Primary mosaic neuroendocrine/adenocarcinoma of the prostate

Kálmán Almási¹, Noémi Kránitz¹, Tamás Kullmann²,*

¹Department of Pathology, PetzAladár Hospital, 9024 Győr, Hungary
²Department of Oncology, PetzAladár Hospital, 9024 Győr, Hungary
*Correspondence
kullmanndoki@hotmail.com
(Tamás Kullmann)

Abstract
Mosaic cancers are composed of two or several genetically different cell populations. Mosaicism has been considered to be rare among solid malignancies, with the exception of the germ cell tumours. Although neuroendocrine foci have been described within adenocarcinomas of the prostate, they have been interpreted as an advanced stage of the disease and the clinical relevance of the histological finding has remained uncertain.

In this communication we give a summary on neuroendocrine prostate cancers. We recall the results of a recent single centre cohort of patients with neuroendocrine prostate cancer that showed higher incidence and better prognosis as expected by the literature. We show for the first time histological images of a primary mosaic neuroendocrine/adenocarcinoma of the prostate. A model explaining the development of mosaic cancers in the prostate is presented. Finally, we discuss the phenomenon of mosaicism related to prostate cancer in general.

Keywords
Neuroendocrine; Mosaic; Mosaicism; Prostate cancer; Immunohistochemistry

1. Mosaicism

Mosaicism is defined as the presence of two or several genomes in an individual or a tissue. Everyone acquires multitudes of genomic mutations during development, growth and aging despite having efficacious DNA repair mechanisms. Earlier these somatic mutations were poorly explored; however, new generation sequencing technologies have allowed the more precise characterisation of somatic mosaicism. The number of mutations increases with aging and so increases the risk of developing malignant transformations [1].

Mosaicism may appear in three different contexts: within a healthy organ, within a cancerous organ and within a cancer itself. Within a healthy organ the multiplication of a cell that has undergone somatic mutation may lead to the formation of mosaic tissue. Cancer cells usually hamper multiple mutations and their multiplication is more rapid compared to the surrounding tissue, so literally every cancerous organ is mosaic. When cancer cells alter further they may form a mosaic cancer [2].

Heterogeneity is a more frequently used and broader term for expressing irregularity of cancers. Beyond genetic differences it refers to epigenetic modifications and also to microenvironmental influences including the pattern of tumour infiltrating immune cells, endothelial cells and stromal cells [3]. The issue of the investigation of cancer heterogeneities would be the identification of therapeutically targetable alterations; hence heterogeneity of cancers has got limited clinical impact so far.

The particularity of prostate cancer is that in most cases it appears to be multifocal and plurimorphic [4]. This means that it may have several synchronous individual localisations and within a single lesion as well as between lesions the cells may show morphologic variability. The topographic heterogeneity explains the necessity of doing cartographic sampling and the morphologic heterogeneity has been recognised for a long time in the Gleason grading system used in the histopathological reports. The Gleason score correlates with the clinical prognosis. Intratumoral heterogeneity can be identified even within single primary tumour foci by whole genome sequencing [5].

Sequencing studies suggest that the clonal diversity is higher within primary tumours than in metastatic samples. Indeed, the more complex the diversity of a primary tumour the higher is the rate of recurrence [4]. Among the multiple clonal varieties only a limited number seem to be capable to form metastases. Therefore, in the metastases of untreated patients the heterogeneity may be lower than in their primary tumours.

Whole exome profiling realised on prostatectomy and pelvic lymph node samples of the same patients found that 87% of the subclones detected in the regional lymph node metastases were genetically related to the primary tumour of the same patient [5].

For distant metastatic prostate cancer the rule is that while initially the disease usually responds to androgen deprivation therapy it becomes castration resistant with time. The resistance mechanisms are subject of intense investigations.
2. Neuroendocrine prostate cancer

Neuroendocrine prostate cancer (NEPC) is considered to be the worst prognostic form of metastatic castration resistant prostate cancer. In some autopsy cohorts the prevalence of NEPC in metastatic castration resistant prostate cancers has been described in up to 20% [3]. However, in the clinical routine the prevalence of NEPC is found to be much lower. In a Surveillance, Epidemiology and End Results (SEER) database report the incidence of NEPC was only 0.04% among all prostate cancer patients [6]. This frequency certainly does not explain the resistance mechanism of the majority of the cases.

Considering the development of NEPC the current hypothesis is that it evolves from a preexisting adenocarcinoma by dedifferentiation under androgen deprivation therapy [4]. Thus, it is assumed to represent a late stage of the disease.

Neuroendocrine cells are normally present in the prostatic tissue [7]. Due to their isolation and rarity their identification in the normal tissue by hematoxylin-eosin staining remains extremely challenging [8]. NEPC may present as small cell or large cell carcinoma. Carcinoid tumours have only rarely been described [9]. Similarly to adenocarcinoma in normal prostatic tissue NEPC may also develop in adenocarcinoma in single or multiple foci. De novo NEPC has also been recognised [9] but only in homogenous small cell cancer like form.

Neuroendocrine cancers may be identified by specific immunohistochemistry (IHC) stainings with synaptophysin, cluster of differentiation 56 (CD56) or chromogranine A. The suspicion of NEPC may be supported if one of the two tumour markers, neuron specific enolase (NSE) and chromogranine A is detected at elevated level [4].

A recent prospective single centre cohort of patients with NEPC showed higher incidence and better prognosis as expected by the literature [10]. 10 prostate cancer patients out of a total of 521 showed neuroendocrine phenomena, so the clinical incidence was over 2%. 5 patients’ survival reached one year, the longest survivor lived over 5 years. 3 patients received 3 lines of palliative chemotherapy. A patient with localised disease underwent prostatectomy after neoadjuvant chemotherapy and was lost of an intercurrent disease without having signs of relapse. Interestingly, patients with favorable outcome had dual elevation of the tumour markers prostate specific antigen (PSA) and neuron specific enolase (NSE) and responded to both anticancer treatments (those usually directed for prostatic adenocarcinoma and those for high grade neuroendocrine cancer) administered in an alternating way.

In this study the neuroendocrine phenomena were tested by IHC (synaptophysin, CD56, chromogranine A) and laboratory chemistry (NSE, chromogranine A) when clinicians suspected the diagnosis. The diagnosis of neuroendocrine cancer was established in two cases: (1) either by a positive IHC staining with neuroendocrine markers (2) or by the observation of a favourable radiologic response of a metastatic disease to platinum-based chemotherapy expressed also by the decrease of elevated neuroendocrine serum markers (Table 1).

The local study was closed at the end of 2018. We have still been diagnosing NEPC in about 2% of all prostate cancer cases and have been having patients with relatively good outcome. We published case reports presenting the pitfalls of the diagnostic and the possibility of several years of survival [11]. Some of our metastatic mosaic neuroendocrine/adenocarcinoma patients did not have androgen deprivation therapy or only for a short period of time. Therefore, we formulated a doubt on the current concept that explains the formation of NEPC with long lasting anti-hormonal treatment.

3. Primary mosaic neuroendocrine adenocarcinoma of the prostate

Here we show—to our knowledge for the first time—images of primary mosaic neuroendocrine/adenocarcinoma of the prostate. The presented superposed images were realised with the Inkscape vector graphic drawing software. The lower layer was the hematoxylin-eosin stained (Merck reagent) in normal mode, the upper layer was the chromogranine A stained (Cell Marque LK2H10, DakoAutostainer, Rocklin, CA, USA) in dark mode.

Fig. 1 represents isolated neuroendocrine cells within an acinar adenocarcinoma (Gleason grade group 3) in a prostate biopsy specimen. The density of the neuroendocrine cells is not higher in the cancer than in the normal prostatic tissue. However, it cannot be excluded that one of them might be the source of a malignant neuroendocrine line. The patient received 74 Gy of radiotherapy to the prostate. A year later he was diagnosed in another hospital with intermediate grade neuroendocrine cancer affecting paraaortic lymph nodes and the liver, localisations of predilection for metastatic NEPC. Since no primary tumour was found the suspicion that the earlier diagnosed prostate cancer be the origin may be raised.

Fig. 2a represents a primary prostate cancer (Gleason grade group 5). Fig. 2b shows positivity of chromogranine A IHC staining. Fig. 2c is the superposition of the two above figures showing clearly that only a part of the malignant cells are stained and thus have neuroendocrine feature. The remaining part is common adenocarcinoma. This figure demonstrates a primary mosaic prostate cancer. The patient underwent prostatectomy after receiving 3 cycles of primary cisplatin-

| TABLE 1. Diagnostic criteria of the neuroendocrine cancer of the prostate. |
|-----------------------------|----------------------------------------------------------------------------|
| Diagnostic criteria of the neuroendocrine cancer of the prostate          |                                                                           |
| Major: positive IHC staining with either CD56, synaptophysin, chromogranine A or NSE |
| Minor: elevation of serum NSE or chromogranine A                          |
| Minor: favourable response to a platinum-based chemotherapy               |

One major or two minor criteria are needed for the retention of the diagnosis. IHC: immunohistochemistry; NSE: neuron specific enolase; CD56: cluster of differentiation 56.
FIGURE 1. Biopsy specimen of a prostatic acinar adenocarcinoma. (a) Haematoxylin-eosin staining. (b) Chromogranine A immunohistochemistry showing a positive cell in the malignant zone. (c) Chromogranine A immunohistochemistry showing two positive cells in the normal glandular zone (The scale bars show 50 µm).
FIGURE 2. Biopsy specimen of a prostatic mosaic neuroendocrine/adenocarcinoma. (a) Haematoxylin-eosin staining. (b) Chromogranine A immunohistochemistry showing numerous positive cells in the malignant zone. (c) Superposition of Fig. 2a,b. Δ showing malignant adenocarcinoma cells, ▲ showing neuroendocrine cells, the yellow circle showing a normal glandular zone. (There are also necrotic zones staining with chromogranine A, e.g., at the bottom in the middle of the slide. The scale bars show 50 µm.).
etoposide treatment. The prostate did not show any positiv-
ity at neuroendocrine IHC, meaning that the neuroendocrine
component was in complete pathological remission after cy-
totoxic therapy. 22 months after prostatectomy, definitive
radiotherapy of the prostatic bed was realised for biochemical
relapse. Prostate specific membrane antigen positron emission
tomography/computed tomography (PSMA PET-CT) showed
only local hyperfixation.

While the parallel presence of neuroendocrine carcinoma
and adenocarcinoma can only be speculated on the first sample
it is clearly documented on the second one. It is not difficult to
imagine that in the absence of preoperative chemotherapy and
prostatectomy the disease would have become metastatic and
it may also be suspected that the two parts might show different
progression rate and also different spreading localisations.

4. A model for the development of
mosaic prostate cancers

Currently, the most efforts of prostate cancer research are
concentrated to the understanding of mechanisms of resis-
tance to castration. The problematic of the pathogenesis of
NEPC is part of these investigations. Some authors pro-
pose the divergent evolution of NEPC from one or more
adenocarcinoma cells, based on results of extensive whole
exome sequencing [12]. NEPC is the sui generis castration
resistant cancer. As mentioned earlier it is usually observed
in late stage of the disease and is generally suspected to arise
by dedifferentiation under long lasting androgen deprivation
therapy [13]. A review published in this Journal recognises
the possibility that neuroendocrine differentiation may appear
in earlier stage of the disease but also supports the hypothesis
that adenocarcinoma cells are the precursors of the malignant
neuroendocrine cells [14]. Contrarily to these theories, our
findings suggest the possibility of parallel evolution of the two
components from the onset on.

For supporting our explanation, we rely on the works of
Blackwood et al. [15]. They demonstrated (through the
observation of the transmission of changes in the mitochondrial
DNA) that different cells of the normal prostatic epithelium
originate from a common epithelial stem cell. With other
words, an epithelial stem cell may supply the whole cellular
population of the acini, i.e., basal cells, luminal cells and
neuroendocrine cells as well.

Although according to the current concept prostate cancer
stem cells are among the luminal cells [16] with the above
model it is enough to assume that prostate cancer may also
arise from epithelial stem cells to explain the parallel develop-
ment of adenocarcinoma and neuroendocrine cancer. The
observation that the prostate cancer specific transmembrane pro-
tease, serin 2-erythroblast transformation-specific related gene
(TMGRSS2-ERG) rearrangement is similarly present in 50%
in adenocarcinomas as well as in neuroendocrine carcinomas
further supports the hypothesis of shared clonal origin [17].
Similarly to non-seminomatous germ cell tumours [18] and
chronic myeloid leukaemia the proliferation of a malignant-
turned stem cell could be responsible of the mosaic pattern.

Furthermore, mosaicism could explain some of the other-
wise not evident observations related to the hormone sensitiv-
ity of prostate cancers. Since the introduction of second line
antihormonal therapies their optimal use has not been estab-
lished. (We prefer using the term “second line” or “androgen
receptor signalling inhibitors” instead of the generally applied
androgen receptor targeted agents abbreviated for “ARTA” as
we find these ones less misleading. Please, consider that there
are medications that act on the androgen receptors still they are
not considered among ARTAs and contrarily, not all ARTAs
act on the androgen receptors.)

First, subsequent studies showed that the more anticancer
drugs were used in parallel from the beginning on for the treatment of a hormone sensitive metastatic prostate cancer
the more additional clinical benefit could be obtained [19, 20].
Second, administration of the second line antihormonal agents
is recommended until clinical or radiological progression. Or,
in a small set of patients of our single centre, patients whose
therapy was stopped at PSA progression did not have a
worse prognosis than those treated several months longer until
clinical or radiological progression [21]. In addition, the same
antidrogenic drug was reintroduced to some patients after
progression under second line chemotherapy by cabazitaxel.
A novel decrease in PSA level could be observed in patients
treated earlier until PSA progression as well as until radi-
ological progression (yet unpublished data). It means that in
these patients the cancer lost its earlier gained resistance to
the antihormonal drug. The most plausible explanation for
this unexpected phenomenon would be the parallel presence
of different cell lines.

Let’s admit that adenocarcinoma cells of different character-
istics may arise from a single malignant-turned stem cell. Even
if these cells have the same phenotype they may differ in terms
of their mechanism of resistance to androgen deprivation.
In this case a treatment line may suppress one cell line more than
the other. Parallel use of the treatments may suppress more
cell lines. Sequential use of the treatments may block a cell
line that was not suppressed by the preceding treatment. The
deductions of the hypothesis correspond exactly to the clinical
observations.

Concerning the reasons for missing the right diagnosis of
NEPC in the clinical routine, the two main reasons are the
rarity of neuroendocrine IHC staining on biopsy samples either
of the prostate or the metastatic laesions (Table 2), and the
erroneous elimination of the potential prostatic origin for a
metastatic disease progression on the basis of a non-elevated
PSA level. Repeating the biopsy of the prostate for a known
prostate cancer patient is uncommon. Similarly, sampling
metastatic sites particularly in the bone is not the rule. Mak-
ing neuroendocrine IHC staining for an adenocarcinoma of
unknown primary is not part of the clinical routine either. Mak-
ing on biomarkers is no more evident. NSE is sensitive
but not specific. Chromogranine A may be difficult to access
in certain laboratories. Strategies to improve the detection rate
of neuroendocrine prostate cancers are summarised in Table 3.

One of the reasons why NEPC has not been in the focus of
clinical interest may be that therapeutic options used to be
limited to platinum-based chemotherapy. Some recent inves-
tigations have promising results. Aurora kinase A (AURKA)
and N-myc have been identified as potential drug targets [17].
Dual AURKA and N-myc inhibitors developed by structure-

TABLE 2. Clinical situations when neuroendocrine cancer of the prostate should be suspected.

<table>
<thead>
<tr>
<th>Clinical situations when neuroendocrine cancer of the prostate should be suspected</th>
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<tbody>
<tr>
<td>Clinical or radiological progression of prostate cancer without PSA rise</td>
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<tr>
<td>Diagnosis of an extended metastatic disease associated to a relatively low PSA</td>
</tr>
<tr>
<td>Presence of any non-pulmonary visceral metastases</td>
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<tr>
<td>Metastatic carcinoma of unknown primary</td>
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</table>

PSA: prostate specific antigen.

TABLE 3. Strategies to improve the detection rate of neuroendocrine prostate cancers.

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<tr>
<td>Avoiding to consider PSA as unique and to 100% sensitive marker of prostate cancer</td>
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<td>Realisation of neuroendocrine IHC staining in the situations summarised in Table 2</td>
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<td>Determination of NSE and/or chromogranine A when biopsy or IHC are not feasible</td>
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IHC: immunohistochemistry; NSE: neuron specific enolase, PSA: prostate specific antigen.

**Conclusions**

Neuroendocrine prostate cancer is underdiagnosed in the clinical routine and its prognosis is underestimated. We presented a case of primary mosaic neuroendocrine/adenocarcinoma of the prostate. This entity underlines the importance of mosaicism in the pathogenesis of prostate cancer.

**AVAILABILITY OF DATA AND MATERIALS**

The main data are presented within this article. Further source data are available on reasonable request from the corresponding author.

**AUTHOR CONTRIBUTIONS**

KA—identified the primary mosaic neuroendocrine/adenocarcinoma of the prostate; prepared the figures. TK—wrote the first version of the text. KA and NK—gave their remarks and made corrections. All authors have read and approved the final version.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**ACKNOWLEDGMENT**

The authors are thankful to all fellow colleagues and laboratory assistants who participated in the patients’ care and the handling of their specimens.

**FUNDING**

This research received no external funding.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.
REFERENCES
