Medical Sciences, Anhui Medical University, Hefei, Anhui, 230032, PR China

ARTICLE INFO

Keywords:
Immunophenotype
Neoadjuvant endocrine therapy
Prostate cancer
Prostate-specific antigen
Progression-free survival

ABSTRACT

Background: To investigate changes in the immunophenotypes of androgen receptor (AR), prostate-specific antigen (PSA), synaptophysin (Syn), chromogranin A (CgA), p53 and Ki-67 after neoadjuvant endocrine therapy (NET) for prostate cancer (PCa) and to analyze their clinical significance.

Methods: Paired paraffin samples were collected from 40 PCa patients before and after NET, and immunohistochemistry were used to detect AR, PSA, Syn, CgA, p53 and Ki-67 expression. Based on The Cancer Genome Atlas (TCGA), Kaplan–Meier survival curves were plotted for analysis of PSA and Ki-67 expression in relation to progression-free survival (PFS).

Results: After NET, the mean scores for PSA and Ki-67 expression in PCa patients were lower than those before NET ($P < 0.05$), while the mean scores for Syn and CgA expression were higher than those before NET ($P < 0.05$). The mean Gleason score and WHO/ISUP (World Health Organization/International Society of Urological Pathology) grade after NET were lower than those before NET ($P < 0.05$). In PCa patients who had not yet received NET, PSA expression correlated positively with Gleason score and WHO/ISUP grade and negatively with Ki-67 expression ($P < 0.05$); p53 expression correlated negatively with Gleason score and WHO/ISUP grade ($P < 0.05$). TCGA showed that PFS was lower in PCa patients with high PSA and Ki-67 expression ($P < 0.05$).

Conclusions: PSA and Ki-67 protein expressions decreased significantly in PCa patients after NET and can be used as biological markers for prognostic assessment of PCa patients. NETs may induce a neuroendocrine (NE) phenotype in PCa. Monitoring the immunophenotypes of PCa patients after NET may inform assessment of efficacy and prognosis.

Abbreviations: AR, androgen receptor; CgA, chromogranin A; ISUP, International Society of Urological Pathology; NE, neuroendocrine; NET, neoadjuvant endocrine therapy; PCa, prostate cancer; PSA, prostate-specific antigen; PFS, progression-free survival; Syn, synaptophysin; TCGA, The Cancer Genome Atlas; WHO, World Health Organization.

* Corresponding author. The First Affiliated Hospital of Anhui Medical University, 218 Jixi Road, Hefei, Anhui 230022; Department of Pathology, Anhui Medical University, 81 Meishan Road, Hefei, Anhui, 230032, PR China.

E-mail addresses: 1278298752@qq.com (M. Li), 2145010070@stu.ahmu.edu.cn (K. Zhong), 18919690338@163.com (G. He), yinyu@ahmu.edu.cn (Y. Yin).

[1] These authors contributed equally.

https://doi.org/10.1016/j.heliyon.2024.e34864
Received 18 January 2024; Received in revised form 16 July 2024; Accepted 17 July 2024
Available online 26 July 2024

1. Introduction

Prostate cancer (PCa) is the most common malignant tumor of the male reproductive system [1] and the second most common cancer in men after nonmelanoma skin cancers such as basal cell carcinoma and squamous cell carcinoma [2]. It is also a highly heterogeneous group of hormone-responsive tumors [3]. Neoadjuvant endocrine therapy (NET) is the standard regimen for preoperative adjuvant therapy and postoperative consolidation in PCa [4], and this therapy induces shrinkage of PCa tumors primarily through degenerative changes or reductions in cytokine proliferative activity [5]. NET response can manifest as an altered tumor immunophenotype [6].

For patients with high-risk, metastatic and refractory advanced PCa, it is important to monitor response to treatment and explore highly effective combination therapies [7]. Use of immunohistochemistry to detect molecular phenotypes of PCa, such as p53, yielded results that are highly consistent with genomic analyses and is able to accurately identify the heterogeneity of PCa [8]. However, there are few reports in the literature on NETs and changes in the PCa immunophenotype. Therefore, this article focuses on changes in androgen receptor (AR), prostate-specific antigen (PSA), synaptophysin (Syn), chromogranin A (CgA), p53 and Ki-67 expression in PCa before and after NET and analyzes their clinical significance, aiming to provide a reference for evaluating the effect of NET as well as the prognosis of PCa patients.

2. Materials and methods

2.1. Collection of clinical samples

Fig. 1 illustrated the flowchart of the study procedure. Paired PCa paraffin specimens from 40 before and after NET cases archived in the Department of Pathology of the First Affiliated Hospital of Anhui Medical University between January 2018 and June 2020 were collected. All participants gave their consent after telephone contact and approval was obtained from the Biomedical Ethics Committee of Anhui Medical University (approval number: 20190406). The patients were 51–82 years old, with a median age of 71.5 years, and their clinical data were complete. All patients were treated with goserelin acetate sustained-release implants in combination with bicalutamide tablets for a median treatment duration of 4 months after diagnosis of PCa by prostate puncture biopsy, followed by radical prostatectomy. Each prostate lesion section was assessed by two pathologists. Pathological diagnosis, Gleason grading and WHO/ISUP (World Health Organization/International Society of Urological Pathology) grouping according to the WHO (2022) classification of tumors [9] showed that the histological type of all 40 PCa cases was prostate adenocarcinoma.

2.2. Immunohistochemistry

The MaxVision immunohistochemical method was used. PCa biopsy tissue and radical specimens were processed into 4 μm-thick tissue sections. Following deparaffinization and rehydration with xylene via graded ethanol, the sections were boiled in 10 mM citrate buffer (pH 6.0) and then treated with 3 % H_2O_2 for 10 min to block endogenous peroxidase activity. Anti-PSA (ZM-0218), anti-Ki-67 (ZM-0167), anti-Syn (ZA-0506), anti-CgA (ZM-0076), anti-p53 (ZM-0408), and anti-AR (ZA-0054) antibodies were added after rinsing

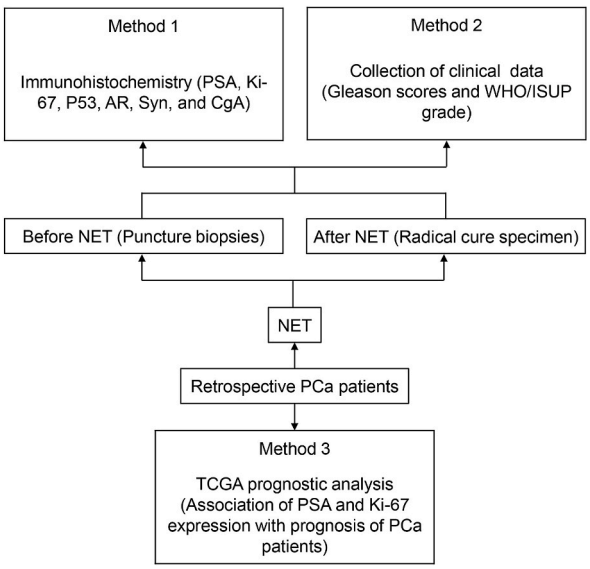


Fig. 1. Flow chart of the study design. NET: neoadjuvant endocrine therapy; PCa: prostate cancer; TCGA: The Cancer Genome Atlas; WHO/ISUP: World Health Organization/International Society of Urological Pathology.

with PBS buffer; the primary antibodies were obtained from Beijing Zhong Shan-Golden Bridge Biological Technology Co., Ltd. (Beijing, China). This was followed by overnight incubation at 4 °C. The next day, after rinsing with PBS, ready-to-use immunohistochemical reagents (MaxVision-HRP mouse/rabbit) from Fuzhou Maixin Biotech Co., Ltd. (Fuzhou, Fujian, China) were added. After incubation, PBS rinsing, DAB color development, hematoxylin restaining, gradient ethanol dehydration, xylene transparency, neutral resin sealing, and microscopy were performed.

2.3. Interpretation of immunohistochemistry results

Positive Ki-67, p53, and AR protein expression was found in the nucleus; positive PSA, Syn, and CgA proteins expression was detected in the cytoplasm. Staining was considered positive when yellowish to brownish in color. The results were interpreted by two pathologists using a double-blind method. Five high-magnification fields of view (400 ×) were randomly selected for each section and scored according to a semiquantitative scoring system: (1) score according to the intensity of staining of positive cells - no staining is 0 points, light yellow is 1 point, yellow is 2 points, and brownish yellow is 3 points; (2) score according to the percentage of positive cells - <1 % is 0 points, 1 %–10 % is 1 point, 11 %–50 % is 2 points, and >50 % is 3 points. The results of the two scores were multiplied: <6 was classified as low expression, and ≥6 was classified as high expression.

2.4. TCGA prognostic analysis

The https://www.xiantaozi.com/database is based on The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/) and the following analyses were performed using this database. RNA sequencing data in level 3 HTSeq-FPKM format from 551 PCa cases in TCGA were analyzed, and survival curves were plotted by the Kaplan–Meier method using the log-rank test and Cox regression for prognostic analysis.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 23.0 software (SPSS Inc; Chicago, IL, USA). Differences between the two sets of qualitative data were compared by the $\chi 2$ test. If $1 \leq T \leq 5$ occurs in a certain lattice, then a continuity correction is required. Fisher's exact test was used for any lattice where $T < 1$ occurred. As the quantitative data did not follow a normal distribution, the rank sum test was used. In all cases, statistical significance was defined as $P < 0.05$.

3. Results

3.1. Changes in PCa immunophenotype after NET

Expression of AR, PSA, Syn, CgA, p53 and Ki-67 in paired tissues of 40 PCa patients before and after NET was examined by the MaxVision immunohistochemical method, which showed Ki-67, p53 and AR positivity in the nucleus and PSA, Syn and CgA positivity in the cytoplasm. After NET, the high expression rates of PSA, Ki-67, and AR showed varying degrees of downregulation, but only downregulation of the high expression rate of Ki-67 was statistically significant. The high expression rates of Syn, CgA, and p53 showed varying degrees of upregulation, but only upregulation of the high expression rate of Syn was statistically significant ($P < 0.05$, Table 1, Fig. 2). Changes in p53 and AR were still not statistically significant, as found by various immunophenotypic scores after NET. In contrast, the decrease in PSA and Ki-67 scores was statistically significant, as was the increase in Syn and CgA scores ($P < 0.05$, Table 2, Fig. 3).

Table 1
Expression of immune indicators before and after NET in PCa patients, [n (%)].

Group	n	PSA		P value	Ki-67		P value	Syn		P value
		Low	High		Low	High		Low	High	
Before	40	9 (22.5)	31 (77.5)	0.446	31 (77.5)	9 (22.5)	0.007[a]	38 (95.0)	2 (5.0)	0.023[a]
After	40	12 (30.0)	28 (70.0)		39 (97.5)	1 (2.5)		31 (77.5)	9 (22.5)	
		CgA			p53			AR		
Group	n	Low	High	P value	Low	High	P value	Low	High	P value
Before	40	32 (80.0)	8 (20.0)	0.431	25 (62.5)	15 (37.5)	0.818	7 (17.5)	33 (82.5)	0.189
After	40	29 (72.5)	11 (27.5)		24 (60.0)	16 (40.0)		12 (30.0)	28 (70.0)	

AR, androgen receptor; CgA, chromogranin A; NET, neoadjuvant endocrine therapy; PCa, prostate cancer; PSA, prostate-specific antigen; Syn, synaptophysin.
[a] *P* value statistically significant.

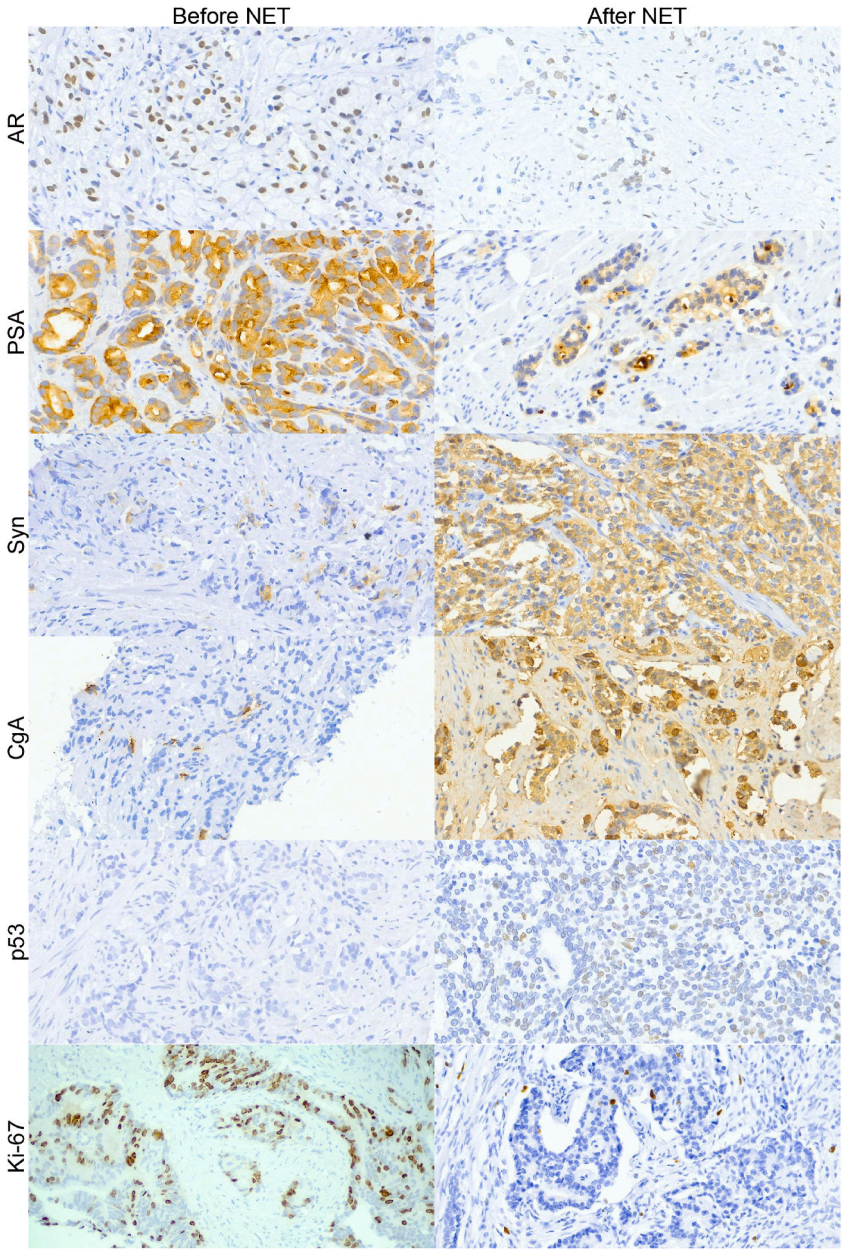


Fig. 2. Expression of various immune indicators in prostate cancer (PCa) patients before and after neoadjuvant endocrine therapy (NET) Protein expression was detected using immunohistochemistry. Ki-67, p53 and AR positivity in the nucleus and PSA, Syn and CgA positivity in the cytoplasm. Staining was considered positive when yellowish to brownish in color. Original magnification, × 200.

Table 2
Comparison of various immunophenotype scores before and after NET in PCa patients.

Group	n	PSA	Ki-67	Syn	CgA	p53	AR
Before	40	6.980 ± 2.567	3.500 ± 1.414	1.150 ± 1.861	1.500 ± 2.253	3.480 ± 3.178	5.430 ± 1.357
After	40	5.900 ± 2.134	1.800 ± 1.636	2.150 ± 2.293	2.500 ± 3.530	3.530 ± 3.130	5.230 ± 1.593
Z value		−2.764	−4.154	−3.749	−3.101	−0.286	−0.415
P value		0.006[a]	<0.001[a]	<0.001[a]	0.002[a]	0.775	0.678

AR, androgen receptor; CgA, chromogranin A; NET, neoadjuvant endocrine therapy; PCa, prostate cancer; PSA, prostate-specific antigen; Syn, synaptophysin.

[a] *P* value statistically significant.

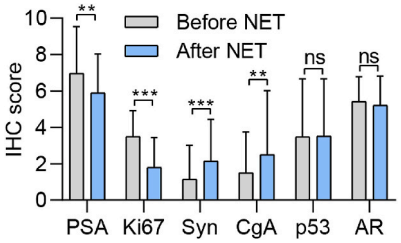


Fig. 3. Comparison of various immunophenotype scores in prostate cancer (PCa) patients before and after neoadjuvant endocrine therapy (NET) *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; NS: not significant. Statistical data using IBM SPSS Statistics 23.0 software. Graphing with GraphPad Prism 8.0.2 software.

3.2. Changes in clinicopathologic features of PCa after NET

Analysis of Gleason scores and WHO/ISUP grade before and after NET showed a decrease in the proportion of PCa patients with Gleason score of 8–10 and WHO/ISUP grade of 4–5 after NET. However, the difference was not statistically significant (Table 3). The numerical decrease in Gleason score and WHO/ISUP grade of PCa patients after NET was statistically significant ($P < 0.05$, Table 4, Fig. 4).

3.3. Relationship between PSA and p53 expression and clinicopathologic features in PCa before NET

Table 5 shows the results after collating the clinical data of the patients. PSA expression correlated positively with Gleason score and WHO/ISUP grade, with coefficients of contingency (r_n) of 0.393 and 0.393. p53 expression correlated negatively with Gleason score and WHO/ISUP grade, with coefficients of contingency (r_n) of −0.406 and −0.455, respectively. PSA correlated negatively with expression of Ki-67, with a coefficient of contingency (r_n) of −0.713. p53 did not correlate significantly with expression of Ki-67.

3.4. Association of PSA and Ki-67 expression with prognosis of PCa patients

The association of PSA and Ki-67 expression with progression-free survival (PFS) in 551 PCa patients in the TCGA database showed that PFS was reduced in patients with high expression of PSA (Fig. 5A) and Ki-67 (Fig. 5B) compared to those with low expression ($P < 0.05$).

4. Discussion

NET with goserelin acetate sustained-release implants in combination with bicalutamide tablets is able to ameliorate moderate to severe lower urinary tract symptoms (LUTS) to some extent in patients with advanced PCa [10]. In patients with high PSA expression after PCa resection, it has been reported that salvage prostate bed radiotherapy (PBRT) combined with NETs significantly prolongs PFS [11]. By compiling clinical data, we found in this study a significant reduction in Gleason score and WHO/ISUP grade of PCa after NET. A previous study has also shown that NETs can achieve tumor downgrade and downstaging, increase tumor resection rates, and reduce the rate of positive resection margins [12]. The serum PSA level is well known to assist in the diagnosis of PCa, but the specificity of determining clinical staging based on serum PSA is poor, and diagnostic bias and overtreatment may occur [13]. PSA does not reliably differentiate between mild and life-threatening disease at the time of diagnosis [14]. The Ki-67 protein has been widely used as a proliferation marker for human tumor cells [15]. It has also been shown that Ki-67 can be used as a prognostic marker after localized PCa [16]. PSA correlated significantly negatively with Ki-67 in this study, which may support the above conclusion about PSA. In this study, we confirmed that PSA and Ki-67 protein expression levels were significantly reduced after NET by detecting the immunophenotype of PCa; TCGA-based prognostic analysis showed prolonged PFS in PCa patients with low PSA and Ki-67 protein expression. These results indicate that NETs inhibit tumor growth, delay disease progression, and improve quality of life in PCa patients.

NETs also induce neuroendocrine (NE) transdifferentiation in PCa by selecting a subpopulation of androgen-independent cells in PCa into a dominant clone [17]. PCa NE transdifferentiation promotes tumor progression by allowing tumor cells to evade the adaptive mechanisms of various therapies, leading to therapy resistance, which promotes tumor progression and is strongly associated with

Table 3
Changes in Gleason score and WHO/ISUP grade in PCa patients after NET, [n (%)].

Group	n	Gleason score		P value	WHO/ISUP grade		P value
		6–7	8–10		1–3	4–5	
Before	40	13(32.5)	27(67.5)	0.070	13 (32.5)	27 (67.5)	0.070
After	40	21 (52.5)	19 (47.5)		21 (52.5)	19 (47.5)	

NET, neoadjuvant endocrine therapy; PCa, prostate cancer.

Table 4
Changes in Gleason score and WHO/ISUP grade in PCa patients after NET, [n (%)].

Group	n	Gleason score	WHO/ISUP grade
Before	40	8.400 ± 1.221	4.125 ± 1.005
After	40	7.700 ± 0.812	3.475 ± 1.072
Z value		−3.196	−3.228
P value		0.001[a]	0.001[a]

NET, neoadjuvant endocrine therapy; PCa, prostate cancer.
[a] *P* value statistically significant.

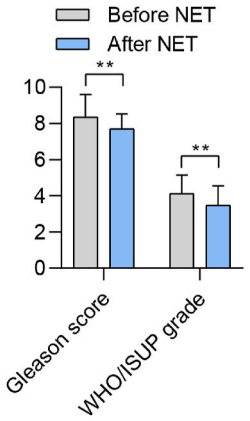


Fig. 4. Changes in some clinicopathologic features of prostate cancer (PCa) patients before and after neoadjuvant endocrine therapy (NET) *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; NS: not significant. Statistical data using IBM SPSS Statistics 23.0 software. Graphing with GraphPad Prism 8.0.2 software.

mortality in PCa patients [18]. NETs have been reported to induce NE transdifferentiation in PCa cell lines associated with down-regulation of AR, PSA, and several miRNAs [18]. Immunohistochemistry staining for NE markers (mainly CgA and Syn) is commonly used for NE differentiation [19]. This study confirmed upregulation of Syn and CgA protein expression in PCa after NET, and the coexpression rate of Syn and CgA increased from 17.5 % to 40.0 %, suggesting that some tumor cells develop a NE phenotype after NET. We will sequence cells exhibiting NET-induced NE differentiation, and subsequent steps will be performed contingent upon sequencing results. MicroRNAs exercise pleiotropic regulation and are integral parts of an extensive array of signaling pathways [20], and a systematic review has postulated microRNAs as key epigenetic players in NEPCa and as diagnostic biomarkers for NEPCa [21]. It is also feasible to conduct additional research on upstream regulatory mechanisms of NEPCa pertaining to microRNAs.

Key genetic alterations during PCa development include *TP53* (Gene ID: 7157) deletion and/or mutation and AR amplification and/or mutation [22]. It has been shown that deletion of *RB1* (Gene ID: 5925) and *TP53* (Gene ID: 7157) genes promotes phenotypic shift in PCa tumor cells and tumor growth by inducing stem cell development and activating the epithelial-mesenchymal transition (EMT) [23]. In this study, we analyzed the relationship between the p53 protein expression level and clinicopathological features of PCa and found that p53 expression correlated significantly negatively with Gleason score and WHO/ISUP grade, a result that also suggests that deletion of the *TP53* (Gene ID: 7157) oncogene may be associated with PCa progression. In addition to the fact that normal development and maintenance of the prostate gland depends on androgens acting through AR, AR continues to play an important role in the development and progression of PCa [24]. Indeed, the transcription factor AR regulates expression of a variety of prostate functional protease genes, including *KLK3* (Gene ID: 354), which encodes PSA, and NET can intervene in posttranslational modification of AR and affect expression of AR and its downstream regulatory molecules [25]. The splice variant of AR possesses weak transcription factor activity, and constitutively active androgen receptor splice variant-7 (AR-V7) has been associated with resistance to endocrine therapy and shorter overall survival in PCa [26]. However, this study did not find a statistically significant decrease in AR after NET. In the future, the sample cohort can be further expanded. This study has some limitations. NET was not universal at the time the experiment was conducted, so the sample size collected was not large enough. In addition, it would have been possible to add a prognostic analysis such as overall survival at the end of this experiment, but the sample has not yet been followed for 5 years. The immunohistochemistry markers in this study may reflect the therapeutic effect of NET in the patient's treatment system, potentially providing more precise directives for subsequent treatment. Specifically, 1) if NET has an effect, is it possible to strengthen the application of endocrine therapy, or some patients are no longer suitable for surgery, then they can rely on endocrine therapy; 2) if NET has no effect, it is necessary to reassess the intensity and duration of radiotherapy and chemotherapy after surgery. In any case, this discovery is beneficial for personalized treatment of tumor patients.

In conclusion, the significant reduction in PSA and Ki-67 protein expression levels after NET for PCa can be used as biological markers for prognostic assessment of patients with PCa, which can provide a reference for clinical individualized treatment and

Table 5
Relationship between PSA and p53 expression and clinicopathologic features in PCa before NET, [n (%)].

Characteristics	n	PSA			p53		
		Low	High	P value	Low	High	P value
Age, yr							
≤70	19	4(21.1)	15 (78.9)	1.000	14 (73.7)	5 (26.3)	0.165
>70	21	5 (23.8)	16 (76.2)		11 (52.4)	10 (47.6)	
Gleason score							
6-7	13	6 (46.2)	7(53.8)	0.037[a]	4(30.8)	9 (69.2)	0.011[a]
8-10	27	3 (11.1)	24 (88.9)		21(77.8)	6 (22.2)	
WHO/ISUP grade							
1-3	13	6 (46.2)	7 (53.8)	0.037[a]	4 (30.8)	9 (69.2)	0.011[a]
4-5	27	3 (11.1)	24 (88.9)		21 (77.8)	6 (22.2)	
Resection margin							
No	17	4 (23.5)	13 (76.5)	1.000	12 (70.6)	5 (29.4)	0.364
Yes	23	5 (21.7)	18 (78.3)		13 (56.5)	10 (43.5)	
Perineural invasion							
No	8	2 (25.0)	6 (75.0)	1.000	5 (62.5)	3 (37.5)	1.000
Yes	32	7 (21.9)	25 (78.1)		20 (62.5)	12 (37.5)	
Lymph node metastasis							
No	30	6 (20.0)	24 (80.0)	0.827	20 (66.7)	10 (33.3)	0.572
Yes	10	3 (30.0)	7 (70.0)		5 (50.0)	5 (50.0)	
Ki-67							
Low	31	4 (12.9)	27 (87.1)	0.025[a]	19 (61.3)	12 (38.7)	1.000
High	9	5 (55.6)	4 (44.4)		6 (66.7)	3 (33.3)	
Syn							
Low	38	9 (23.7)	29 (76.3)	1.000	24 (63.2)	14 (36.8)	1.000
High	2	0 (0.0)	2 (100.0)		1 (50.0)	1 (50.0)	
CgA							
Low	32	7 (21.9)	25 (78.1)	1.000	22 (68.8)	10 (31.3)	0.221
High	8	2 (25.0)	6 (75.0)		3 (37.5)	5 (62.5)	
AR							
Low	7	2 (28.6)	5 (71.4)	1.000	6 (85.7)	1 (14.3)	0.334
High	33	7 (21.2)	26 (78.8)		19 (57.6)	14 (42.4)	

AR, androgen receptor; CgA, chromogranin A; NET, neoadjuvant endocrine therapy; PCa, prostate cancer; PSA, prostate-specific antigen; Syn, synaptophysin.
[a] *P* value statistically significant.

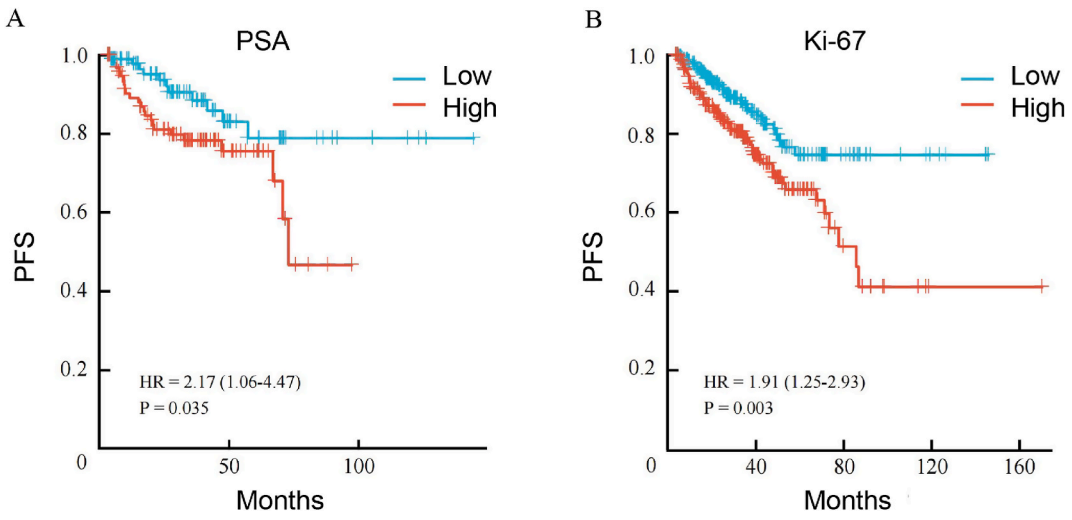


Fig. 5. Association of PSA and Ki-67 expression with prognosis of prostate cancer (PCa) patients Analysis of the effect of (A) PSA and (B) Ki-67 expression on progression-free survival (PFS) in PCa patients in the Cancer Genome Atlas (TCGA).

prognostic risk assessment. In addition, NETs may lead to emergence of a NE phenotype of PCa, and further explorations are needed regarding the molecular mechanisms by which NETs alter the immune phenotype of PCa.

5. Conclusions

PSA and Ki-67 protein expressions decreased significantly in PCa patients after NET and can be used as biological markers for prognostic assessment of PCa patients. NETs may induce a NE phenotype in PCa. Monitoring the immunophenotypes of PCa patients after NET may inform assessment of efficacy and prognosis.

Ethics approval

This study was approved by the Biomedical Ethics Committee of Anhui Medical University (approval number: 20190406). This study is an observational study, and the study followed STROBE guidelines.

Informed consentpatien

All participants gave their consent.

Registry and the registration no. of the study/trial

N/A.

Animal studies

N/A.

Data availability statement

Data associated with the study has not been deposited into a publicly available repository and data will be made available on request.

CRediT authorship contribution statement

Mei Li: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Kun Zhong:** Writing – review & editing, Software, Methodology, Investigation. **Guifang He:** Methodology, Investigation. **Yu Yin:** Writing – review & editing, Methodology, Investigation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:YuYin reports financial support was provided by National Natural Science Foundation of China and Key Natural Science Research Project of Anhui Universities. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***.

	Item No	Recommendation	**Page** No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7
Data sources/ measurement	8[a]	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6, 7*
Bias	9	Describe any efforts to address potential sources of bias	NA

(*continued on next page*)

(*continued*)

	Item No	Recommendation	Page No
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7, 8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13[a]	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	8, 9
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	27
Descriptive data	14[a]	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, 9
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15[a]	Report numbers of outcome events or summary measures	8, 9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95 % confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12, 13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Acknowledgement

The authors wish to acknowledge the financial support from the National Natural Science Foundation of China (grant no. 81972414) and the Key Natural Science Research Project of Anhui Universities (grant no. 2023AH053332).

References

[1] R.L. Siegel, K.D. Miller, N.S. Wagle, et al., Cancer statistics, 2023, Ca - Cancer J. Clin. 73 (1) (2023) 17–48, https://doi.org/10.3322/caac.21763.
[2] M. Nguyen-Nielsen, M. Borre, Diagnostic and therapeutic strategies for prostate cancer, Semin. Nucl. Med. 46 (6) (2016) 484–490, https://doi.org/10.1053/j.semnuclmed.2016.07.002.
[3] G. Wang, D. Zhao, D.J. Spring, et al., Genetics and biology of prostate cancer, Genes Dev. 32 (17–18) (2018) 1105–1140, https://doi.org/10.1101/gad.315739.118.
[4] A.M. Horning, Y. Wang, C.K. Lin, et al., Single-cell RNA-seq reveals a subpopulation of prostate cancer cells with enhanced cell-cycle-related transcription and attenuated androgen response, Cancer Res. 78 (4) (2018) 853–864, https://doi.org/10.1158/0008-5472.Can-17-1924.
[5] T. Yanagisawa, P. Rajwa, C. Thibault, et al., Androgen receptor signaling inhibitors in addition to docetaxel with androgen deprivation therapy for metastatic hormone-sensitive prostate cancer: a systematic review and meta-analysis, Eur. Urol. 82 (6) (2022) 584–598, https://doi.org/10.1016/j.eururo.2022.08.002.
[6] M.S. Soloway, R. Watson, The role of induction androgen deprivation prior to radical prostatectomy, Eur. Urol. 29 (Suppl 2) (1996) 114–118, https://doi.org/10.1159/000473850.
[7] D. Prezioso, T. Lotti, R. Montironi, et al., Role of neoadjuvant treatment in clinically confined prostate cancer. Takeda NHT Italian Group, Eur. Urol. 35 (Suppl 1) (1999) 17–21, https://doi.org/10.1159/000052302. ; discussion 22.
[8] E. Abdulfatah, Z.R. Reichert, M.S. Davenport, et al., De novo neuroendocrine transdifferentiation in primary prostate cancer-a phenotype associated with advanced clinico-pathologic features and aggressive outcome, Med. Oncol. 38 (3) (2021) 26, https://doi.org/10.1007/s12032-021-01473-2.
[9] S.H. Kim, W.S. Park, B.R. Park, et al., PSCA, Cox-2, and Ki-67 are independent, predictive markers of biochemical recurrence in clinically localized prostate cancer: a retrospective study, Asian J. Androl. 19 (4) (2017) 458–462, https://doi.org/10.4103/1008-682x.180798.
[10] J. Cyrta, D. Prandi, A. Arora, et al., Comparative genomics of primary prostate cancer and paired metastases: insights from 12 molecular case studies, J. Pathol. 257 (3) (2022) 274–284, https://doi.org/10.1002/path.5887.
[11] G.J. Netto, M.B. Amin, D.M. Berney, et al., The 2022 World Health organization classification of tumors of the urinary system and male genital organs-Part B: prostate and urinary tract tumors, Eur. Urol. 82 (5) (2022) 469–482, https://doi.org/10.1016/j.eururo.2022.07.002.
[12] M. Mason, P. Richaud, Z. Bosnyak, et al., Degarelix versus goserelin plus bicalutamide in the short-term relief of lower urinary tract symptoms in prostate cancer patients: results of a pooled analysis, Low. Urin. Tract. Symptoms 9 (2) (2017) 82–88, https://doi.org/10.1111/luts.12114.

[13] A. Pollack, T.G. Karrison, A.G. Balogh, et al., The addition of androgen deprivation therapy and pelvic lymph node treatment to prostate bed salvage radiotherapy (NRG Oncology/RTOG 0534 SPPORT): an international, multicentre, randomised phase 3 trial, Lancet 399 (10338) (2022) 1886–1901, https://doi.org/10.1016/s0140-6736(21)01790-6.
[14] A. Bourefis, H. Berredjem, O. Djeffal, et al., HSP27/Menin expression as new prognostic serum biomarkers of prostate cancer aggressiveness independent of PSA, Cancers 14 (19) (2022), https://doi.org/10.3390/cancers14194773.
[15] J. Tosoian, S. Loeb, PSA and beyond: the past, present, and future of investigative biomarkers for prostate cancer, Sci. World J. 10 (2010) 1919–1931, https://doi.org/10.1100/tsw.2010.182.
[16] X. Sun, P.D. Kaufman, Ki-67: more than a proliferation marker, Chromosoma 127 (2) (2018) 175–186, https://doi.org/10.1007/s00412-018-0659-8.
[17] T.C. Yuan, S. Veeramani, M.F. Lin, Neuroendocrine-like prostate cancer cells: neuroendocrine transdifferentiation of prostate adenocarcinoma cells, Endocr. Relat. Cancer 14 (3) (2007) 531–547, https://doi.org/10.1677/erc-07-0061.
[18] J.T. Dankert, M. Wiesehöfer, E.D. Czyrnik, et al., The deregulation of miR-17/CCND1 axis during neuroendocrine transdifferentiation of LNCaP prostate cancer cells, PLoS One 13 (7) (2018) e0200472, https://doi.org/10.1371/journal.pone.0200472.
[19] J.Y. Tsang, G.M. Tse, Breast cancer with neuroendocrine differentiation: an update based on the latest WHO classification, Mod. Pathol. 34 (6) (2021) 1062–1073, https://doi.org/10.1038/s41379-021-00736-7.
[20] K. Uray, E. Major, B. Lontay, MicroRNA regulatory pathways in the control of the actin-myosin cytoskeleton, Cells 9 (7) (2020), https://doi.org/10.3390/cells9071649.
[21] A. Sreekumar, S. Saini, Role of MicroRNAs in neuroendocrine prostate cancer, Noncoding RNA 8 (2) (2022), https://doi.org/10.3390/ncrna8020025.
[22] W. Abida, J. Cyrta, G. Heller, et al., Genomic correlates of clinical outcome in advanced prostate cancer, Proc. Natl. Acad. Sci. U. S. A. 116 (23) (2019) 11428–11436, https://doi.org/10.1073/pnas.1902651116.
[23] S.Y. Ku, S. Rosario, Y. Wang, et al., Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance, Science 355 (6320) (2017) 78–83, https://doi.org/10.1126/science.aah4199.
[24] C.A. Heinlein, C. Chang, Androgen receptor in prostate cancer, Endocr. Rev. 25 (2) (2004) 276–308, https://doi.org/10.1210/er.2002-0032.
[25] K.H. Stopsack, S. Nandakumar, A.G. Wibmer, et al., Oncogenic genomic alterations, clinical phenotypes, and outcomes in metastatic castration-sensitive prostate cancer, Clin. Cancer Res. 26 (13) (2020) 3230–3238, https://doi.org/10.1158/1078-0432.Ccr-20-0168.
[26] A. Sharp, I. Coleman, W. Yuan, et al., Androgen receptor splice variant-7 expression emerges with castration resistance in prostate cancer, J. Clin. Invest. 129 (1) (2019) 192–208, https://doi.org/10.1172/jci122819.