Asian Journal of Andrology (2014) 16, 541–544

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INVITED REVIEW

Prostate Disease

Androgen-deprivation therapy-induced aggressive prostate cancer with neuroendocrine differentiation

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Most prostate cancers (PCas) are classified as acinar type (conventional) adenocarcinoma which are composed of tumor cells with luminal differentiation including the expression of androgen receptor (AR) and prostate-specific antigen (PSA). There are also scattered neuroendocrine (NE) cells in every case of adenocarcinoma. The NE cells are quiesecent, do not express AR or PSA, and their function remains unclear. We have demonstrated that IL8-CXCR2-P53 pathway provides a growth-inhibitory signal and keeps the NE cells in benign prostate and adenocarcinoma quiescent. Interestingly, some patients with a history of adenocarcinoma recur with small cell neuroendocrine carcinoma (SCNC) after hormonal therapy, and such tumors are composed of pure NE cells that are highly proliferative and aggressive, due to P53 mutation and inactivation of the IL8-CXCR2-P53 pathway. The incidence of SCNC will likely increase due to the widespread use of novel drugs that further inhibit AR function or intratumoral androgen synthesis. A phase II trial has demonstrated that platinum-based chemotherapy may be useful for such therapy-induced tumors.
Asian Journal of Andrology (2014) **16**, 541–544; doi: 10.4103/1008-682X.123669; published online: 21 February 2014

Keywords: neuroendocrine; prostate cancer; small cell carcinoma; therapy

INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed malignancy and the second leading cause of cancer-related death in western countries. Its incidence is also increasing rapidly in countries such as China where PCa had traditionally been considered relatively uncommon.

Low-grade, organ-confined PCa is curable by surgery or radiation therapy. When local therapies can no longer be used in patients with advanced or metastatic PCa, hormonal therapy, by inhibiting androgen production and/or blocking androgen receptor (AR) function, is the treatment of choice. The molecular basis for hormonal therapy is that PCa is a hormonally-regulated cancer. Tumor cells express AR and androgen is required for the survival of tumor cells. As a result, hormonal therapy achieves therapeutic effect in nearly all patients. Unfortunately, this therapy is not curative and the cancer nearly always recurs after an initial period of response. The recurrent tumor after hormonal therapy is known as castration resistant prostate cancer (CRPC). Detailed histologic and molecular studies of CRPC have rarely been performed because biopsy or resection is rarely performed in such a clinical setting. Nonetheless, CRPC is still a form of adenocarcinoma and the tumor cells still show nuclear localization of AR and PSA production, suggesting that AR signaling is still active and likely critical at this stage of the disease.[1,2] A smaller percentage of patients will recur with small cell neuroendocrine carcinoma (SCNC) which has a different phenotype.[3] Because most CRPCs still appear to be AR-driven, newer agents have been developed to inhibit intratumoral androgen production (e.g. abiraterone acetate)[4] or more effectively block AR function (e.g. enzalutamide)[5] and they have shown clinical benefits by extending survival in patients who have exhausted therapeutic options.[2] Despite their proven efficacy, resistance to these drugs occurs quickly[6] and more importantly, a significant portion of the patients recur with SCNC.[7] In this review article, we will discuss the cellular heterogeneity of benign prostate and PCa, and the potential molecular mechanisms and therapeutic options for therapy-induced SCNC.

CELLULAR HETEROGENEITY IN BENIGN PROSTATE

The prostate gland is an epithelial organ composed of epithelia and stroma (**Figure 1a**). The stroma is complex consisting of smooth muscle cells, fibroblasts, blood vessels, nerves, inflammatory cells and so on. The pioneering work from Dr. Cunha's laboratory has demonstrated that stroma plays a critical role in the development, function, and carcinogenesis of the prostate.[8,9] The epithelial compartment of the prostate has three cell types: (i) secretory cells (or luminal cells) that produce secreted proteins including prostate-specific antigen (PSA), (ii) basal cells that likely function as reserve cells and (iii) neuroendocrine (NE) cells[10–12] that have neuronal morphology and endocrine function. The NE cells are a minor component of the prostate epithelia and comprise no more than 1% of the total epithelial cell population. They contain intracytoplasmic dense-core secretory granules under electron microscopy. It is difficult to identify NE cells on hematoxylin and eosin stained slides under light microscopy. Immunohistochemistry (IHC) with antibodies against NE markers such as chromogranin A, synaptophysin or CD56 can be used

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Received: 01 October 2013; Revised: 03 December 2013; Accepted: 03 December 2013

which demonstrates the presence of the scattered NE cells among the more abundant basal and luminal cells.[10–12] NE cells are also present in mice. However, unlike the wide distribution pattern seen in human prostate, NE cells are concentrated around the proximal urethra of the mice, but are not commonly seen in the different prostate lobes. The consistent presence of NE cells in every human prostate suggests that they likely play important roles in prostate development and function. However, little detail is known regarding their function.

CELLULAR HETEROGENEITY OF PROSTATIC ADENOCARCINOMA

A large number of men develop malignancy in the prostate, and in the majority of the patients, prostate carcinogenesis is a long process that often takes years or decades. It is widely accepted that prostate malignancy starts as prostatic intraepithelial neoplasia (PIN) which is characterized histologically by the appearance of malignant-appearing luminal cells without stromal invasion (**Figure 1b**).[13] In this regard, PIN is similar to carcinoma *in situ* seen in many other organs; but unlike carcinoma *in situ* in most organs (e.g. uterine cervix and urinary bladder), it takes years for PIN to progress to an invasive malignancy. Therefore, the presence of focal PIN alone in older men poses little risk to one's quality of life or life expectancy. Prostatic adenocarcinoma usually develops in a background of high grade PIN. In addition to having malignant luminal-type epithelial cells, invasive adenocarcinoma is characterized by the absence of basal cells. Therefore, on hematoxylin and eosin stained tissue sections, the tumor appears to be composed of malignant luminal cells only (**Figure 1c**). However, if the tumor is stained by IHC for NE markers, every case will have some scattered NE tumor cells.[10–12] It is debatable if the number of NE cells in prostatic adenocarcinoma correlates with tumor grade and stage. However, they possess unique features such as being negative for AR and PSA,[14] making them distinct from the bulk luminal type tumor cells. It is widely believed that because they do not express AR, they are androgen-independent, survive hormonal therapy and may contribute to the development of CRPC.[10–12]

PROSTATE CARCINOMA WITH NE DIFFERENTIATION

Although the vast majority of malignancies seen in the prostate are prostatic acinar type adenocarcinomas, occasionally tumors composed of pure NE cells are encountered clinically. The most common type of NE tumor is classified as SCNC with histologic features quite different from those of adenocarcinoma (**Figure 1d**).[10,15,16] In contrast to adenocarcinoma that forms glandular structures, SCNC grows as individual cells, cords or solid sheets. While cells of adenocarcinoma have abundant cytoplasm, relatively low nucleus/cytoplasm ratio, vacuolated nuclei with coarse chromatin pattern and prominent nucleoli, those of SCNC have scant cytoplasm, high nucleus/cytoplasm ratio, fine chromatin pattern and no nucleoli. SCNC also tends to have frequent mitotic figures and areas of necrosis, which are rarely seen with adenocarcinoma. Immunohistochemically, adenocarcinoma is characterized by the expression of luminal differentiation markers such as CK8, CK18, AR and PSA (**Figure 2**), while SCNC shows focal, perinuclear staining pattern for cytokeratin, negative staining for AR and PSA and positive staining for NE markers chromogranin A (**Figure 2**), synaptophysin and CD56.[10,15,16] SCNC is also positive for P53[17] and CD44,[18] and occasionally TTF-1.[10,15,16,19] However, in daily clinical practice, we have encountered cases that have only some of the histological and immunohistochemical features of SCNC as described above. Some cases with classic morphology of SCNC are negative for all the NE markers (**Figure 3**), while other cases that have histologic features in between adenocarcinoma and SCNC express NE markers strongly and diffusely. Such variations can cause difficulties and inconsistencies in pathologic diagnosis.

At least some of the cases with strong expression of NE markers, but morphologically imperfect for SCNC may be classified as large cell neuroendocrine carcinoma, which is very rarely diagnosed pathologically. The largest series of large cell neuroendocrine carcinoma was reported by Evans *et al*. who collected seven cases.[20] Histologically, large cell neuroendocrine carcinoma contained solid sheets and ribbons of cells with abundant pale to amphophilic cytoplasm, large nuclei with coarse chromatin and prominent nucleoli along with brisk mitotic activity and foci of necrosis. Their cases were strongly positive for CD56, CD57, chromogranin A, synaptophysin and P504S/alpha methylacyl CoA racemase. They also exhibited strong bcl-2 overexpression, expression of MIB1, and p53 in > 50% of nuclei, as well as focally positive staining for PSA and prostatic acid phosphatase and negative AR staining.[20] Six of six patients with available follow-up information died with metastatic disease at a mean of 7 months after platinum-based chemotherapy,[20] suggesting a clinical behavior similar to SCNC.

We have seen in consultation a case of carcinoid tumor (well-differentiated NE carcinoma) that is morphologically identical to carcinoid tumors seen in lung or the gastrointestinal tract. The patient was found to have a firm nodule on digital rectal exam, but had low serum PSA levels. Prostate biopsy showed that the tumor was composed of round and regular NE cells growing in a nested and tubular pattern. No mitotic figures or necrosis were present. The tumor quickly metastasized to bilateral lungs with numerous small nodules.

MOLECULAR MECHANISMS AND TREATMENT OF THERAPY-INDUCED SCNC

Although SCNC can arise *de novo*, it usually occurs as recurrent tumors in men who have received hormonal therapy for prostatic adenocarcinoma,[21–23] suggesting that the NE phenotype is driven by the hostile environment created by hormonal therapy. The relationship of these NE tumors to adenocarcinoma is also highlighted by the finding that most SCNC coexist with a component of conventional adenocarcinoma, although pure forms of SCNC are found occasionally.[24] Significant progress has been made in understanding the molecular mechanism of SCNC development. We reported that in benign prostate and adenocarcinoma, the IL-8-CXCR2-P53 pathway provides a strong growth inhibitory signal that keeps NE cells quiescent.[17,25] P53 mutation, likely a result of environmental pressure from hormonal therapy, inactivates this pathway and leads to hyperproliferation and aggressive behavior of the NE cells, resulting in the development of SCNC.[17] These findings are consistent with a clonal selection model and indicate that clones of NE cells gain a proliferative advantage in an androgen-deprived environment through P53 mutation. Rubin's group identified gene amplification and overexpression of Aurora A kinase and MYCN in a subset of such tumors, and the former may represent a potential therapeutic target.[26] Collin's group found that decreased expression of REST transcription complex may drive the emergence of the NE phenotype, favoring a model of adenocarcinoma trans-differentiating to SCNC.[27]

Accurate diagnosis of SCNC is important in determining the appropriate therapy, but it requires biopsy or resection of the primary or metastatic tumor followed by histologic examination and possibly IHC staining. Since patients who have failed hormonal therapy often have multiple metastases and poor prognosis, an invasive procedure (biopsy or resection) may not be practical. Therefore, many cases of SCNC that appear after hormonal treatment were simply considered to be CRPC, making this an underdiagnosed entity.[28,29]

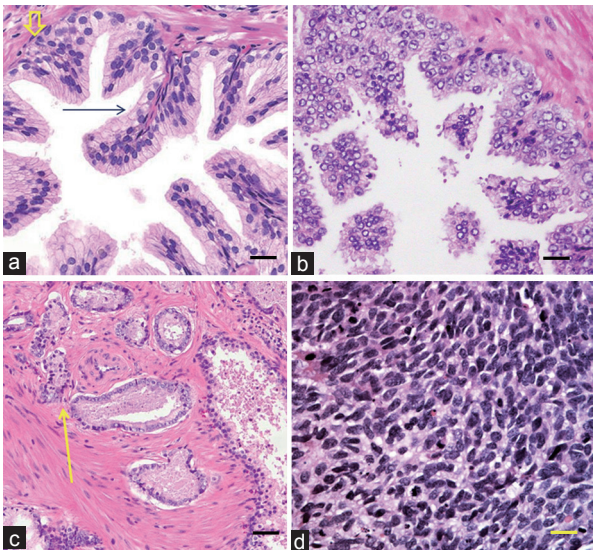

Figure 1: Histologic features of benign prostate gland, high grade PIN, adenocarcinoma and SCNC. (**a**) A high power view of benign prostate gland showing an inner luminal cell layer (long arrow) and an outer basal cell layer (short arrow). (**b**) High grade PIN showing malignant luminal cells without invasion of the stroma. (**c**) Prostatic adenocarcinoma showing proliferation of small, compact malignant glands (arrow). (**d**) SCNC which is composed of pure NE tumor cells without glandular formation. NE: neuroendocrine; PIN: prostatic intraepithelial neoplasia; SCNC: small cell neuroendocrine carcinoma.

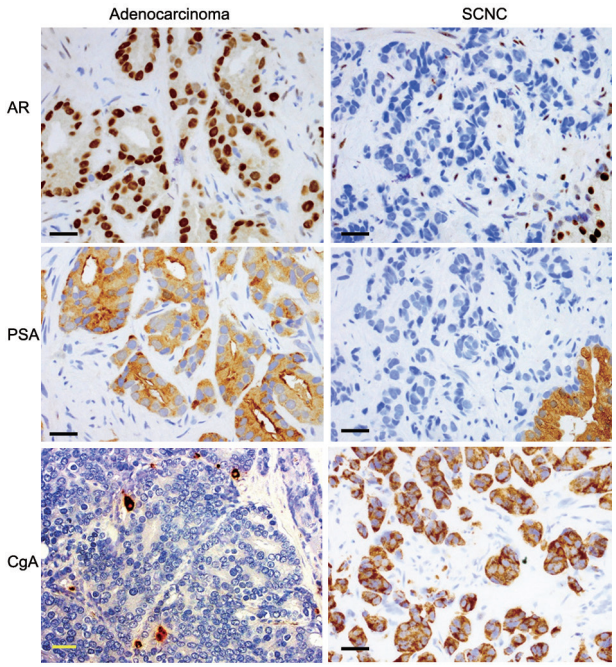

Figure 2: Immunohistochemical characteristics of prostatic adenocarcinoma *vs* SCNC. Adenocarcinoma expresses luminal differentiation markers AR and PSA. Tumor cells are negative for NE marker CgA. SCNC is negative for AR and PSA, but expresses CgA. AR: androgen receptor; CgA: chromogranin A; NE: neuroendocrine; PSA: prostate-specific antigen; SCNC: small cell neuroendocrine carcinoma.

Certain clinical features of SCNC may help to distinguish SCNC from adenocarcinoma. Patients with SCNC usually have low serum PSA relative to tumor burden and are not responsive to hormonal therapy. SCNC is highly aggressive and often shows early metastasis. In contrast to adenocarcinoma that has a propensity to metastasize to lymph nodes and bone, SCNC tends to metastasize to visceral organs such as liver, lung and brain. Aparicio *et al*.[30] prospectively studied cases that had SCNC histology or clinically met the "anaplastic" criteria. Their criteria included exclusive visceral metastases, radiographically predominant lytic bone metastases, bulky lymphadenopathy or bulky high-grade tumor mass in prostate/pelvis, low PSA at initial presentation plus high volume bone metastases, presence of NE markers on histology or in serum at initial diagnosis or at progression and short interval to androgen-independent progression following the initiation of hormonal therapy. A total of 120 patients who met at least one of the criteria were treated with first-line carboplatin and docetaxel and second-line etoposide and cisplatin in a phase II trial. Seventy-four of 113 (65.4%) and 24 of 71 (33.8%) were progression free after four cycles of carboplatin and docetaxel and etoposide and cisplatin, respectively. Median overall survival was 16 months. Of the seven 'anaplastic' criteria, bulky tumor mass was significantly associated with poor outcome. Lactic acid dehydrogenase strongly predicted for overall survival and rapid progression. Serum carcinoembryonic antigen concentration strongly predicted overall survival, but not rapid progression. NE markers did not predict outcome or response to therapy.[30]

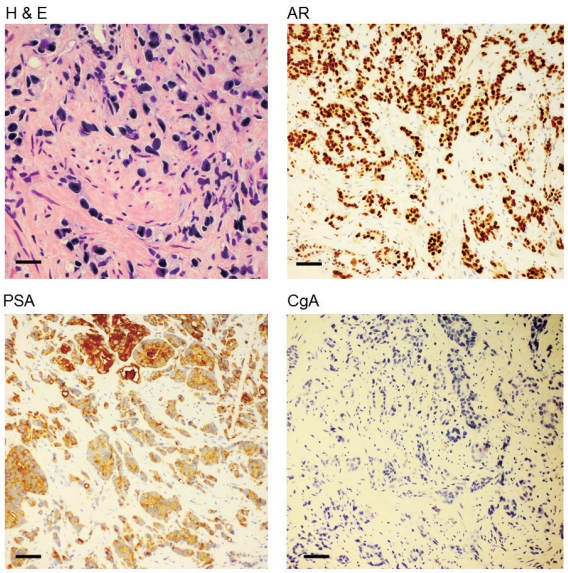

Figure 3: Some treated prostate cancers have classic morphology for SCNC, but not typical IHC profile. The case demonstrates histologic features of SCNC in that tumor cells do not form glandular structures. They are small with scant cytoplasm, darkly-stained nuclei with homogeneous chromatin pattern and no nucleoli. However, the tumor cells still express luminal differentiation markers AR and PSA and negative for NE marker CgA. AR: androgen receptor; CgA: chromogranin A; H and E: hematoxylin and eosin; IHC: immunohistochemistry; NE: neuroendocrine; PSA: prostate-specific antigen; SCNC: small cell neuroendocrine carcinoma.

CONCLUDING REMARKS

NE differentiation is a unique feature of PCa, particularly after treatment targeting androgen production or AR signaling. We must differentiate between adenocarcinoma with focal NE cells from SCNC. The former is fundamentally an adenocarcinoma with luminal differentiation which should respond to hormonal therapy.

The number of NE cells in adenocarcinoma varies from case to case but these cells are quiescent and their prognostic role remains uncertain. Conversely, SCNC is a tumor composed of aggressive and highly proliferative NE cells which does not respond to hormonal therapy and should be treated with chemotherapy. We discourage using the term 'neuroendocrine carcinoma' because it can potentially mix adenocarcinoma containing abundant NE cells with SCNC. With the widespread use of novel androgen axis-targeting drugs such as abiraterone acetate and enzalutamide, we have already witnessed a rapid rise in the incidence of SCNC which will become a main challenge in managing these patients. Therefore, studying the molecular mechanisms, recognizing the disease early and accurately, and developing novel therapies for SCNC remain important tasks for PCa researchers.

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How to cite this article: Lipianskaya J, Cohen A, Chen CJ, Hsia E, Squires J, Li Z, Zhang Y, Li W, Chen X, Xu H, Huang J. Androgen-deprivation therapy-induced aggressive prostate cancer with neuroendocrine differentiation. *Asian J Androl.* doi: 10.4103/1008-682X.123669. 21 February 2014. [Epub ahead of print]