Predicting clinical outcome of therapy-resistant prostate cancer

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Clinical Course of Prostate Cancer and Tumor Histology

Prostate cancer (PCa) is among the most common malignancies in men and a leading cause of cancer-related death. Due to its high incidence, men generally undergo annual screening for serum levels of prostate-specific antigen (PSA). Those with elevated serum PSA are usually referred to urologists and often undergo multiparametric MRI diagnosis and core needle biopsy of the prostate. Due to PSA's poor specificity, only a fraction of the men with elevated PSA will be diagnosed with PCa. Tumors diagnosed through PSA screening are biologically heterogeneous and clinical outcomes vary significantly from patient to patient. Many such tumors are indolent, do not impact patients' quality of life or life expectancy, and require no treatment. Low-grade and low-stage tumors can be cured by surgery or radiation. Unfortunately, many men with high-grade and high-stage tumors will experience tumor recurrence after local treatment. The heterogeneity in the biological behavior of PCa makes this a unique tumor among human malignancies. Over the years, a number of factors have been found useful in predicting the biologic behavior of PCa, such as preoperative PSA levels, findings by multiparametric MRI, Gleason grade of the tumor, and the number of positive biopsy cores. In prostatectomy specimens, tumor grade, tumor stage, and the status of resection margins predict clinical outcome. PCa that cannot be controlled by local therapies is treated with hormonal therapy that blocks androgen production and/or the activity of the androgen receptor (AR). Although hormonal treatment is effective initially, the tumor always recurs and becomes castration resistant (CR). Abiraterone and enzalutamide, which are next-generation AR signaling inhibitors (ARSI), can extend lives for patients with CR PCa (CRPC), but the tumor will eventually progress. Importantly, at the CRPC stage, the prognosis varies significantly from case to case, with some patients surviving for years and others progressing

rapidly. A study in PNAS by Abida et al. (1) identifies RB1 genomic alteration as a potent predictor of poor outcome.

CRPC has traditionally been used to represent a clinical stage of PCa progression after hormonal therapy. Until recently, the histology and genomic alterations of CRPC were not well understood because biopsy or resection of the tumor was very rarely performed at this disease stage since the diagnosis was not an issue and no further treatment could be offered. The landscape of CRPC has changed due to the work done by 2 large multidisciplinary teams—the International Dream Team and the West Coast Dream Team. The 2 teams performed biopsies of metastatic CRPC (mCRPC) in hundreds of patients who have received AR-targeted therapies. The studies from the 2 teams have uncovered the genomic landscape and begun to reveal histologic features of CRPC. AR pathway aberrations were found to be frequent events, including gene amplification and mutation, again supporting the notion that AR is required throughout different stages of PCa. An extremely interesting and important finding is that a significant portion of CRPC cases are histologically classified as small cell neuroendocrine carcinoma (SCNC) with important genomic and transcriptional alterations.

Histologic studies of PCa have played important roles in our understanding of the disease. Benign prostate glands are composed of basal cells expressing P63 and high-molecular-weight cytokeratin and luminal cells expressing the luminal differentiation markers AR and PSA. A third, minor epithelial cell type, known as neuroendocrine (NE) cells, expresses the classic NE markers chromogranin A and synaptophysin but is negative for AR and PSA. The function of NE cells in benign prostate remains poorly understood. The development of PCa undergoes distinct steps that can be readily identified by histologic examination of the tissue. The first step is known as high-grade prostatic intraepithelial neoplasia, in which luminal epithelial cells have become malignant but

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the basal cells are still present, at least partially. Eventually, the malignant luminal cells will break through the basal layer to form cancerous glands of PCa in prostate stroma. As a result, the vast majority of primary PCa cases are characterized by the absence of basal cells and the proliferation of malignant tumor cells with luminal phenotype (AR+, PSA+) with various degrees of glandular formation. Such tumors are histologically classified as adenocarcinoma. Interestingly, every case of adenocarcinoma contains a minor component of NE cells that usually comprises no more than 1% of the tumor cell population (2). Unlike the bulk, luminal-type tumor cells, NE cells in prostatic adenocarcinoma do not express AR and PSA, are positive for NE markers, and are quiescent (2). A small number of patients (no more than 1%) may present with SCNC, which is a histologic variant of PCa. SCNC is composed of pure NE cells but, unlike those present in adenocarcinoma, NE cells in SCNC are highly proliferative and biologically aggressive, leading to early metastasis and death (2).

It is now firmly established that CRPC as a clinical entity is histologically heterogeneous. While the majority of CRPC cases still maintain histologic features of adenocarcinoma composed of bulk luminal tumor cells and a smaller NE tumor cell component, a proportion of the CRPC cases are histologically SCNC (3). Because such SCNC cases arise after AR-targeted hormonal therapy, it has been suggested that they be designated therapy-induced SCNC, or t-SCNC. Studies have shown that t-SCNC has histologic features identical to primary SCNC of the prostate, and the same diagnostic criteria can be applied in both settings for pathologic diagnosis. Interestingly, the study performed by the West Coast Dream Team suggests that t-SCNC may be molecularly different from primary SCNC. While it is generally accepted that primary SCNC is negative for AR (2), t-SCNC has amplified AR and expresses AR protein to a similar extent as what is seen in adenocarcinoma (3). Interestingly, although AR protein is highly expressed in t-SCNC, its transcription signature is very low, indicating that AR may not be functional or may function differently in this setting (3).

Molecular Mechanisms of Therapy-Induced Small Cell Neuroendocrine Carcinoma of the Prostate

In nearly all of the patients with t-SCNC, the original primary tumors in the prostates were adenocarcinoma. How the tumor changes from one histologic type to another remains a topic of intense investigation. In cultured PCa cell lines such as LNCaP, androgen withdrawal in the culture media leads to the disappearance of the luminal phenotype and the appearance of an NE phenotype, suggesting that luminal-type PCa cells possess lineage plasticity and can become NE tumor cells through a process known as transdifferentiation. Experimental evidence has been published that seems to support this model (4, 5), but whether this happens in patients remains unknown. It is important to point out that in the cultured LNCaP cell model, the NE-like cells that appear after androgen withdrawal proliferate very slowly, if at all, which is different from the highly proliferative NE tumor cells in t-SCNC.

Clonal expansion remains a plausible mechanism to explain the transformation of adenocarcinoma to t-SCNC after hormonal therapy. NE cells in adenocarcinoma are negative for AR and thus insensitive to AR-targeted hormonal therapies. Hormonal therapy, while inhibiting AR+ luminal-type tumor cells, would spare NE tumor cells and enrich them. In support of this notion, it has been reported that adenocarcinoma in the CRPC setting generally contains more abundant NE tumor cells. Our laboratory has shown that

NE cells in benign prostate and prostatic adenocarcinoma express cytokine IL-8 and its receptor CXCR2 (6), and an autocrine activation of CXCR2 leads to activation of the TP53 pathway, resulting in inhibition of proliferation of the NE cells (7). It is thus possible that inactivation of this pathway through molecular events such as TP53 mutation would knock out this inhibitory signal, resulting in hyperproliferation of the NE cells and the

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development of t-SCNC (7). Consistent with this model, recent studies have shown that TP53 mutation is rare in primary prostatic adenocarcinoma, more common in CRPC adenocarcinoma (CRPC-Adeno), and most frequent in t-SCNC (3, 8–10). We have also shown that TP53 inactivation potentiates prostate cancer cells' growth and confers an adaption to the castration environment (11). Therefore, an interesting hypothesis is that mutations in luminal-type tumor cells of prostatic adenocarcinoma during hormonal therapy would lead to CRPC-Adeno, while similar mutations in NE tumor cells of adenocarcinoma would result in the expansion of the NE tumor cells and t-SCNC.

Predicting Clinical Outcome of Therapy-Resistant Prostate Cancer

The genomic landscape of mCRPC has been reported. Studies over the past several years have provided insights into the genomics of t-SCNC. RB and TP53 mutations are frequent events in t-SCNC, similar to what has been observed in SCNC of other organs (12). Importantly, studies in animal models have shown that loss of function of RB and TP53 is sufficient to induce SCNC and their mutations are driver mutations (13, 14). There are also significant overexpression and gene amplification of *AURKA* and *MYCN* in SCNC (15), and N-Myc can similarly drive the development of SCNC (16).

Multiple studies have attempted to discover predictive and prognostic factors in CRPC. Histologic examination alone provides important prognosis information, as patients with t-SCNC have significant shorter survival than those without SCNC (3). It has been reported that the expression of a splicing variant of AR (AR-V7) in circulating tumor cells of men with mCRPC predicts poor response to abiraterone and enzalutamide and a worse prognosis, while the patients seem to benefit from taxane treatment (17). Alterations in DNA repair genes predict good response to PARP inhibitors (18), and biallelic loss of *CDK12* may predict good response to checkpoint inhibitors (19). The article in PNAS by Abida et al. (1) correlates contemporaneously obtained comprehensive genomic profiles of a large number of mCRPCs with clinical outcome. The authors discover that genomic alterations in *RB1* show the strongest discrimination for shorter time on ARSI and survival; alterations in *TP53* and *AR* are also associated with shorter time on ARSI (though there is no association with overall survival); and aneuploid chromosomal status is associated with worse overall survival and time on treatment compared with diploid status (1).

In conclusion, significant advances have been made in understanding therapy-resistant PCa in areas of histology, genomics, and transcriptomics, resulting in increased ability in disease prognosis and therapy-response prediction. It is expected that we

will continue to learn more in these areas as biopsy of mCRPC becomes more common and technologies continue to advance, resulting in better patient management and better clinical outcome.

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