Clinicopathological and Genetic Analyses of Small Cell Neuroendocrine Carcinoma of the Prostate: Histological Features for Accurate Diagnosis and Toward Future Novel Therapies

Short Running Title: Characteristics of Prostatic Small Cell Neuroendocrine Carcinoma

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Abstract

Differentiating small cell neuroendocrine carcinoma (SCNC) of the prostate from prostatic cancer with diffuse neuroendocrine (NE) differentiation based on morphological features alone can be challenging. Given that treatment strategies vary depending on histological type, accurate diagnosis is critical. This study firstly aimed to identify the accurate diagnostic factors for primary prostate NE tumors. Furthermore, the possibility of novel treatment strategies through genetic analysis is also an aim. Specifically, prostate biopsies conducted in our hospital between January 2017 and May 2020 were included. As a result seven cases of SCNC and four cases of prostatic cancer with diffuse NE differentiation were identified. No significant differences in serum neuron-specific enolase (NSE), pro-gastrin-releasing peptide (ProGRP), and prostate-specific antigen (PSA) levels were observed between SCNC and adenocarcinoma with diffuse NE differentiation. The Ki-67 labeling index was significantly higher and PSA immunoreactivity tended to be lower in SCNC. Genetic analysis did not detect any *ALK* mutations but confirmed several other mutations, including those of *PIK3CA* and *TP53*. In conclusion, given the heterogeneity in serum NE markers in SCNC, diagnosis based on these markers alone is difficult. A high Ki-67 labeling index and low PSA immunoreactivity may be useful for diagnosing, but p53 immunoreactivity is insufficient to distinguish these tumors. Although further studies are required to interpret the results of the genetic analysis involving *ALK*, *PIK3CA*, and *TP53* mutations, the results of our genetic analysis suggest that *PIK3CA* mutations in SCNC of the prostate may provide a novel therapeutic strategy.

Introduction

Adenocarcinoma constitutes the majority of histological types of prostate cancer (1). Primary neuroendocrine (NE) tumors of the prostate have also been identified and can be divided into four types based on the World Health Organization classification: adenocarcinoma with NE differentiation, well-differentiated NE tumor, small cell NE carcinoma (SCNC), and large cell NE carcinoma (2). Of these, adenocarcinoma with NE differentiation and SCNC occur at a certain frequency (2). Approximately 0.5% to 2% of cases of primary SCNC of the prostate have been reported among prostate malignancies (3), in which morphological features are essential for the diagnosis of this tumor (4). However, diagnosis based on morphological features alone is sometimes challenging. Moreover, positive immunoreactivity for NE markers does not always provide a definitive diagnosis of NE carcinoma of the prostate due to the concept of prostate adenocarcinoma with NE differentiation (5). Given that therapeutic strategies differ depending on histological type, diagnoses are critical (4, 6).

An aggressive variant of SCNC occurs in castration-resistant prostate cancer (CRPC) after hormonal therapy and taxane chemotherapy (7-9), referred to as therapy-related NE prostate cancer (t-NEPC) (8). Genomic and transcriptional alterations are known to occur in CRPC cases, resulting in SCNC (10). De novo type SCNC may occur, and there are several reports on the frequency of de novo SCNC and t-NEPC cases. A high proportion of t-NEPC cases among patients with SCNC of the prostate has been reported (11), whereas other studies have indicated that SCNC cases constitute approximately equal proportions of t-NEPC and de novo types (8). In this regard, various mechanisms, including the presence or absence of a history of therapy, may be associated with SCNC of the prostate (12).

Recently, the concept of prostate cancer with diffuse NE differentiation has been reported, raising concerns regarding its differentiation from SCNC (5). Here, we first aimed to clarify the characteristics of prostate NE tumors diagnosed by biopsy and determine factors contributing to an accurate diagnosis. Moreover, the presence of *ALK* mutation (ALK F1174C) in SCNC of the prostate was reported, highlighting its potential use as a novel therapeutic target (13). Therefore, we further explored the possibility of future novel therapeutic strategies.

Materials And Methods

Study design

This study included cases biopsied after January 2017 due to the shift from systemic prostate needle biopsies to the addition of extra biopsies based on pre-biopsy magnetic resonance imaging findings at our institute. As per the previous methodology of our institution, Primary pathological findings were first recorded by YO and reviewed by a second pathologist (YM) according to the 2014 International Society of Urological Pathology system (14-16).

In the event of disagreement on diagnostic findings, another trained pathologist was recruited for discussion until a final consensus was reached. From January 2017, clinical information, including age, body mass index, and pre-biopsy serum prostate-specific antigen (PSA) levels, and pathological findings, such as the presence of tumor lesions, histological type (including suspected NE tumors), number of positive scores, highest Gleason grade (only for adenocarcinoma cases), lesion length, and immunohistochemical confirmation of NE marker expression, were recorded by YO for database generation. Cases were extracted from the database if NE tumors were suspected based on morphological findings and immunohistochemical confirmation of NE markers, such as synaptophysin and chromogranin A. Subsequently, cases were re-reviewed using actual specimens. Serum neuron-specific enolase (NSE) and pro-gastrin-releasing peptide (ProGRP) levels were confirmed in the most recent values at the time of biopsy. Metastasis to other organs and prognosis were confirmed using electronic medical records. The diagnostic criteria for small cell neuroendocrine carcinoma (SCNC) of the prostate were based on the World Health Organization classification (2), with emphasis on morphology and immunoreactivity of NE markers. Given that prostate cancer can exhibit partial NE differentiation, adenocarcinoma with NE in this study was defined as cases with diffuse immunoreactivity for NE markers (prostatic carcinoma with diffuse NE differentiation), based on a previous report (5). The definition of "diffuse" is controversial, but in this study, we included cases in which at least 50% were positive. In each of the cases in this study, only a prostate biopsy was conducted, and no additional surgically resected specimens were obtained.

Immunohistochemical analyses

Immunohistochemical examinations were performed on available paraffin blocks using primary antibodies against the following markers: androgen receptor (AR; Nichirei, clone AR441; 1:1 dilution), chromogranin A (clone 1E2; 1:5 dilution), Ki-67 (Dako, clone MIB-1; 1:50 dilution), prostein (Dako, clone 10E3; 1:100 dilution), PSA (Dako, clone ER-PR8; 1:50 dilution), p53 (Nichirei, clone DO-7; 1:1 dilution), and synaptophysin (Roche, clone 4B5; 1:1 dilution). CD56 was not

assessed in this study due to its low specificity (17). The H-score was used for immunohistochemical evaluation and was calculated by multiplying the staining intensity (0, negative; 1, weakly positive; 2, moderately positive; and 3, strongly positive) by the percentage of cells exhibiting that staining intensity (0–100), resulting in a score in the range of 0–300 (18). For the Ki-67 labeling index, hotspots were assessed (19–21). Areas containing a high number of Ki-67-positive cells (at least 500 cells) were selected, and the percentage of positive cells was calculated. For cases of mixed SCNC-adenocarcinoma, immunostaining for adenocarcinoma and small cell carcinoma areas was performed.

Examination of hotspot driver mutations using a gene panel

To explore the possibility of novel therapeutic strategies, we examined hotspot driver mutations using the Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHPv2, Thermo Fisher Scientific, Inc.). Next-generation sequencing (NGS) was performed as described previously (22–24). Tumor tissues were obtained from thin sections (4 µm thickness per slice) prepared from formalin-fixed and paraffin-embedded (FFPE) tissue blocks by manually dissecting the tumor area. DNA was extracted from FFPE tissues using the GeneRead FFPE Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and was quantified using Qubit 2 (Thermo Fisher Scientific, Inc., Waltham, MA). DNA (10 ng) was applied to library construction with the CHPv2 based on Ion Torrent AmpliSeq™ technology (Thermo Fisher Scientific, Inc.) and subjected to sequencing with the Ion PGM next-generation sequencer (Thermo Fisher Scientific, Inc.). The CHPv2 for NGS is a gene panel for analyzing well-characterized mutation hotspots of the following 50 cancer-related genes: *ABL1*, *AKT1*, *ALK*, *APC*, *ATM*, *BRAF*, *CDH1*, *CDKN2A*, *CSF1R*, *CTNNB1*, *EGFR*, *ERBB2*, *ERBB4*, *EZH2*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *FLT3*, *GNA11*, *GNAQ*, *GNAS*, *HNF1A*, *HRAS*, *IDH1*, *IDH2*, *JAK2*, *JAK3*, *KDR*, *KIT*, *KRAS*, *MET*, *MLH1*, *MPL*, *NOTCH1*, *NPM1*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *PTPN11*, *RB1*, *RET*, *SMAD4*, *SMARCB1*, *SMO*, *SRC*, *STK11*, *TP53*, and *VHL*. The sequenced data were processed and analyzed using Torrent Suite 5.12.2 and Ion Reporter version 5.16 (Thermo Fisher Scientific Inc.). Quality control reports were acquired from Torrent Suite. To identify somatic variants, single nucleotide variations and insertion or deletion mutations with a coverage of 20 base pairs and encoding amino acid sequence substitutions comparable to the UCSC hg19 reference genome sequence were first selected. Next, single-nucleotide polymorphisms (SNPs) were excluded using these sequences as queries against COS-MIC (<http://cancer.sanger.ac.uk/cosmic>), dbSNPs (NCBI, NIH; <https://www.ncbi.nlm.nih.gov/projects/SNP/>), and other publicly accessible databases. Finally, mutations with >5% allele frequency were defined as somatic nonsynonymous mutations and further evaluated. The biological significance of the identified somatic mutations was assessed using COSMIC, ClinVar (NCBI; <https://www.ncbi.nlm.nih.gov/clinvar/>).

Statistical analyses

Statistical analyses were performed using International Business Machines (IBM) Statistical Package for the Social Sciences Statistics version 25 (IBM Corp., Armonk, NY, USA). Serum NSE, ProGRP, and PSA levels and the H-score and Ki-67 labeling index were subjected to statistical analysis. Student's t-test was used to compare two groups. One-way analysis of variance (one-way ANOVA) and the Bonferroni post hoc test were used to compare three groups. A p-value <0.05 was considered statistically significant.

Results

Clinicopathological results

Between January 2017 and May 2021, 1014 prostate biopsies were conducted at our hospital, among which 654 (64.5%) prostate cancer cases were confirmed. After primary evaluation of hematoxylin and eosin-stained specimens, immunohistochemistry of synaptophysin and chromogranin A was conducted in 32 cases (32/654, 4.9%) because there were cases suspected of neuroendocrine tumors but could not be determined by morphological images alone. Components positive for both synaptophysin and chromogranin A were identified in 11 cases. Seven cases (7/654, 1.1%) contained SCNC components, two of which were pure SCNC and five were mixed SCNC-adenocarcinoma. Four of the 11 cases (4/654, 0.6%) were prostatic carcinoma with diffuse NE differentiation (Figure 1). Although t-NEPC accounts for a subset of SCNC of the prostate (10, 11), cases in this study were treatment-naïve, with the exception of a patient in one case who had been treated with hormonal therapy (Case 6) for 18 months before the biopsy.

All prostatic carcinomas with diffuse NE differentiation were Gleason Grade 5 and were difficult to differentiate from SCNC morphologically. In contrast to SCNC, these cases exhibited the following features: nuclear structures with densely stained nuclei and inconspicuous nucleoli observed at high magnification, lower nuclear-to-cytoplasmic ratio, and foamy cytoplasm, consistent with previous reports (2, 5). Age, histological type, serum NSE, and ProGRP and PSA levels; organs with metastasis; and outcomes are summarized in Table 1. The mean ($\pm$ standard deviation) serum NSE, ProGRP, and PSA values in SCNC were 51.9 $\pm$ 78.7 ng/mL, 2381.3 $\pm$ 5086.2 pg/mL, and 31.3 $\pm$ 56.7 ng/mL, respectively, whereas these values in prostate cancer with diffuse NE differentiation were 10.8 $\pm$ 5.1 ng/mL, 50.5 $\pm$ 35.3 pg/mL, and 1603.7 $\pm$ 2396.5 ng/mL, respectively (PSA values in the case with hormonal therapy and unmeasured values were excluded). There were no significant differences in serum NSE, Pro GRP, and PSA levels between SCNC and prostate cancer with diffuse NE differentiation (Student's t-test, $p=0.257$, $p=0.313$, and $p=0.281$, respectively).

Immunohistochemical results

One case with a history of hormonal therapy before the biopsy was excluded. Immunohistochemical results of the remaining 10 cases are summarized in Tables 2 and 3 and Figures 2 and 3. Compared with adenocarcinoma components, SCNC exhibited a significantly higher Ki-67 labeling index in mixed SCNC-adenocarcinoma (one-way ANOVA, $p<0.01$) and prostatic carcinoma with diffuse NE differentiation (one-way ANOVA, $p<0.01$). Immunohistochemical PSA positivity in SCNC demonstrated a trend of lower H-scores, although this did not reach statistical significance ($p=0.09$ and $p=0.1$ for adenocarcinoma components in mixed SCNC-adenocarcinoma and prostatic carcinoma with diffuse NE differentiation, respectively). Cases with extremely high positivity (overexpression) or complete negativity (null-type) for p53 were identified in both SCNC and prostatic carcinoma with diffuse NE differentiation (Figures 4 and 5). No significant differences were noted in any immunohistochemical finding pertaining to adenocarcinoma components between mixed SCNC-adenocarcinoma and prostatic carcinoma with diffuse NE differentiation.

Hotspot driver mutations

Hotspot driver mutations were evaluated in available cases, including one of the two pure SCNCs, three of the four prostatic carcinomas with diffuse NE differentiation, and four of the five mixed SCNC-adenocarcinoma cases. Both SCNC and adenocarcinoma components were examined in mixed SCNC-adenocarcinoma case. The one pure SCNC was subjected to the FoundationOne CDx assay (Foundation Medicine Inc., Cambridge, MA). In summary, *ALK* mutation was not detected, whereas *TP53* mutation was the most frequently identified. In one case of SCNC, a mutation in *PIK3CA* was also detected. Results are summarized in Table 4.

Discussion

SCNC of the prostate is a rare but highly aggressive cancer (8). In this study, seven cases of SCNC (including two pure cases and five mixed SCNC-adenocarcinoma cases) were identified with a frequency of approximately 1%. Further, four cases of prostatic carcinoma with diffuse NE differentiation were observed. Generally, prostatic carcinoma with diffuse NE differentiation does not differ from the conventional type of prostate adenocarcinoma with similarly high GG in prognosis (17), although this remains controversial (2). Further, the difference in prognosis between SCNC and prostatic carcinoma with diffuse NE differentiation remains controversial (2, 4, 6). In this study, two of the four patients with prostatic carcinoma with diffuse NE differentiation died (although one death was due to a complicated gastric cancer), and three of the seven patients with SCNC died. However, cases of prostate cancer with diffuse NE differentiation in this study were limited to those with suspected SCNC morphologically and confirmed immunoreactivity for NE markers, all of which were aggressive cases corresponding to GG5. Thus, assessing the true difference in prognosis between SCNC and prostatic carcinoma with diffuse NE differentiation remains challenging. Lymph nodes and bones are well-established metastatic sites in prostate cancer (14, 25-27). Apart from these organs, one case of liver metastasis was identified among the four cases of adenocarcinoma (Case 1), whereas distant metastases were identified in three of the seven cases of SCNC (Cases 5–7, brain, liver, lung, and skin). Although the number of cases should be considered, SCNC exhibited a tendency of distant metastases to sites other than lymph nodes or bones, consistent with previous reports (8, 28, 29). Clinical data revealed a trend of low serum PSA levels and extremely high serum ProGRP levels in SCNC cases. However, one case of SCNC with high PSA levels was identified, and four of the seven SCNC cases did not exhibit high ProGRP and NSE levels. Furthermore, the mean serum NSE, ProGRP, and PSA values were different between SCNC and prostatic carcinoma with diffuse NE differentiation, although the differences were not statistically significant. Although the small number of cases should be considered, the heterogeneity of serum NSE and ProGRP levels in SCNC cases may also be relevant. Although extremely high serum NSE and ProGRP values suggest the presence of SCNC, it may be difficult to completely distinguish between SCNC and diffusely NE-differentiated prostate cancer based on serum PSA and NE marker values alone.

ALK mutation was not detected in this study. Including the FoundationOne CDx assay results of one case, only five SCNC cases were genetically analyzable (Cases 4–7 and Case 11); therefore, further investigations are warranted to elucidate the profile of targetable mutations, such as *ALK*F1174C in SCNC of the prostate. Comparison of gene mutations between the SCNC and adenocarcinoma components from the same mixed SCNC-adenocarcinoma was possible for two cases. No mutations were identified in the adenocarcinoma component, and *ERBB2* mutation in the SCNC component was noted in one case.

The other case revealed truncating loss-of-function mutations in the tumor suppressor genes *PTEN* and *TP53* in the adenocarcinoma component. Notably, the SCNC component of this case shared the same *TP53* mutation at the nucleotide alteration level (c.820_821delGT; p.Val274fs), implying the existence of a common precursor lesion committed to tumorigenesis (30, 31). The SCNC component did not contain *PTEN* mutations. *PTEN* mutations appeared at or after the splitting of the two histologic types. Two cases of *TP53* truncating mutations exhibited a null-type pattern on immunostaining for p53. Consistent with reports on other cancers (32), nonsynonymous amino acid-replacing *TP53* mutations were identified in two cases of prostate cancer with diffuse NE differentiation and one case of pure SCNC with high expression of p53. Moreover, a *PIK3CA* mutation (p. His1047Arg) was detected in one case of SCNC (Case 7). *PIK3CA* mutations have been identified in various types of cancers (33) and have also been reported to be a genetic driver of prostate cancer (34). However, to the best of our knowledge, this mutation has not been reported in prostate SCNC. A combination of *PIK3CA* inhibitors, alpelisib and fulvestrant, has been proposed as a novel therapy for breast cancer (35-37). Our genetic findings suggest that the presence of this *PIK3CA* mutation in SCNC may offer a novel therapeutic target.

Histopathologically, the basic morphological features of SCNC are relatively small tumor cells with inconspicuous nucleoli, densely stained nuclei with increased chromatin, a high nuclear-to-cytoplasmic ratio, and stretchability often resulting in nuclear lines (38). The significance of these morphological features has increased due to the establishment of the concept of prostatic carcinoma with diffuse NE differentiation (2). However, distinguishing SCNC from prostatic carcinoma with diffuse NE differentiation based on morphology and immunohistochemistry for NE markers alone can be challenging. The H-score for chromogranin A tended to be higher for SCNC than for prostatic carcinoma with diffuse NE differentiation, although this difference was not statistically significant. Further, no significant difference in immunoreactivity for synaptophysin was noted between the two tumor types, highlighting the difficulty in differentiating SCNC from prostatic carcinoma with diffuse NE differentiation based solely on immunoreactivity to NE markers. Although high p53 immunoreactivity has been reported in SCNC of other organs (39-41), both SCNC and adenocarcinoma with diffuse NE differentiation of the prostate frequently exhibited a mutated *TP53* pattern, that is, overexpression or null-type, which precluded these tumor types from being distinguished. In particular, both prostate cancer with diffuse NE differentiation and SCNC may exhibit overexpression or null-type immunoreactivity for p53. Pathologists, urologists, and oncologists should therefore be aware that p53 immunoreactivity is insufficient to distinguish between these two histological types. In contrast, the Ki-67 labeling index, a well-known indicator of malignant potential (42-45), was significantly different between the two histologic types. The average labeling index for SCNC was 70%, which was approximately three times significantly higher than that for prostatic carcinoma with diffuse NE differentiation. With regard to prostatic epithelial cell markers, a degree of AR and prostein immunoreactivity was observed in SCNC, whereas less PSA immunoreactivity was noted, although this did not reach statistical significance. In SCNC of the prostate, AR and prostein are not useful in differentiating prostate cancer, as they maintain immunoreactivity. However, PSA, in addition to the Ki-67 labeling index, might be an auxiliary marker in the diagnosis of SCNC, because a considerable, although not statistically significant, reduction in PSA immunoreactivity was observed in SCNC.

Further studies are warranted, our findings suggest that a high Ki-67 labeling index and low PSA immunoreactivity may contribute to an accurate diagnosis of SCNC. Although other novel markers for SCNCs have been reported (46, 47), an advantage of our approach is that it can be applied using antibodies commonly employed in the diagnostic pathology department. In addition, immunohistochemistry for prostate markers other than PSA, such as AR and prostein (48-50), may be suitable for excluding metastatic SCNC from other organs. Nevertheless, we once more wish to state the limitations of this study. The study includes seven cases of SCNC and 4 cases of prostatic carcinoma with diffuse NE differentiation. Therefore, we acknowledge that the small number of cases is one of the limitations of this study.

Conclusion

Our study provides insight into histological characteristics that may facilitate the diagnosis of SCNC of the prostate. Moreover, genetic analysis findings suggest that the presence of *PIK3CA* mutation in this tumor may offer a novel therapeutic target.

Abbreviations

ANOVA, analysis of variance; AR, androgen receptor; CRPC, castration-resistant prostate cancer; FFPE, formalin-fixed and paraffin-embedded; NE, neuroendocrine; NGS, next-generation sequencing; NSE, neuron-specific enolase; ProGRP, pro-gastrin-releasing peptide; PSA, prostate-specific antigen; SCNC, small cell neuroendocrine carcinoma; SNPs, single-nucleotide polymorphism; t-NEPC, therapy-related NE prostate cancer

Declarations

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Conflicts of interest/Competing interests

All authors declare that there is no conflict of interest regarding the publication of this paper.

Data availability

The dataset supporting the conclusions of this article is included within the article and all material is available upon request.

Authors' contributions

Arika Ida: Gathering of the required information from the database, Reassessment of the specimens with Yoichiro Okubo, Genetic analysis with Rika Kasajima, Producing a draft of the manuscript. Yoichiro Okubo: Review of the specimens, Construction of the database, Integration of the data, Final revisions of the manuscript. Rika Kasajima: Genetic analysis, Interpretation of the results with Arika Ida, Yoichiro Okubo, and Yohei Miyagi. Kota Washimi, Shinya Sato, and Emi Yoshioka: Participation in some of the reviews of cases with unclear diagnoses and provision of advice on the results of genetic analysis. Kimito Osaka and Takahisa Suzuki: Review, organization, and provision of the clinical data to Arika Ida and Yoichiro Okubo, Confirmation of the patients' prognoses by contacting other facilities as necessary. Yayoi Yamamoto: Provision of advices on tumor spread, especially distant metastasis. Tomoyuki Yokose: Revision of some parts of the manuscript pertaining to pathological diagnosis from the perspective of a senior pathologist and provision of advice on the results of genetic analysis. Takeshi Kishida: Provision of detailed clinical information to Yoichiro Okubo, Revision of some parts of the manuscript. Yohei Miyagi: Review and reassessment of the specimens and revision of the manuscript as the senior pathologist. All authors have read and approved the final version of the manuscript.

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Ethics approval and consent participate

Informed consent was obtained from the patients for research and publication. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Review Committee of the Kanagawa Cancer Center (approval number: 2019-36, June 26, 2019).

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Tables

Table 1. Clinicopathological data for each case

Case	Age (years)	Histological type	Serum NSE level (ng/mL)	Serum ProGRP level (pg/mL)	Serum PSA level (ng/mL)	GG	Metastasis	Outcome (months)
1	89	Prostatic carcinoma with diffuse NE differentiation	16.1	84.6	51.5	5	Bone, liver, lymph node	DOD 19
2	65	Mixed SCNC-adenocarcinoma	7.1	32.1	11.1	5	Lymph node	AWD 46
3	80	Prostatic carcinoma with diffuse NE differentiation	10.2	52.8	435.8	5	Lymph node	DOC 13 (gastric cancer)
4	84	Mixed SCNC-adenocarcinoma	Not measured	Not measured	146.3	5	none	AWD 37
5	67	SCNC (pure type)	47.3	1440.0	0.5	Not applicable	Bone, lung, lymph node	DOD 9
6	78	Mixed SCNC-adenocarcinoma (A case after hormonal therapy)	15.2	44.6	0.0	5	Bone, brain, liver, lung, lymph node, skin	DOD 19
7	76	SCNC (pure type)	210.0	12700.0	0.6	Not applicable	Liver, lung	DOD 9
8	77	Mixed SCNC-adenocarcinoma	13.5	35.5	15.8	5	None	AWD 15
9	60	Prostatic carcinoma with diffuse NE differentiation	Not measured	Not measured	754.8	5	Bone, lymph node	AWD 16
10	77	Prostatic carcinoma with diffuse NE differentiation	6.0	14.2	5172.5	5	Bone	AWD 15
11	78	Mixed SCNC-adenocarcinoma	18.5	35.7	13.6	5	None	AWD 9

Abbreviations: NSE, neuron-specific enolase; NE, neuroendocrine; ProGRP, pro-gastrin-releasing peptide; PSA, prostate-specific antigen; GG, Gleason Grade; SCNC, small cell neuroendocrine carcinoma; DOD, died of disease; AWD, alive with disease; DOC, died of other causes.

The means±standard deviations of serum NSE, ProGRP, and PSA values in SCNC were 51.9±78.7 ng/mL, 2381.3±5086.2 pg/mL, and 31.3±56.7 ng/mL, respectively, whereas these values in prostate cancer with diffuse NE differentiation were 10.8±5.1 ng/mL, 50.5±35.3 pg/mL, and 1603.7±2396.5 ng/mL, respectively (PSA values in the one case with hormonal therapy and unmeasured values were excluded). No significant differences (Student's t-test) in serum NSE, ProGRP, and PSA levels were observed between small cell carcinoma and prostatic carcinoma with diffuse NE differentiation (excluding cases treated with hormonal therapy). All of the adenocarcinomas confirmed in this study were classified as Gleason Grade 5.

Table 2. H-score and Ki-67 labeling index of various immunohistochemical markers for each case

Cases	Histological type	AR	Chromogranin A	Ki-67 labeling index	Prostein	PSA	p53	Synaptophysin
1	Prostatic carcinoma with diffuse NE differentiation	270 (3×90)	4 (2×2)	40.9%	300 (3×100)	10 (1×10)	30 (2×15)	150 (2×75)
2	Adenocarcinoma area in mixed SCNC-adenocarcinoma	300 (3×100)	1 (1×1)	26.8%	240 (3×80)	80 (2×40)	300 (3×100)	120 (2×60)
3	Prostatic carcinoma with diffuse NE differentiation	300 (3×100)	2 (2×1)	28.1%	30 (1×30)	40 (2×20)	180 (3×60)	160 (2×80)
4	Adenocarcinoma area in mixed SCNC-adenocarcinoma	270 (3×90)	1 (1×1)	10.5%	300 (3×100)	160 (2×80)	1 (1×1)	120 (2×60)
6	Adenocarcinoma area in mixed SCNC-adenocarcinoma	160 (2×80)	180 (3×60)	0.2%	40 (2×20)	180 (2×90)	3 (1×3)	40 (2×20)
8	Adenocarcinoma area in mixed SCNC-adenocarcinoma	120 (2×60)	40 (2×20)	22.7%	270 (3×90)	0 (0×0)	20 (2×10)	210 (3×70)
9	Prostatic carcinoma with diffuse NE differentiation	285 (3×95)	1 (1×1)	25.1%	240 (3×80)	160 (2×80)	10 (2×5)	140 (2×70)
10	Prostatic carcinoma with diffuse NE differentiation	300 (3×100)	90 (3×30)	15.3%	120 (2×60)	100 (2×50)	240 (3×80)	210 (3×70)
11	Adenocarcinoma area in mixed SCNC-adenocarcinoma	300 (3×100)	1 (1×1)	32.9%	270 (3×90)	80 (2×40)	0 (0×0)	160 (2×80)
2	SCNC area in mixed SCNC-adenocarcinoma	270 (3×90)	1 (1×1)	71.7%	100 (2×50)	40 (2×20)	300 (3×100)	120 (2×60)
4	SCNC area in mixed SCNC-adenocarcinoma	270 (3×90)	90 (3×30)	33%	270 (3×90)	10 (1×10)	5 (1×5)	9 (3×3)
5	SCNC (pure type)	60 (2×30)	180 (3×60)	79.7%	0 (0×0)	0 (0×0)	210 (3×70)	270 (3×90)
6	SCNC area in mixed SCNC-adenocarcinoma	10 (1×10)	240 (3×80)	76.5%	0 (0×0)	5 (1×5)	50 (1×50)	100 (2×50)
7	SCNC (pure type)	0 (0×0)	40 (2×20)	88.6%	0 (0×0)	0 (0×0)	0 (0×0)	90 (1×90)
8	SCNC area in mixed SCNC-adenocarcinoma	270 (3×90)	90 (3×30)	59.7#	240 (3×80)	0 (0×0)	40 (2×20)	90 (1×90)
11	SCNC area in mixed SCNC-adenocarcinoma	0 (0×0)	60 (2×30)	85.9%	0 (0×0)	0 (0×0)	0 (0×0)	80 (1×80)

Abbreviations: AR, androgen receptor; NE, neuroendocrine; PSA, prostate-specific antigen; SCNC, small cell neuroendocrine carcinoma

Staining intensity (0–3) and staining percentage (0–100) are provided in parentheses for the H-scores. In practice, these values were multiplied to calculate the H-score. Incidentally, Case 6 was a reference because hormonal therapy was performed during the biopsy.

Table 3. H-score and Ki-67 labeling index for each immunohistochemical marker

	SCNC (H-score, mean±SD)	Adenocarcinoma area in mixed SCNC-adenocarcinoma (H-score, mean±SD)	Prostatic carcinoma with diffuse NE differentiation (H-score, mean±SD)
AR	145±138.7	247.5±86.2	288.8±14.4
chromogranin A	76.8±60.6	10.8±19.5	24.3±43.9
Ki-67 labeling index	69.8±20.8	23.2±9.5	27.3±10.5
prostein	101.7±125.3	270±24.5	172.5±120.9
PSA	1.7±4.1	80±65.3	77.5±66.5
p53	92.5±129.9	80.3±146.8	115±112.7
Synaptophysin	134.8±109	152.5±42.7	165±31.1

Abbreviations: AR, androgen receptor; NE, neuroendocrine; PSA, prostate-specific antigen; SCNC, small cell neuroendocrine carcinoma; SD, standard deviation

Immunohistochemical results for small cell neuroendocrine carcinoma (SCNC, n=6, comprising two cases of pure SCNC and four cases of mixed SCNC-adenocarcinoma), adenocarcinoma areas in cases of mixed SCNC-adenocarcinoma (n=4), and prostatic carcinoma with diffuse NE differentiation (n=4), excluding one case in which hormonal therapy was performed. In principle, H-scores were employed in the assessments. Only the Ki-67 labeling index was based on the percentage of hotspots.

Table 4. Results of hotspot driver mutations

Case	Histological type	<i>EGFR</i>	<i>ERBB2</i>	<i>FLT3</i>	<i>NOTCH1</i>	<i>PIK3CA</i>	<i>PTEN</i>	<i>SGK1*</i>	<i>SMAD4</i>
3	Prostatic carcinoma with diffuse NE differentiation								
4	Adenocarcinoma area in mixed SCNC-adenocarcinoma								
4	SCNC area in mixed SCNC-adenocarcinoma		p.Val859Asp						
5	SCNC (pure type)	p.Gly873Arg		p.Ser684Pro	p.His1601Leu				
6	SCNC area in mixed SCNC-adenocarcinoma								
7	SCNC (pure type)					p.His1047Arg		p.Thr321fs*25	
8	Adenocarcinoma area in mixed SCNC-adenocarcinoma								
9	Prostatic carcinoma with diffuse NE differentiation								
10	Prostatic carcinoma with diffuse NE differentiation								p.Gly358Arg
11	Adenocarcinoma area in mixed SCNC-adenocarcinoma						p.Gln245ter		
11	SCNC area in mixed SCNC-adenocarcinoma								

Abbreviations: NE, neuroendocrine; SCNC, small cell neuroendocrine carcinoma

Results of hotspot driver mutations for each case. Case 7 data were based on a FoundationOne CDx assay report.

*Since *SGK* mutations are unavailable in the Ion AmpliSeq Cancer Hotspot Panel v2, they were analyzed only in Case 7, in which the FoundationOne CDx assay was applied. *SGK* mutations were not analyzed in other cases.

Figures

Figure 1

Histopathology of prostatic carcinoma with diffuse neuroendocrine differentiation. (A) Adenocarcinoma with cribriform structures. A low nuclear-to-cytoplasmic ratio and foamy cytoplasm are observed (hematoxylin-eosin staining; original magnification, ×100). (B) Immunostaining with synaptophysin. Moderate but diffusely stained carcinoma cells are noted (original magnification, ×100).

Figure 2

Ki-67 labeling index for each group. Ki-67 labeling index for the small cell neuroendocrine carcinoma (SCNC) group (n=6), adenocarcinoma area in mixed SCNC-adenocarcinoma group (n=4), and prostatic carcinoma with diffuse neuroendocrine (NE) differentiation group (n=4) (excluding one case with hormonal therapy). The Ki-67 labeling index was higher in the SCNC group than in the other two groups (one-way analysis of variance and the Bonferroni post hoc test. P-values were <0.01, respectively).

Figure 3

H-score for various immunohistochemical analyses. Immunohistochemical analyses (H-score) for small cell neuroendocrine carcinoma (SCNC, n=6), adenocarcinoma area in mixed SCNC-adenocarcinoma (n=4), and prostatic carcinoma with diffuse neuroendocrine (NE) differentiation (n=4) (excluding one case with hormonal therapy). No significant differences were observed between the three groups (one-way analysis of variance). Abbreviations: AR, androgen receptor; PSA, prostate-specific antigen.

Figure 4

p53 expression in small cell neuroendocrine carcinoma. (A) Diffusely growing small cell neuroendocrine carcinoma (SCNC) with high nuclear-to-cytoplasmic ratio and small nuclei exhibiting dense chromatin staining (hematoxylin-eosin (HE) staining; original magnification, $\times 100$). (B) Carcinoma cells exhibiting p53 expression, with the exception of slight degeneration (original magnification, $\times 100$). (C) Confirmation of SCNC with diffuse proliferation (HE staining; original magnification, $\times 100$). (D) Carcinoma cells lacking immunoreactivity for p53, referred to as null-type (original magnification, $\times 100$).

Figure 5

p53 expression in prostate cancer with diffuse neuroendocrine differentiation. (A) Adenocarcinoma exhibiting predominantly solid growth. The luminal structure is obscure, the nuclear-to-cytoplasmic ratio is low, and the cytoplasm is relatively pale and foamy (hematoxylin-eosin (HE) staining; original magnification, $\times 100$). (B) Carcinoma cells exhibiting p53 expression (original magnification, $\times 100$). (C) Adenocarcinoma with evidence of cribriform and luminal structures (HE staining; original magnification, $\times 100$). (D) Carcinoma cells lacking immunoreactivity for p53, referred to as null-type (original magnification, $\times 100$).