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Review

Neuroendocrine differentiation in prostate cancer: Novel morphological insights and future therapeutic perspectives

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ABSTRACT

Neuroendocrine prostate cancer (NEPC) is an aggressive variant of prostate cancer that commonly arises in later stages of castration resistant prostate cancer (CRPC) The detection of NEPC has clinical implications as these patients are often treated with platinum chemotherapy rather than with androgen receptor targeted therapies. The poor molecular characterization of NEPC accounts in part for the lack of disease specific therapeutics. Several mechanisms are involved in NE differentiation, including inflammation and autophagy, and may actually represent future therapeutic targets for advanced NEPC patients. Furthermore, a growing body of evidence suggests a potential role of circulating tumor cells in the early diagnosis and treatment of NEPC.
Here we summarize the recent findings on NEPC pathogenesis and we discuss the ongoing clinical trials and future perspectives for the treatment of NEPC patients.

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Contents

1. Introduction

Prostate cancer is a leading cause of cancer-death, even if good response to androgen deprivation therapy (ADT) is often observed. ADT

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is the base of the treatment of advanced prostate cancer, being the possibility that the tumor evolves to an androgen refractory phenotype. Several mechanisms are involved in the development of androgen independence, including androgen receptor (*AR*) gene amplification and mutations, involvement of coregulators, ligand-independent activation of the AR, and the involvement of the neuroendocrine (NE) component among adenocarcinoma (PCa) cells [1]. The NE cells are defined immunohistochemically by the presence in the cytoplasm of markers, such as synaptophysin, chromogranin A (CgA) and neuron-specific enolase (NSE), and show dense-core granules at the ultrastructural level.

Diagnosis depends on histological confirmation, which is essential considering that NE in advanced prostate cancer is considered an aggressive phenotype with poor outcome and limited treatment options [2].

Almost all prostate cancers show focal NE differentiation, although the majority show only rare or sparse single NE cells as demonstrated by NE markers. In 5–10% of PCa there are zones with a large number of single or clustered NE cells detected by CgA immunostaining [3]. However, it is controversial whether NE differentiation in typical PCa worsens prognosis [4–7]. Serum CgA levels and potentially other markers such as pro-gastin-releasing peptide [8] may be diagnostically and prognostically useful, particularly in PSA negative, androgen independent carcinomas [9].

In this review, we summarize the recent findings on NEPC pathogenesis and morphologic classification. In addition, we discuss the ongoing clinical trials and future perspectives for the treatment of NEPC patients.

2. The origin of NE cells and their contribution to prostate carcinogenesis

In the last years, the mechanisms involved in the NE differentiation have been highly debated. Ousset and her colleagues evaluated the contribution of multipotent and unipotent progenitors to physiological prostate postnatal development. They found that keratin 5 (K5) and keratin 14 (K14) expressing multipotent basal progenitors contribute to the expansion of basal and luminal and NE lineages. Otherwise luminal progenitors contribute to the expansion of luminal lineage cells expressing AR [10].

The role of basal cells as progenitors during prostate development is further supported by the model proposed by Pignon et al., in which luminal cells form from multipotent ΔNp63-expressing progenitor cells, while the transition to unipotency and luminal cells of ΔNp63-positive basal cells acquire self-renewal capacities [11].

Furthermore, Wang et al. identified a rare luminal population of castration-resistant NKX3.1-expressing cells (CARNs) that were able to give rise to basal, luminal and NE cells [Wang 2009]. *Nkx3.1* is a member of the NK subfamily of homeobox genes that were first identified in *Drosophila* [12] and are involved in processes of cell fate specification and organogenesis in many species [13]. In adult mouse prostate, *Nkx3.1* is expressed by all luminal cells and by almost 10% of basal cells [14]. Its expression is androgen-dependent [15,16] and is lost after castration but can be rapidly restored by androgen readministration. However, Nkx3-1 expression is retained in a small percentage of epithelial cells in the anterior prostate of androgen-deprived patients, called castration-resistant Nkx3-1-expressing cells (CARNs). In the study by Wang et al., CARNs were able to reconstitute prostate tissue in grafts formed from single or multiple lineage-marked CARNs. Thus, the grafts generated prostatic ducts with epithelial cells expressing luminal markers, such as E-cadherin, CK18 and AR, basal markers, such as p63 and CK5, and NE markers (synaptophysin) [17].

In 2013, the same group showed that about 4% of basal cells present graft-forming activity which allows basal cells to give rise to prostatic ducts with luminal, basal and synaptophysin-positive NE cells [18]. Taken together, these data suggest a model in which distinct cells of origin generate the different molecular subtypes of PCa.

The mechanisms by which NE cells influence prostate carcinogenesis are not fully understood. It has been reported that NE cells support prostate carcinogenesis through the production of several paracrine growth factors. DaSilva et al. showed that selective inhibitors of insulin-like growth factor 1 receptor (IGF-1R), EGFR or Src cause a nearly complete blockade of prostate cancer cell survival due to NE secretions [19]. The cross-talk between NE-derived factors and IGF-1R seems to be mediated, in part, by protein tyrosine phosphatase receptor type F (PTPRF) [19]. As for macrophage migration inhibitory factor (MIF), it is a pro-inflammatory cytokine involved in prostate carcinogenesis. In vitro studies reported that NE differentiation of LNCaP cells is associated with markedly increased MIF release, which promotes the proliferation of PCa cells through the stimulation of the AKT and ERK1/2 signaling pathways [20].

An extremely small proportion of PCa patients present with “de novo” NE tumors. Indeed, the transdifferentiation process from an epithelial-like to a NE-like phenotype can be considered a consequence of the selective pressure induced by all treatments causing a fall in androgen levels. The driving events in the pathogenesis of NEPC include loss of AR and androgen-regulated protein expression, induction of NE and neural programs, loss of tumor suppressors (TP53, RB1, PTEN, as seen in CRPC), activation of mitotic programs, and genomic instability [21]. RB1 and TP53 are tumor suppressors that are dysfunctional in several tumors including NEPC and a subset of castration resistant prostate cancer (CRPC). The combined deficiency of RB1 and TP53 promotes the transformation to NEPC, as reported in conditional mouse models of NEPC [22,23]. Recently, several cell-cycle genes have been shown to be frequently amplified and/or overexpressed in NEPC, thus supporting their role in driving uncontrolled growth and disease progression. This list includes UBE2C, cyclin D1, Aurora kinase A (AURKA) and B (AURKB), and polo-like kinase PLK1 [24]. Furthermore, RE1-silencing transcription factor (REST, also known as neuron-restrictive silencer factor [NRSF]) is essential in controlling NE phenotype of both Small cell carcinoma (SmCC) and focal NE in PCa [25].

3. Novel morphologic classification

Recently, Epstein et al. have proposed a novel morphologic classification of NEPC [26]. This list includes: (1) Usual PCa with NE differentiation; (2) PCa with Paneth cell NE differentiation; (3) Carcinoid tumor; (4) SmCC; (5) Large cell neuroendocrine carcinoma (LCNEC); and (6) Mixed NE carcinoma (SmCC or LCNEC)–acinar adenocarcinoma (Fig. 1).

Paneth cell-like change of the prostatic epithelium is considered to be a distinct form of NE differentiation characterized by isolated cells or small groups of cells with prominent eosinophilic cytoplasmic granules [27]. At present, the strict application of the Gleason grading to the areas showing Paneth cell-like cells is still controversial. Indeed, these areas should be graded as Gleason pattern 5, which seems to not reflect the frequent association of Paneth cell-like cells with lower-grade conventional PCa and limited disease [26].

The presence of Paneth cell-like change seems to be not associated with PSA levels, tumor grade or stage [28]. Data on the prognostic significance of Paneth cell-like differentiation are still controversial. In fact, it seems to be correlated with favorable prognosis [29], although a positive correlation between Paneth cell-like change and cribriform pattern, which has been recently recommended as Gleason pattern 4 [30], has been also reported [28].

Otherwise, true carcinoid tumors of the prostate are exceedingly rare, occur predominantly in elderly men, and are discovered incidentally [31]. They should present diffuse NE differentiation, such as CgA and synaptophysin immunoreactivity, as well as they should be essentially negative for prostate specific antigen (PSA) and prostatic acid phosphatase (PAP), not closely related to normal PCa and originate from prostatic parenchyma [26].

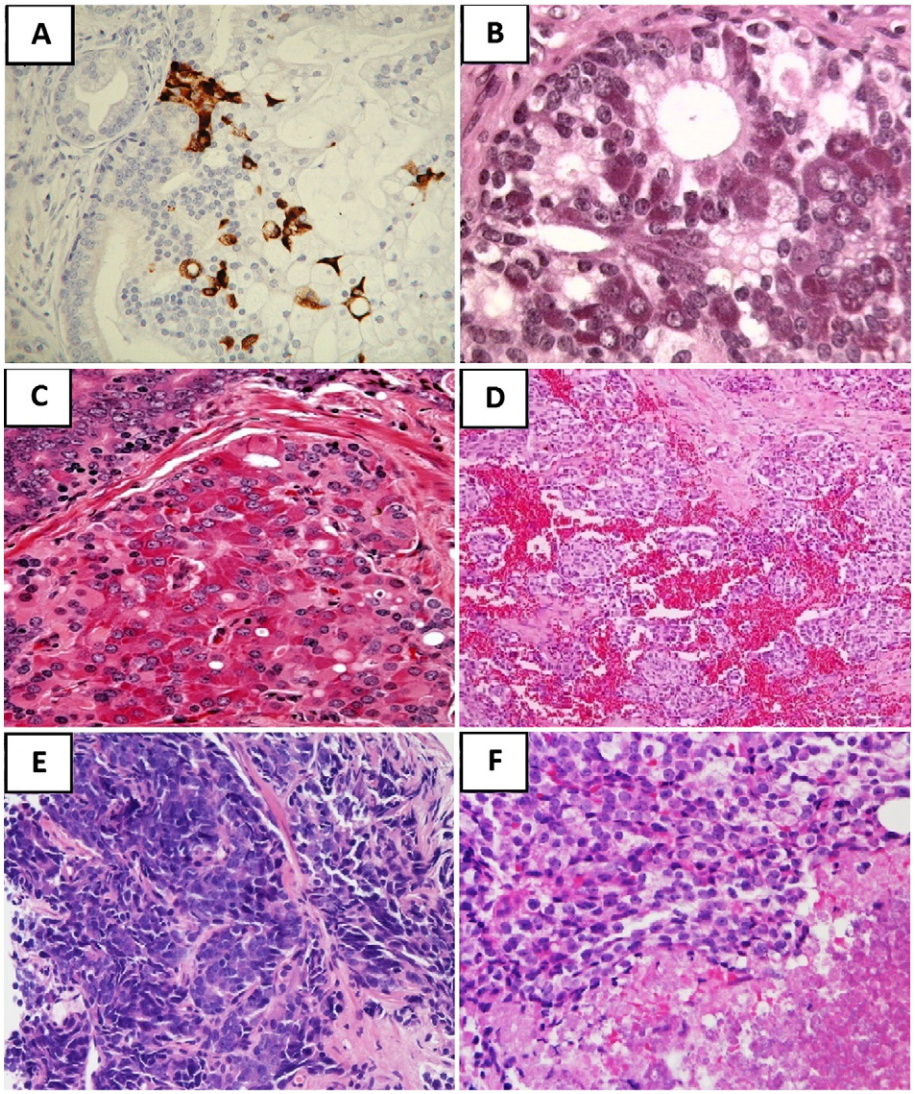

Fig. 1. Neuroendocrine prostate tumors. A — Immunohistochemical demonstration of the NE component with CgA; B — Localization of NE cells in prostatic acinar tissue; C — Paneth-like cells; D — Carcinoid of the prostate; E — Small cells carcinoma (SmCC) of the prostate; F — Large cells carcinoma of the prostate (LCNEC).

The term "carcinoid-like tumors" has been used to identify tumors with nested architecture and uniform nuclei, most of which refer to ordinary acinar PCa with an organoid appearance and focal NE differentiation. Differently from true carcinoids, they are usually positive for PSA. They may also be variants of Paneth cell-like NE differentiation [32].

Patients with prostatic carcinoids may present with hematuria, burning nicturia, urinary frequency, oliguria or symptoms of urinary retention. On the other hand, patients with "carcinoid-like" tumors are usually asymptomatic, although some cases of Cushing syndrome induced by the production of adrenocorticotropic hormone have been reported [33].

Data on the prognosis and standard treatment for these patients are limited by the small number of cases reported.

Prostatic SmCC is likely to become increasingly common with recent advances in pharmacologic ADT [34]. It represents between 1 and 5% of all prostatic malignancies [35] and it may occur de novo or as a recurrent tumor in patients who received ADT. SmCC is a high grade tumor characterized by typical nuclear features, such as the lack of prominent nucleoli, nuclear molding, fragility, and crush artifact [26]. In approximately 50% of the cases, the tumors are mixed SmCC and PCa of the prostate. The appearance of a small cell component within the course of PCa of the prostate usually indicates an aggressive terminal phase of the disease. In the case of predominant SmCC component, serum PSA level falls and may be undetectable, whereas clinically evident ACTH or antidiuretic hormone production may be observed.

Several mechanisms are involved in the pathogenesis of SmCC. *ERG* (*ETS-related gene*) and *TMPRSS2* are 2 of the 29 members of the ETS family of genes, which plays a crucial role in the development of different tissues as well as cancer progression. Approximately half of the usual PCa and SmCC present *TMPRSS2-ERG* gene fusion [36] or other ERG gene rearrangements [36–38]. Tan and colleagues evaluated the status of RB1, TP53, and PTEN in SmCC. They reported that retinoblastoma (Rb) protein is lost in 90% of small cell carcinoma cases, with RB1 allelic loss in 85% of the cases [39]. These data support the notion that Rb loss represents a crucial step in the development of SmCC, suggesting for a potential diagnostic and therapeutic role. Interestingly, Rb loss has been proposed as a gatekeeper to hormone independence in prostate cancer [40], may be explaining the scarce rate of response to ADT in patients with SmCC.

As for p53, a growing body of evidences show that p53 mutation may lead to inactivation of the Interleukin-8 (IL-8)-CXCR2-p53 signaling pathway. This event results in the loss of a growth suppressing mechanism and in the hyper-proliferation of NE cells in SmCC [41]. Furthermore, strong and diffuse membrane staining for CD44, a cell-surface molecule proposed to identify cancer stem/progenitor cells in prostate cancer, has been shown in 100% of the prostatic SmCC, whereas in

usual prostatic adenocarcinomas only rare positive scattered tumor cells are CD44 positive [42].

The average survival according to the SEER database from 1973 to 2004 is less than two years [43]. There is no difference in prognosis between patients with pure SmCC and those with mixed glandular and SmCC. SmCC has the tendency to systemically metastasize, especially to the lymph nodes, liver, bone, and lungs [44]. Histologically, prostatic SmCC is identical to the SmCC of the lung and should be considered in the differential diagnosis [45]. They are also similar in terms of aggressiveness and rate of occult metastases, supporting the use of platinum-based combination chemotherapy with or without ADT in patients with prostatic SmCC [46,47]. However, in the study led by Mackey et al., surgery but not cisplatin chemotherapy was associated with longer survival in patients with genitourinary SmCC [48].

LCNEC of prostate is exceptionally rare [49]. It is a high-grade tumor that consists of large nests and ribbons of cells with abundant pale to amphiphilic cytoplasm, large nuclei and prominent nucleoli along with high mitotic activity and foci of necrosis [23,35]. LCNEC is strongly positive for CD56, CD57, CgA, synaptophysin, and P504S/alpha methylacyl CoA racemase. There is strong bcl-2 overexpression, expression of MIB1, and p53 in >50% of nuclei, focally positive staining for PSA and PAP, and negative AR staining.

Mixed NE carcinoma is characterized by the concomitant presence of admixed components of NE (SmCC or LCNEC) and usual conventional acinar PCa. Most of patients with mixed SmCC and PCa present with metastatic CRPC. These patients are often treated with both ADT and platinum-based chemotherapy [26]. A better knowledge of the biological scenario underlying the concomitant presence of SmCC and PCa is markedly needed in order to improve the outcome of these patients.

4. Future therapeutic perspectives for patients with NEPC

NE differentiation is a multifactorial process that involves several events, such as inflammation, that represent potential therapeutic targets in advanced NEPC.

The somatostatin receptor activation has been shown to play a role in the regulation of PCa cell proliferation [50]. Octreotide is an octapeptide that mimics natural somatostatin pharmacologically, but with a longer half-life. Interestingly, a phase II trial on the use of somatostatin analog octreotide acetate in patients with non-metastatic CRPC was stopped early after a pre-planned interim analysis showed no prostate specific antigen (PSA) declines after 3 cycles of treatment among the first 13 patients enrolled [51]. On the other hand, the combination of somatostatin analog, dexamethasone, and zoledronate showed superior objective and palliative clinical responses than zoledronate alone in patients with bone metastases from CRPC [52].

Based on these data, the use of somatostatin analogs in patients affected by Usual PCa with NE differentiation seems to merit further investigations, especially in patients with advanced stages or bone involvement.

Here we report the emerging targets in the treatment of NEPC (Fig. 2) as well as the evidences on the role of NE differentiation in the development of resistance to emerging agents in patients with prostate cancer.

4.1. Antiangiogenetic agents

Complete androgen blockade before surgery seems to downregulate the expression of vascular endothelial growth factor (VEGF) and to decrease vascularization, except in the cell areas with NE features [53].

At present, several trials are in course to assess the efficacy and safety of antiangiogenetic drugs in patients with prostate cancer (Table 1). Among them, two studies are ongoing to evaluate bevacizumab, an anti-VEGF monoclonal antibody, in combination with temozolomide (NCT00137774) or HER1/HER2 inhibitor pertuzumab and sandostatin (NCT01121939) in patients with advanced NE tumors. Moreover, a phase II randomized study of docetaxel with or without low-dose, short course sunitinib, a VEGF receptor tyrosine kinase inhibitor, is in course in patients with advanced solid tumors (NCT01803503) (Table 1).

Among novel drugs, Cabozantinib (XL184), a small-molecule kinase inhibitor with potent activity toward MET and VEGF receptor 2 (VEGFR2), is emerging as a promising agent active in advanced PCa. At present, a phase II study in evaluating Cabozantinib in patients with

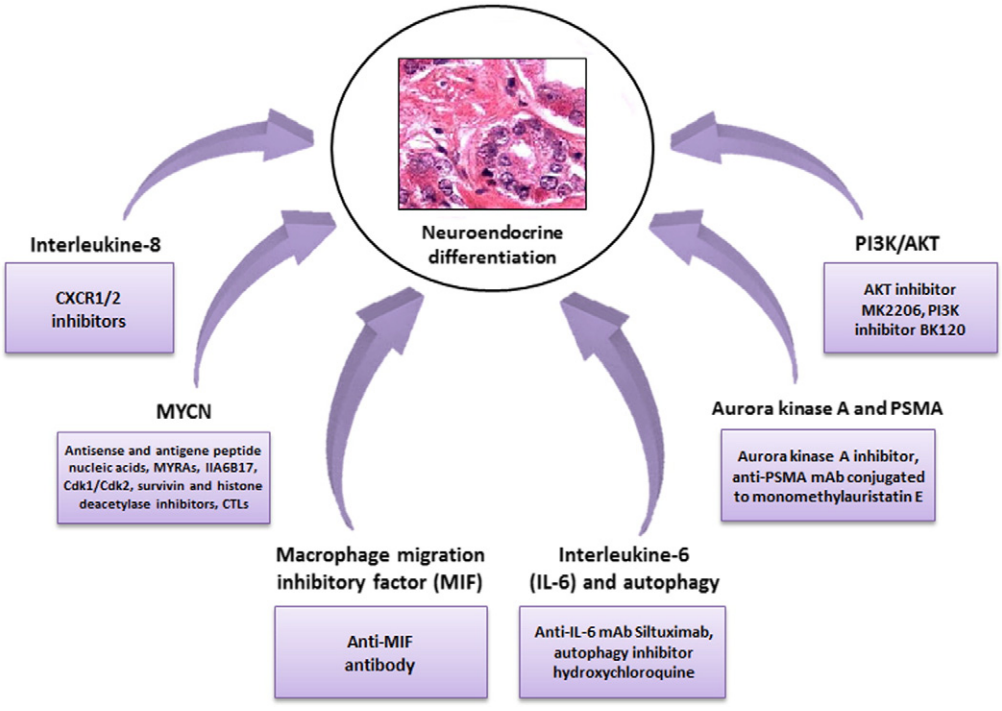

Fig. 2. Mechanisms of NE differentiation in prostate tumors and potential therapeutic approaches. CTLs = cytotoxic T cells; mAb = monoclonal antibody; PI3K = phosphoinositide 3-kinase; PSMA = Prostate specific membrane antigen.

Table 1
Clinical trials on potentially effective agents in patients with NEPC. Data on clinical trials from https://clinicaltrials.gov.

Trial ID number	Phase	Agent description and Study design
NCT01765790	I	Anti-MIF antibody in patients with advanced solid tumors
NCT01480154	I	Autophagic inhibitor hydroxychloroquine in combination with Akt Inhibitor MK2206 in treating patients with advanced solid tumors, melanoma, prostate or kidney cancer
NCT00137774	II	Anti-VEGF mAb bevacizumab in combination with temozolomide in patients with advanced NE tumors
NCT01121939	II	HER1/HER2 inhibitor pertuzumab and sandostatin in patients with advanced NE tumors
NCT01803503	II	Docetaxel with or without low-dose, short course sunitinib, a VEGF receptor TKI, in patients with advanced solid tumors
NCT01466036	II	Cabozantinib in patients with carcinoid tumors or well/moderately differentiated pancreatic NE tumors
NCT01799278	II	Aurora kinase A inhibitor MLN8237 in patients with SmCC and other NE subtypes as well as metastatic CRPC
NCT00786682	II	Autophagic inhibitor hydroxychloroquine in combination with docetaxel in patients with metastatic prostate cancer
NCT01828476	II	Autophagic inhibitor hydroxychloroquine in combination with abiraterone and Bcl-2 family inhibitor ABT-263 in patients with advanced prostate cancer
NCT01251861	II	Bicalutamide with or without Akt Inhibitor MK2206 in patients with previously treated prostate cancer
NCT01385293	II	PI3K inhibitor BK120 in patients with metastatic CRPC
NCT01695473	II	PI3K inhibitor BK120 as neoadjuvant therapy in high-risk prostate cancer
NCT02035124	II	PI3K inhibitor BK120 in combination with cabazitaxel in patients with prostate cancer pretreated with docetaxel in patients with metastatic CRPC previously treated with docetaxel
NCT02020135	II	PSMA ADC in subjects with metastatic CRPC

ADC: antibody–drug conjugated; CRPC: castration resistant prostate cancer; mAb: monoclonal antibody; MIF: macrophage migration inhibitory factor; NE: Neuroendocrine; PSMA: Prostate specific membrane antigen; SmCC: small cell carcinoma; TKI: tyrosine kinase inhibitor; VEGF: Vascular Endothelial Growth Factor.

carcinoid tumors or well/moderately differentiated pancreatic NE tumors (NCT01466036). The estimated enrollment is 70 patients, and the results are still awaited.

4.2. Interleukin-6, phosphoinositide 3-kinase/AKT and autophagy

Interleukin-6 (IL-6) is a multifunctional cytokine involved in regulation of growth of various malignant tumors. IL-6 exerts its biological activities through two molecules, IL-6 receptor (IL-6R) and gp130, and activates pathways of signal transducers and activators of transcription and mitogen-activated protein kinases (MAPKs). IL-6 activates several signaling pathways, including JAK-STAT3 [54] and phosphoinositide 3-kinase (PIK3)-Akt [55,56], which are also involved in IL-6 induced NE differentiation in PCa cells. Recently, Zhu et al. showed that IL-6 induces NE differentiation by suppressing RE-1 silencing transcription factor (REST), a neuronal gene-specific transcriptional repressor that is involved in autophagy activation, in LNCaP cells. In this study, overexpression of exogenous REST abrogated IL-6-induced NE differentiation in prostate cancer cells, suggesting a major role of REST in IL-6 induced NE differentiation [57].

Interestingly, IL-6 levels are significantly increased in patients undergoing ADT and seem to be frequently higher in patients with CRPC and metastatic PCa [58]. A phase I/II study with multiple-dose of anti-IL-6 monoclonal antibody Siltuximab in patients with advanced solid tumors, including prostate cancer has been performed. Although this agent was well tolerated, they showed that no objective responses occurred in patients treated with Siltuximab [59].

Delk et al. showed that IL-6 secreted by bone marrow stromal cells induces NE differentiation and autophagy in bone metastatic PCa cells through an STAT3-independent pathway [60]. In the same view, Chang et al. [61] showed that autophagy is involved in PCa progression. In addition, autophagy seems to play a cytoprotective role in IL-6 induced NE differentiation, and inhibition of autophagy by knockdown of beclin1 or Atg5 sensitizes NE differentiated LNCaP cells to chemotherapy with etoposide. These data reveal that targeting autophagy may represent a future promising strategy in patients with NEPC.

Presently, autophagic inhibitor hydroxychloroquine is under evaluation in combination with docetaxel (NCT00786682) or Akt Inhibitor MK2206 (NCT01480154) or abiraterone and ABT-263 (NCT01828476), a potent and orally bioavailable Bcl-2 family inhibitor, in patients with metastatic prostate cancer (Table 1).

4.3. Interleukin-8 and macrophage migration inhibitory factor

The human prostate cancer cells over-expressing Interleukin-8 (IL-8) are highly tumorigenic and metastatic with associated increased angiogenesis [62]. Serum levels of IL-8 have been found to be markedly elevated in patients with prostate cancer and correlate with the stage of disease [63].

IL-8 and its receptors CXCR1 and CXCR2 are involved in the NE differentiation process and may represent potential therapeutic targets in patients with NEPC. CXCR1 is overexpressed in PCa cells, and it has been hypothesized that paracrine activation of CXCR1 by IL-8 may contribute to androgen-independent proliferation of prostate cancer in patients treated with ADT [61]. Moreover, NE cells express IL-8 and CXCR2, as observed by staining serial sections of PCa tissue [64]. Notably, G31P, a CXCR1/2 inhibitor, has been shown to inhibit prostate cancer cell growth in vitro and in nude mouse xenografts [58]. In this study, G31P inhibited tumor tissue vascularization by decreasing the expression of VEGF and NF-κB in an orthotopic xenograft tissue [65]. Currently, no trials on IL-8 or CXCR1/2 are in course in patients with NEPC.

Another important cytokine involved in the NE differentiation process appear to be the macrophage migration inhibitory factor (MIF). MIF, through the binding to its receptor CD74, is a regulator of innate immunity and has been implicated in the development and progression of multiple types of tumors [66–68]. Tawadros and his group investigated the regulation of MIF expression during NE transdifferentiation of PCa cells. NE differentiation of LNCaP cells was obtained by increasing intracellular levels of cAMP or by culturing cells in an androgen-depleted medium and was associated with increased MIF release. The addition of exogenous recombinant MIF to LNCaP and PC-3 cells stimulated the AKT and ERK1/2 signaling pathways and promoted proliferation and resistance to paclitaxel and thapsigargin-induced apoptosis [69]. Currently, a phase I study is testing the use of an anti-MIF antibody in patients with advanced solid tumors (NCT01765790, Table 1). This study will be completed within the end of 2015.

4.4. AURKA and MYCN

The genes *AURKA* and *MYCN* are also involved in the NE process. Aurora kinases are serine/threonine kinases that are essential for cell proliferation. As for *MYCN*, it is a cellular proto-oncogene of the MYC family of transcription factors. AURKA amplification has been reported

in over 60% of PCa from patients that developed treatment-related NEPC and in 86% of metastases, whereas concurrent amplification of MYCN was present in 69% of treated PCAs and 83% of metastases [70].

In the study by Beltran et al. [21], 10 patients with metastatic NEPC underwent metastatic tumor biopsy and blood collection for CTC analysis utilizing the Epic Sciences platform. Patients were subsequently treated with platinum (4 patients), *AURKA* inhibitor (4 patients), or hormonal therapy (2 patients). *AURKA* and *MYCN* were amplified in 4 out of 7 evaluated tumors. CTCs showed epithelial plasticity, likely arising from epithelial–mesenchymal transition (EMT), and low AR expression. Based on these findings, Epic CTCs seem to be useful in the earlier detection of NEPC, suggesting further investigations in this setting.

In 2013, a phase II trial of danusertib, an AURKA/B/C inhibitor, has been performed, showing minimal efficacy in patients with mCRPC [71]. At present, a phase II trial is in course to assess the efficacy and safety of the Aurora Kinase A Inhibitor MLN8237 in patients with SmCC and other NE subtypes as well as metastatic CRPC (NCT01799278). The study will be completed at February 2016.

Targeting MYC or the MYC pathway has emerged as a very attractive approach to search for cancer intervention. Different strategies have been proposed [72], including the use of MYCN antisense and antigene peptide nucleic acids, small-molecule inhibitors (MYRAs, IIA6B17), Cdk1/Cdk2 and survivin inhibitors and histone deacetylase inhibitors. In addition, priming cytotoxic T cells (CTLs) to specifically target and kill MYCN-amplified cells may also represent may also represent an effective approach in MYCN-amplified tumors including NEPC.

4.5. Prostate specific membrane antigen (PSMA)

Several androgen-regulated proteins, such as PSA and prostate specific membrane antigen (PSMA), are variably expressed in NEPC. PSMA is a unique membrane bound glycoprotein, overexpressed on prostate cancer as well as neovasculature of most of the solid tumors [73]. PSMA can serve as target for delivery of therapeutic agents such as antibody–drug conjugated (ADC) therapy. PSMA ADC consists of a fully human anti-PSMA monoclonal antibody conjugated to monomethylauristatin E through a valine–citrulline linker.

Recently, Petrylak et al. [74] presented the results from a phase II trial on the use of PSMA-ADC at 2.5 mg/kg in 34 patients with taxane-refractory metastatic CRPC. Of them, 39% had both docetaxel and cabazitaxel, while 58% had received both abiraterone and enzalutamide. Dosing was initiated at 2.5 mg/kg and adjusted at 2.3 mg/kg for tolerability. They showed that PSA decline of ≥30% was noted in 36% (2.3 mg/kg) and 16% (2.5 mg/kg), while circulating tumor cell (CTC) decline of ≥50% was reported in 74% patients in both 2.3 and 2.5 mg/kg. Duration of therapy on 2.3 mg/kg was longer than on 2.5 mg/kg, as well as the rate of serious adverse events (37 vs 59%). Interestingly, PSA and CTC responses were associated with higher PSMA + CTC, while PSA responses alone were correlated with lower NE markers, suggesting that NE differentiation may play a role in this context. Based on these results, this study has been extended and is currently enrolling metastatic CRPC patients (NCT02020135) (Table 1).

4.6. Circulating tumor cells

Currently, there are no reliable serum markers to identify patients that are transforming to NEPC. The enumeration and molecular characterization of circulating tumor cells (CTCs) has been recently proposed in this setting. CTCs seem to be a potential marker of drug-induced changes and mechanisms of drug resistance in patients with CRPC, but are not well described in NEPC.

Kaur et al. evaluated CTC count in 61 patients, 21 NEPC 40 patients with CRPC [75]. The median age was 73.7 years in NEPC patients and 73.9 years in CRPC patients. The median OS was, respectively, 22.6 and 20.7 months in patients with CRPC and NEPC. The rate of detectable CTC counts was similar for the two groups (47.6% of NEPC and 55% of CRPC). CTC counts were prognostic for both groups. Indeed, the detection of 0 to 4 CTCs in NEPC were associated with a median OS of 22.6 versus 6.6 months in patients with ≥5 CTCs ($p < 0.001$). Similarly, CRPC patients with 0 to 4 CTCs had a higher median OS (not reached, mean 40.6 months) compared to those with ≥5 CTCs (11.2 months, $p < 0.001$). These results suggest that patients with NEPC show similar frequency of detectable and elevated CTC counts compared to an overall CRPC population. Moreover, CTC counts seem to be prognostic for both NEPC and CRPC groups. Further attempts are required to validate CTC count as an early signal of NE differentiation in order to optimize the management of these patients.

4.7. NE differentiation as a mechanism of drug-resistance

NE differentiation has been proposed as a mechanism for hormonal escape or AR independence [74]. However, the mechanisms by which NE differentiation arises after ADT, and the effect of targeting these cell populations remain uncertain.

Terry et al. studied the potential relationship between the AR axis and a novel putative marker of NE differentiation, the human male protocadherin-PC (PCDH-PC) [76]. They found that PCDH-PC overexpression attenuated the ligand-dependent activity of the AR. The acquisition of a NE phenotype by PCa cells positively correlated with resistance to cytotoxic agents including docetaxel, as demonstrated by the evidence that knockdown of PCDH-PC in NE cells partially re-sensitized cells to this drug [77].

In 2013, Hirano et al. published their results on the relationship between the clinical efficacies of secondary hormone therapy for CRPC following first line hormone therapy and NE differentiation. Data on CgA kinetics were available in 35 patients. Among them they found that CgA levels before and at 3 months during the treatment were similar. However, a tendency for worse survival was reported in 8 patients with ≥25% increase in CgA level from baseline (63% vs 84% at 5 years, $p = 0.0507$) compared with the remaining patients [78].

Androgen receptor splice variants (ARSVs) have been proposed as a potential mechanism of resistance to ADT. ARSVs are commonly present in CRPC tissue/cells where they mediate resistance to anti-androgens and androgen synthesis inhibitors by regulation of a mitosis-dependent ARSV-driven transcriptome program. Ferrari et al. [79] presented the results on the in vitro activity of enzalutamide in androgen dependent LNCaP cells, isogenic androgen-independent (AI) cells and AI CW22RV1 (RV1) cells. They observed that after an effective interval of enzalutamide, a precipitous increase of pathogenic ARSV7 occurred in AD LNCaP cells concurrent with the acquisition of AI/CR PC characteristics. These changes included downregulation of AR-regulated transcripts (KLK3, TMPRSS), a biphasic response of cell cycle genes (CDK1, CCNA2, UBE2C) (culture passage [cp] 3 downregulation, cp16 upregulation) and upregulation of NE genes (AGR2,AURKA, SSTR2) at cp 16.

5. Discussion

In the last years, several step forwards have been provided in understanding the biologic mechanisms driving progression toward the NE phenotype. Due to a high prevalence of NE differentiation in patients who receive prolonged ADT, the real incidence of NEPC remains controversial. At 2014 ASCO Genitourinary Cancers Symposium, Perez et al. investigated for the presence of NE differentiation in a series of 450 patients with PCa. Only three patients presented NE differentiation [80]. On the other hand, Jimenez and his colleagues [81] evaluated the NE differentiation patterns in 237 metastatic sites from 187 patients with PCa or poorly differentiated carcinomas. The list of metastases included the bone (43%), lung (17%), liver (17%), lymph node (9%), bladder (6%), soft tissue (5%), brain (2%), and others (2%). Ninety-two metastatic biopsies from 79 patients showed positive NE differentiation. Seven patients who had, at first, negative biopsies on metastatic sites

had, successively, positive biopsies. NE differentiation was found in 41% bone sites, compared to 53% of non-bone sites. Among the 72 patients with known clinical history, 47 underwent metastatic biopsy during hormone sensitive stage (HSPC) and 25 during CRPC stage. They reported NE differentiation in 21 (44%) and 14 (56%) cases, respectively, suggesting that patterns of NE differentiation may vary within the same patient during the natural history of PCa.

6. Conclusions

NEPC represents a highly heterogeneous disease. Despite the recent findings described in these review, the management of patients with NEPC still remains a challenge for oncologists. Data gathered from ongoing trials will surely improve the management of NEPC patients, even if it is difficult to define the relevance of each one's individual contribution. However, preliminary results from clinical trials thus constitute a basis for moderate enthusiasm, but a dramatic improvement of NEPC patient outcomes seems still so far.

References

[1] K.J. Pienta, D. Bradley, Mechanisms underlying the development of androgen-independent prostate cancer, Clin. Cancer Res. 12 (2006) 1665–1671.
[2] A. Komiya, K. Yasuda, A. Watanabe, Y. Fujiuchi, T. Tsuzuki, H. Fuse, The prognostic significance of loss of the androgen receptor and neuroendocrine differentiation in prostate biopsy specimens among castration-resistant prostate cancer patients, Mol. Clin. Oncol. 1 (2013) 257–262.
[3] P.A. di Sant'Agnese, Neuroendocrine differentiation in carcinoma of the prostate: diagnostic, prognostic, and therapeutic implications, Cancer 70 (1992) 254–268.
[4] A. Komiya, K. Yasuda, A. Watanabe, Y. Fujiuchi, T. Tsuzuki, H. Fuse, The prognostic significance of loss of the androgen receptor and neuroendocrine differentiation in prostate biopsy specimens among castration-resistant prostate cancer patients, Mol Clin Oncol. 1 (2) (2013) 257–262.
[5] D.G. Bostwick, J. Qian, A. Pacelli, et al., Neuroendocrine expression in node positive prostate cancer: correlation with systemic progression and patient survival, J. Urol. 168 (2002) 1204–1211.
[6] D. Theodorescu, S.R. Broder, J.C. Boyd, et al., Cathepsin D and chromogranin A as predictors of long term disease specific survival after radical prostatectomy for localized carcinoma of the prostate, Cancer 80 (1997) 2109–2119.
[7] M.H. Weinstein, A.W. Partin, R.W. Veltri, et al., Neuroendocrine differentiation in prostate cancer: enhanced prediction of progression after radical prostatectomy, Hum. Pathol. 27 (1996) 683–687.
[8] M. Tarle, M.Z. Ahel, K. Kovacić, Acquired neuroendocrine-positivity during maximal androgen blockade in prostate cancer patients, Anticancer Res. 22 (2002) 2525–2529.
[9] A. Berruti, L. Dogliotti, A. Mosca, et al., Effects of the somatostatin analog lanreotide on the circulating levels of chromogranin-A, prostate-specific antigen, and insulin-like growth factor-1 in advanced prostate cancer patients, Prostate 47 (2001) 205–211.
[10] M. Ousset, A. Van Keymeulen, G. Bouvencourt, et al., Multipotent and unipotent progenitors contribute to prostate postnatal development, Nat. Cell Biol. 14 (2012) 1131–1138.
[11] J.C. Pignon, C. Grisanzio, Y. Geng, J. Song, R.A. Shivdasani, S. Signoretti, p63-expressing cells are the stem cells of developing prostate, bladder, and colorectal epithelia, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 8105–8110.
[12] Y. Kim, M. Nirenberg, Drosophila NK-homeobox genes, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 7716–7720.
[13] C. Abate-Shen, M.M. Shen, E. Gelmann, Integrating differentiation and cancer: the Nkx3.1 homeobox gene in prostate organogenesis and carcinogenesis, Differentiation 76 (2008) 717–727.
[14] H. Chen, L.N. Mutton, G.S. Prins, C.J. Bieberich, Distinct regulatory elements mediate the dynamic expression pattern of Nkx3.1, Dev. Dyn. 234 (2005) 961–973.
[15] P.J. Sciavolino, E.W. Abrams, L. Yang, L.P. Austenberg, M.M. Shen, C. Abate-Shen, Tissue-specific expression of murine Nkx3.1 in the male urogenital system, Dev. Dyn. 209 (1997) 127–138.
[16] C.J. Bieberich, K. Fujita, W.-W. He, G. Jay, Prostate-specific and androgen dependent expression of a novel homeobox gene, J. Biol. Chem. 271 (1996) 31779–31782.
[17] X. Wang, M. Kruithof-de Julio, K.D. Economides, D. Walker, H. Yu, M.V. Halili, Y.P. Hu, S.M. Price, C. Abate-Shen, M.M. Shen, A luminal epithelial stem cell that is a cell of origin for prostate cancer, Nature 461 (2009) 495–500.
[18] Z.A. Wang, A. Mitrofanova, S.K. Bergren, et al., Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity, Nat. Cell Biol. 15 (2013) 274–283.
[19] J.O. DaSilva, G.P. Amorino, E.V. Casarez, B. Pemberton, S.J. Parsons, Neuroendocrine-derived peptides promote prostate cancer cell survival through activation of IGF-1R signaling, Prostate 73 (2013) 801–812.
[20] T. Tawadros, F. Alonso, P. Jichlinski, N. Clarke, T. Calandra, J.A. Haefliger, T. Roger, Release of macrophage migration inhibitory factor by neuroendocrine-differentiated LNCaP cells sustains the proliferation and survival of prostate cancer cells, Endocr. Relat. Cancer 20 (1) (2013) 137–149.
[21] H. Beltran, S. Tomlins, A. Aparicio, V. Arora, D. Rickman, G. Ayala, J. Huang, L. True, M.E. Gleave, H. Soule, C. Logothetis, M.A. Rubin, Aggressive variants of castration-resistant prostate cancer, Clin. Cancer Res. 20 (2014) 2846–2850.
[22] Z. Zhou, A. Flesken-Nikitin, D.C. Corney, W. Wang, D.W. Goodrich, P. Roy-Burman, et al., Synergy of p53 and Rb deficiency in a conditional mouse model for metastatic prostate cancer, Cancer Res. 66 (2006) 7889–7898.
[23] T. Chiaverotti, S.S. Couto, A. Donjacour, J.H. Mao, H. Nagase, R.D. Cardiff, et al., Dissociation of epithelial and neuroendocrine carcinoma lineages in the transgenic adenocarcinoma of mouse prostate model of prostate cancer, Am. J. Pathol. 172 (2008) 236–246.
[24] H. Beltran, D.S. Rickman, K. Park, S.S. Chae, A. Sboner, T.Y. MacDonald, et al., Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets, Cancer Discov. 1 (2011) 487–495.
[25] J.M. Coulson, J.L. Edgson, P.J. Woll, J.P. Quinn, A splice variant of the neuron-restrictive silencer factor repressor is expressed in small cell lung cancer: a potential role in derepression of neuroendocrine genes and a useful clinical marker, Cancer Res. 60 (2000) 1840–1844.
[26] J.I. Epstein, M.B. Amin, H. Beltran, T.L. Lotan, J.M. Mosquera, V.E. Reuter, B.D. Robinson, P. Troncoso, M.A. Rubin, Proposed morphologic classification of prostate cancer with neuroendocrine differentiation, Am. J. Surg. Pathol. 38 (2014) 756–767.
[27] M.G. Weaver, F.W. Abdul-Karim, J. Srigley, et al., Paneth cell-like change of the prostate gland. A histological, immunohistochemical, and electron microscopic study, Am. J. Surg. Pathol. 16 (1992) 62–68.
[28] H. Adlakha, D.G. Bostwick, Paneth cell-like change in prostatic adenocarcinoma represents neuroendocrine differentiation: report of 30 cases, Hum. Pathol. 25 (2) (1994) 135–139.
[29] E.F. Tamas, J.I. Epstein, Prognostic significance of paneth cell-like neuroendocrine differentiation in adenocarcinoma of the prostate, Am. J. Surg. Pathol. 30 (2006) 980–985.
[30] B.C. Sarbay, G. Kir, C.S. Topal, E. Gumus, Significance of the cribriform pattern in prostatic adenocarcinomas, Pathol. Res. Pract. (2014), http://dx.doi.org/10.1016/j.prp.2014.03.003 (pii:S0344-0338(14)00090-9).
[31] R. Murali, K. Kneale, N. Lalak, W. Delprado, Carcinoid tumors of the urinary tract and prostate, Arch. Pathol. Lab. Med. 130 (2006) 1693–1706.
[32] A. Reyes, C.A. Moran, Low-grade neuroendocrine carcinoma (carcinoid tumor) of the prostate, Arch. Pathol. Lab. Med. 128 (2004) e166–e168.
[33] V.S. Ghali, R.L. Garcia, Prostatic adenocarcinoma with carcinoidal features producing adrenocorticotropic syndrome. Immunohistochemical study and review of the literature, Cancer 54 (1984) 1043–1048.
[34] J. Lipianskaya, A. Cohen, C.J. Chen, E. Hsia, J. Squires, Z. Li, Y. Zhang, W. Li, X. Chen, H. Xu, J. Huang, Androgen-deprivation therapy-induced aggressive prostate cancer with neuroendocrine differentiation, Asian J. Androl. (Feb 21 2014), http://dx.doi.org/10.4103/1008-682X.123669.
[35] R. Mazzucchelli, D. Morichetti, A. Lopez-Beltran, L. Cheng, M. Scarpelli, Z. Kirkali, R. Montironi, Neuroendocrine tumours of the urinary system and male genital organs: clinical significance, BJU Int. 103 (2009) 1464–1470.
[36] C.C. Guo, J.Y. Dancer, Y. Wang, et al., TMPRSS2-ERG gene fusion in small cell carcinoma of the prostate, Hum. Pathol. 42 (2011) 11–17.
[37] T.L. Lotan, N.S. Gupta, W. Wang, et al., ERG gene rearrangements are common in prostatic small cell carcinomas, Mod. Pathol. 24 (2011) 820–828.
[38] L. Wang, S.R. Williamson, S. Zhang, J. Huang, R. Montironi, D.D. Davison, M. Wang, J.L. Yao, A. Lopez-Beltran, A.O. Osunkoya, G.T. Maclennan, L.A. Baldridge, X. Du, L. Cheng, Increased androgen receptor gene copy number is associated with TMPRSS2-ERG rearrangement in prostatic small cell carcinoma, Mol. Carcinog. (2014), http://dx.doi.org/10.1002/mc.22162.
[39] H.L. Tan, A. Sood, H.A. Rahimi, W. Wang, N. Gupta, J. Hicks, S. Mosier, C.D. Gocke, J.I. Epstein, G.J. Netto, W. Liu, W.B. Isaacs, A.M. De Marzo, T.L. Lotan, Rb loss is characteristic of prostatic small cell neuroendocrine carcinoma, Clin. Cancer Res. 20 (2014) 890–903.
[40] K.F. Macleod, The RB, tumor suppressor: a gatekeeper to hormone independence in prostate cancer? J. Clin. Invest. 120 (2010) 4179–4182.
[41] H. Chen, Y. Sun, C. Wu, C.E. Magyar, X. Li, L. Cheng, J.L. Yao, S. Shen, A.O. Osunkoya, C. Liang, J. Huang, Pathogenesis of prostatic small cell carcinoma involves the inactivation of the P53 pathway, Endocr. Relat. Cancer 19 (2012) 321–331.
[42] R.A. Simon, P.A. di Sant'agnese, L.S. Huang, et al., CD44 expression is a feature of prostatic small cell carcinoma and distinguishes it from its mimickers, Hum. Pathol. 40 (2009) 252–258.
[43] S. Deorah, M.B. Rao, R. Raman, et al., Survival of patients with small cell carcinoma of the prostate during 1973–2003: a population based study, BJU Int. 109 (2012) 824–830.
[44] J.H. Rubenstein, M.J. Katin, M.M. Mangano, et al., Small cell anaplastic carcinoma of the prostate: seven new cases, review of the literature, and discussion of a therapeutic strategy, Am. J. Clin. Oncol. 20 (1997) 376–380.
[45] W. Wang, J.I. Epstein, Small cell carcinoma of the prostate. A morphologic and immunohistochemical study of 95 cases, Am. J. Surg. Pathol. 32 (2008) 65–71.
[46] R.J. Amato, C.J. Logothetis, R. Hallinan, et al., Chemotherapy for small cell carcinoma of prostatic origin, J. Urol. 147 (1992) 935–937.
[47] M. Ruscica, M. Arvigo, F. Gatto, E. Dozio, D. Feltrin, M.D. Culler, F. Minuto, M. Motta, D. Ferone, P. Magni, Regulation of prostate cancer cell proliferation by somatostatin receptor activation, Mol. Cell. Endocrinol. 315 (2010) 254–262.
[48] A.M. Aparicio, A.L. Harzstark, P.G. Corn, et al., Platinum-based chemotherapy for variant castrate-resistant prostate cancer, Clin. Cancer Res. 19 (2013) 3621–3630.
[49] J.R. Mackey, H.J. Au, J. Hugh, P. Venner, Genitourinary small cell carcinoma: determination of clinical and therapeutic factors associated with survival, J. Urol. 159 (1998) 1624–1629.

[50] A.J. Evans, P.A. Humphrey, J. Belani, T.H. van der Kwast, J.R. Srigley, Large cell neuroendocrine carcinoma of the prostate: a clinicopathologic summary of 7 cases of a rare manifestation of advanced prostate cancer, Am. J. Surg. Pathol. 30 (2006) 684–693.
[51] T.W. Friedlander, V.K. Weinberg, E.J. Small, J. Sharib, A.L. Harzstark, A.M. Lin, L. Fong, C.J. Ryan, Effect of the somatostatin analog octreotide acetate on circulating insulin-like growth factor-1 and related peptides in patients with non-metastatic castration-resistant prostate cancer: results of a phase II study, Urol. Oncol. 30 (2012) 408–414.
[52] C.S. Mitsiades, J. Bogdanos, D. Karamanolakis, C. Milathianakis, T. Dimopoulos, M. Koutsilieris, Randomized controlled clinical trial of a combination of somatostatin analog and dexamethasone plus zoledronate vs. zoledronate in patients with androgen ablation-refractory prostate cancer, Anticancer Res. 26 (2006) 3693–3700.
[53] R. Mazzucchelli, R. Montironi, A. Santinelli, G. Lucarini, A. Pugnaloni, G. Biagini, Vascular endothelial growth factor expression and capillary architecture in high-grade PIN and prostate cancer in untreated and androgen-ablated patients, Prostate 45 (2000) 72–79.
[54] M.T. Spiotto, T.D. Chung, STAT3 mediates IL-6-induced neuroendocrine differentiation in prostate cancer cells, Prostate 42 (2000) 186–195.
[55] Y. Qiu, D. Robinson, T.G. Pretlow, H.J. Kung, Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3′-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 3644–3649.
[56] S. Xie, H.K. Lin, J. Ni, L. Yang, L. Wang, et al., Regulation of interleukin-6-mediated PI3K activation and neuroendocrine differentiation by androgen signaling in prostate cancer LNCaP cells, Prostate 60 (2004) 61–67.
[57] Y. Zhu, C. Liu, Y. Cui, N. Nadiminty, W. Lou, A.C. Gao, Interleukin-6 induces neuroendocrine differentiation (NED) through suppression of RE-1 silencing transcription factor (REST), Prostate (2014), http://dx.doi.org/10.1002/pros.22819.
[58] D.E. Drachenberg, A.A. Elgamal, R. Rowbotham, M. Peterson, G.P. Murphy, Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer, Prostate 41 (1999) 127–133.
[59] E. Angevin, J. Tabernero, E. Elez, et al., A phase I/II, Multiple-dose, dose escalation study of siltuximab, an anti-interleukin-6 monoclonal antibody, in patients with advanced solid tumors, Clin. Cancer Res. 20 (2014) 2192–2204.
[60] N.A. Delk, M.C. Farach-Carson, Interleukin-6: a bone marrow stromal cell paracrine signal that induces neuroendocrine differentiation and modulates autophagy in bone metastatic PCa cells, Autophagy 8 (2012) 650–663.
[61] P.C. Chang, T.Y. Wang, Y.T. Chang, C.Y. Chu, C.L. Lee, H.W. Hsu, T.A. Zhou, Z. Wu, R.H. Kim, S.J. Desai, S. Liu, H.J. Kung, Autophagy pathway is required for IL-6 induced neuroendocrine differentiation and chemoresistance of prostate cancer LNCaP cells, PLoS One 9 (2014) e88556.
[62] K. Inoue, J.W. Slaton, B.Y. Eve, S.J. Kim, P. Perrotte, M.D. Balbay, S. Yano, M. Bar-Eli, R. Radinsky, C.A. Pettaway, C.P. Dinney, Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer, Clin. Cancer Res. 6 (2000) 2104–2119.
[63] R.W. Veltri, M.C. Miller, G. Zhao, A. Ng, G.M. Marley, G.L. Wright Jr., R.L. Vessella, D. Ralph, Interleukin-8 serum levels in patients with benign prostatic hyperplasia and prostate cancer, Urology 53 (1999) 139–147.
[64] Zhen Li, Clark J. Chen, Jason K. Wang, Elaine Hsia, Wei Li, Jill Squires, Yin Sun, Jiaoti Huang, Neuroendocrine differentiation of prostate cancer, Asian J. Androl. 15 (2013) 328–332.
[65] X. Liu, J. Peng, W. Sun, S. Yang, G. Deng, F. Li, J.W. Cheng, J.R. Gordon, G31P, an antagonist against CXC chemokine receptors 1 and 2, inhibits growth of human prostate cancer cells in nude mice, Tohoku J. Exp. Med. 228 (2012) 147–156.
[66] E. Verjans, E. Noetzel, N. Bektas, A.K. Schütz, H. Lue, B. Lennartz, A. Hartmann, E. Dahl, J. Bernhagen, Dual role of macrophage migration inhibitory factor (MIF) in human breast cancer, BMC Cancer 9 (2009) 230.
[67] Y.C. Chen, X.W. Zhang, X.H. Niu, D.Q. Xin, W.P. Zhao, Y.Q. Na, Z.B. Mao, Macrophage migration inhibitory factor is a direct target of HBP1-mediated transcriptional repression that is overexpressed in prostate cancer, Oncogene 29 (2010) 3067–3078.
[68] E. Girard, C. Strathdee, E. Trueblood, C. Quéva, Macrophage migration inhibitory factor produced by the tumour stroma but not by tumour cells regulates angiogenesis in the B16-F10 melanoma model, Br. J. Cancer 107 (2012) 1498–1505.
[69] T. Tawadros, F. Alonso, P. Jichlinski, N. Clarke, T. Calandra, J.A. Haefliger, T. Roger, Release of macrophage migration inhibitory factor by neuroendocrine-differentiated LNCaP cells sustains the proliferation and survival of prostate cancer cells, Endocr. Relat. Cancer 20 (2013) 137–149.
[70] J.M. Mosquera, H. Beltran, K. Park, T.Y. MacDonald, B.D. Robinson, S.T. Tagawa, S. Perner, T.A. Bismar, A. Erbersdobler, R. Dhir, J.B. Nelson, D.M. Nanus, M.A. Rubin, Concurrent AURKA and MYCN gene amplifications are harbingers of lethal treatment-related neuroendocrine prostate cancer, Neoplasia 15 (2013) 1–10.
[71] H.J. Meulenbeld, J.P. Bleuse, E.M. Vinci, E. Raymond, G. Vitali, A. Santoro, L. Dogliotti, R. Berardi, F. Cappuzzo, S.T. Tagawa, C.N. Sternberg, M.G. Jannuzzo, M. Mariani, A. Petroccione, R. de Wit, Randomized phase II study of danusertib in patients with metastatic castration-resistant prostate cancer after docetaxel failure, BJU Int. 111 (2013) 44–52.
[72] A. Albihn, J.I. Johnsen, M.A. Henriksson, MYC in oncogenesis and as a target for cancer therapies, Adv. Cancer Res. 107 (2010) 163–224.
[73] A. Ghosh, W.D. Heston, Tumor target prostate specific membrane antigen (PSMA) and its regulation in prostate cancer, J. Cell. Biochem. 91 (2004) 528–539.
[74] D.P. Petrylak, D.C. Smith, L.J. Appleman, M.T. Fleming, A. Hussain, R. Dreicer, A.O. Sartor, N.D. Shore, N.J. Vogelzang, H. Youssoufian, V.A. DiPippo, N. Stambler, K. Huang, R.J. Israel, A phase 2 trial of prostate-specific membrane antigen antibody drug conjugate (PSMA ADC) in taxane-refractory metastatic castration-resistant prostate cancer (mCRPC), 2014 ASCO Annual Meeting, 2014 (abstract 5023).
[75] G. Kaur, B. Singh, H. Beltran, N.H. Akhtar, D.M. Nanus, S.T. Tagawa, Circulating tumor cell (CTC) enumeration in patients with metastatic neuroendocrine prostate cancer (NEPC) and castration-resistant prostate cancer (CRPC), J. Clin. Oncol. 32 (2014) (abstr 204).
[76] T. Jiborn, A. Bjartell, P.A. Abrahamsson, Neuroendocrine differentiation in prostatic carcinoma during hormonal treatment, Urology 51 (1998) 585-9.
[77] S. Terry, P. Maillé, H. Baaddi, L. Kheuang, P. Soyeux, N. Nicolaiew, J. Ceraline, V. Firlej, H. Beltran, Y. Allory, A. de la Taille, F. Vacherot, Cross modulation between the androgen receptor axis and protocadherin-PC in mediating neuroendocrine transdifferentiation and therapeutic resistance of prostate cancer, Neoplasia 15 (2013) 761–772.
[78] D. Hirano, R. Hasegawa, K. Satoh, J. Mochida, Y. Yamanaka, H. Hirakata, K. Yamaguchi, S. Sugimoto, N. Kawata, S. Takahashi, Prospective study on the relationship between clinical efficacy of secondary hormone therapy with flutamide and neuroendocrine differentiation in patients with relapsed prostate cancer after first line hormone therapy, Scand. J. Urol. 48 (5) (2014) 436–444.
[79] A.C. Ferrari, X.-M. Liu, M. Alshalalfa, A. Gomez-Pinillos, T. Sierocinski, E. Davicioni, Effect of enzalutamide (EZ) exposure time on androgen receptor (AR) splice variant (SV) expression and gene transcription correlates in androgen-dependent (AD) prostate cancer (PC) cells, 2014 ASCO Annual Meeting, 2014 (abstract e22066).
[80] D. Perez, E. Linares, E. Almagro, B. Cantos, M. Mendez, C. Maximiano, F. Franco, J. Rubio, M. Palka, V. Calvo, P. Ramirez, C. Salas, J. Carballido, M. Provencio, Neuroendocrine differentiation in carcinoma of the prostate: an institutional review, 2014 ASCO Annual Meeting, 2014 (abstract e16109).
[81] R.E. Jimenez, D. Nandy, R. Qin, R. Carlson, W. Tan, M. Kohli, Neuroendocrine differentiation patterns in metastases from advanced prostate cancer, 2014 ASCO Annual Meeting, 2014 (abstract 5085).