Chinese Journal of New Clinical Medicine, July 2021, Volume 14, Number 7

专家述评

Novel therapeutic avenues for therapy-resistant prostate cancer: a review

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Funding: This study was financially supported by National Natural Science Foundation of China(86902611)

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HUANG Jiao-ti, MD, PhD, professor. Dr HUANG earned his medical degree from Anhui Medical University, and obtained a master's degree in Institute of Radiation Medicine, Academy of Military Medical Sciences. He came to the United States in 1987 and earned his PhD in Molecular and Cellular Biology from New York University (NYU) School of Medicine in 1991. He was a postdoctoral fellow at NYU and Yale University during 1991 and 1995. He did his residency in the Department of Anatomic and Clinical Pathology of NYU and did his fellowship in the Department of Oncologic Surgical Pathology of Memorial Sloan-Kettering Cancer Center of New York during 1995 and 2000. He became an assistant professor at University of Rochester in July 2000 and rose to the rank of full professor in 2007. Dr HUANG moved to University of California, Los Angeles in 2008 to be a professor of pathology/urology and later director of Department of Urologic Pathology, professor of Jonsson Cancer Center and Broad Stem Cell Institute. He arrived at Duke at the beginning of 2016 to be chairman of Department of Pathology of Duke University. Dr HUANG's clinical expertise is pathology of genitourinary tumor. Dr HUANG was awarded numerous grants from National Cancer Institute, American Cancer Society, Prostate Cancer Research Program of Department of Defense and Prostate Cancer Foundation. Dr HUANG has published research papers as first author in *Proceedings of the National Academy of Sciences*(*PNAS*), *Cell* and *the New England Journal of Medicine*(*NEJM*) since early 1990s. Dr HUANG and his collaborators have published more than 200 papers focusing on molecular mechanisms of carcinogenesis and tumor progression. In his laboratory, the research on the function of prostatic small cell neuroendocrine carcinoma is at the world's leading level. In addition to patient care and research, he has trained numerous residents, fellows, graduate students and postdocs. Dr HUANG is also the board member and reviewer of many international journals.

[**Abstract**] Although hormonal therapy is effective initially for metastatic prostate cancer(PCa), therapy resistance invariably occurs. Our team has been dedicated to investigating potential mechanisms and exploiting novel therapeutic managements for those advanced patients who have run out of treatment of choice for decades. Our study scopes mainly focus on tumor biomarker identification, neuroendocrine differentiation and tumor metabolism. This review summarizes some of our key findings to advance understandings of how PCa progresses and what potential treatment regimens are.

[**Key words**] Prostate cancer; Therapeutic resistance; Tumor biomarker; Tumor metabolism

[Chinese library classification number] R 737.25 [Document code] A

[Article ID] 1674 - 3806(2021)07 - 0642 - 05

doi:10.3969/j.issn.1674 - 3806.2021.07.02

1 INTRODUCTION

Prostate cancer(PCa) is one of the most common non-cutaneous malignancies worldwide, particularly in developed countries[1]. Although most men with primary PCa have a good clinical outcome, diagnostic and therapeutic challenges still remain. For example, in spite of its high sensitivity, prostate-specific antigen(PSA) screening has been debated for years as it may lead to overtreatment in patients who would otherwise have an indolent disease course and benefit from simple active surveillance[2]. For patients with advanced PCa, commonly used hormonal therapy is unable to provide a permanent cure as all the patients eventually develop disease recurrence where treatment options remain extremely limited[3]. The molecular basis for hormonal therapy is based on the fact that the bulk luminal-type cells in malignant prostate glands express high levels of androgen receptor(AR). Therefore, conventional androgen deprivation therapy(GnRH releasing hormone agonists and antagonists), as well as second-generation hormonal therapies(enzalutamide, abiraterone acetate) are commonly used to slow disease progression[4]. However, despite the initial efficacy, tumor cells eventually acquire resistance by either undergoing AR genetic alterations or transdifferentiating to become neuroendocrine(NE) cells,which do not express any luminal markers (such as AR and PSA) and instead express NE markers such as chromogranin A(CgA) and synaptophysin(SYP)[5]. All these advanced PCa subtypes are resistant to both first and second generation of hormonal therapies, and present a significant challenge in clinical management. To this end, exploring novel diagnostic and therapeutic markers in addition to AR signaling is needed to improve therapeutic efficacy for advanced PCa. For many years, our team has been dedicated to understanding the molecular dynamics of how therapy resistance occurs as well as the discovery of therapeutic approaches to target these important mechanisms. This review summarizes several breakthroughs resulted from our recently published studies.

2 NOVEL NE BIOMARKERS AND THERAPEUTIC TARGETS

PCa is a heterogeneous cancer type with two distinct cellular components: a large amount of luminal-type cells(~99%) and a small portion of NE cells(~1%). Although NE cells are indolent in primary tumors, about 20% of hormonally treated tumors recur as small cell neuroendocrine prostate cancer(SCNC), which consists entirely of NE cells with a high proliferation index and significant metastatic potential. SCNC is the most lethal histological variant and carries the worst prognosis compared with all other prostate tumors. In past decades, identifying novel NE biomarkers has been a main research goal to achieve a more precise diagnosis and a better prognosis. Several classical markers, such as CgA and SYP, as well as newly revealed NE contributors [e.g ONECUT2[6-7], Mucin 1(MUC1-C)[8], Forkhead Box A2(FOXA2)[9], etc], have displayed a certain degree of sensitivity and specificity for detecting SCNC or played a critical role in NE transdifferentiation. However, no NE-specific cell surface markers have been reported. Our team has demonstrated that C-X-C motif chemokine receptor 2(CXCR2), a G protein-coupled receptor of angiogenic CXC chemokine family members, is exclusively expressed in prostatic NE tumor cells through the examination of multiple cases of human PCa tissues[10]. In follow-up studies, we comprehensively characterized the molecular features and biological functions of CXCR2-positive NE cells by employing our unique tumor procurement technique where we successfully obtained pure NE tumor cells directly from fresh prostatectomy samples[11]. Various transcriptomic analyses demonstrated that the fluorescence-activated cell sorting(FACS)-sorted CXCR2-positive NE population transcriptionally resembles SCNC with distinct stem-like, tumorigenic, metastatic, epithelial-mesenchymal transition(EMT)-like, and neuronal properties. More importantly, CXCR2 is able to drive NE phenotype and therapeutic resistance to hormonal therapy, potentially implicating it in lineage plasticity as well. Since hormonal therapy only targets the AR-positive luminal cells, it is conceivable that CXCR2 may represent a potential target for the NE population, which is spared by hormonal therapy. Indeed, targeting CXCR2 significantly results in tumor regression in advanced PCa models. A synergistic combination of AR-targeted therapy and CXCR2 inhibition achieves more profoundly inhibitory effect than either treatment alone, suggesting that targeting cellular heterogeneity is necessary to block tumor progression and improve the patients' long-term outcomes[11].

Large sequencing data and preclinical models have showed that MYCN is amplified in human SCNC and can be a critical driver for the emergence of NE differentiation following hormonal therapy[12-14]. In addition to these findings, our team further discovered an important mechanism for which N-Myc participates in driving therapy-resistant PCa[15]. Through studying both primary and recurrent tumors, a disease stage-dependent role of N-Myc in regulation of ataxia-telangiectasia mutated(ATM) was discovered. In the reported study, we uncovered a previously unappreciated role of ATM whose canonical function has been implicated in the field of DNA damage repair. Specifically, in the hormone-sensitive stage, N-Myc suppresses ATM expression via upregulation of microRNA-421, which leads to alleviation of hormonal therapy-induced cellular senescence. By contrast, after the disease progresses to the castration-resistant stage, N-Myc elevates ATM expression to promote the migration and invasion of tumor cells. We further demonstrated that inhibition of ATM through either genetic or pharmacological approach re-sensitizes tumor cells to anti-androgen treatment. This therapeutic approach may represent a treatment strategy for patients at risk for developing SCNC due to elevated N-Myc[15].

3 METABOLIC IMPLICATIONS IN THERAPY-RESISTANT PCa

Metabolic reprogramming has long been recognized as a profound hallmark of cancer initiation and progression[16]. Since tumor cells often alter their metabolism to support increased proliferation and metastasis, we hypothesize that targeting these metabolic changes might achieve greater efficacy with less side effects in contrast to targeting other cellular mechanisms.

Glucose and glutamine are the two major nutrients used for energy supply and biomass synthesis[17]. Unlike normal prostatic epithelium that employs comparatively glycolytic metabolism to sustain physiological citrate secretion, prostate tumor cells consume citrate to power oxidative phosphorylation and fuel lipogenesis[18]. Specifically, a significant reprogramming of glucose metabolism in cancer cells has been well described where glucose primarily contributes to lactate generation rather than entering the tricarboxylic acid(TCA) cycle, a phenomenon known as the Warburg effect[19]. Our team has yielded two publications that consistently demonstrate a glycolytic propensity of advanced PCa[20-21], where therapy-resistant PCa cells have been observed to have greater glucose consumption and lactate secretion compared with early stage PCa cells. Mechanistically, CD44 and ATM have been characterized as the key modulators, the alteration of which imposes a marked impact on glucose metabolism in PCa. Li et al[20] suggest that the exclusive expression of CD44 in NEPC dramatically elevates the level of PFKFB4, one of the rate-limited enzymes for the glycolysis pathway, while Xu et al[21] demonstrate that ATM mutation, a frequent genetic event observed in recurrent PCa, upregulates the expression lactate dehydrogenase A(LDHA), the key enzyme converting pyruvate to lactate. Inhibiting CD44 has been shown to increase the sensitivity of SCNC to chemotherapy. Similarly, targeting LDHA by disrupting the connection between ATM alteration and LDHA activation might be an approach for Poly (ADP-ribose) polymerase(PARP) inhibitor-resistant PCa tumors.

Interestingly, although glucose is largely shunted away from the TCA cycle for lactic acid fermentation in advanced PCa, the mitochondrial activity is still highly maintained. This fact impels us to explore another readily available nutrient source which might be responsible for the maintenance of the TCA cycle. Second to glucose, glutamine is the most abundant amino acid in the blood with pleiotropic functions in energy generation and macromolecular synthesis[22]. More importantly, through catabolism by glutaminase-1(GLS1), glutamine can serve as a carbon source to help fuel the TCA cycle and maintain cellular energy. In agreement with this notion, one of our recent publications has fully characterized the metabolic consequences of glutaminolysis in PCa and its potential impact on therapy resistance and disease progression[23]. In comparison to hormone-sensitive PCa, therapy-resistant PCa is more addicted to glutamine and utilizes more of the amino acid to support cellular proliferation. This distinct glutamine dependency is due to the differential expression of the two isoforms of GLS1, kidney-type glutaminase(KGA) and glutaminase C(GAC). KGA is the dominant variant in primary tumors while GAC, the more potent isoform, predominates in therapy-resistant PCa. More interestingly, KGA is an AR-regulated isoform while GAC is

not. Therefore, during hormonal therapy, KGA activity is suppressed because of the inhibition of AR. GAC then becomes the major GLS1 isoform and helps tumor cells evade hormonal therapy, where they become dependent on glutamine instead of androgen. Therapeutically, GLS1 inhibitor, CB-839, displays a strong inhibitory effect on GAC, resulting in tumor regression independent of AR-targeted therapy[23].

4 CONCLUSION

The above accomplishments recapitulate our efforts to better understand the molecular and metabolic basis through which PCa acquires therapy resistance and becomes highly lethal. We believe that the knowledge gained from these studies will benefit patients who have run out of treatment of choice and improve their long-term outcomes.

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Chinese Journal of New Clinical Medicine, July 2021, Volume 14, Number 7

激素抵抗型前列腺癌的治疗新策略

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基金项目：国家自然科学基金项目（编号：81902611）

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［**摘要**］ 转移性前列腺癌通过激素剥夺疗法大多可获得良好的治疗效果，然而肿瘤细胞最终产生激素抵抗，对治疗无应答。该研究团队长期致力于探索前列腺肿瘤的激素抵抗的产生机制并寻求新的治疗策略，研究范围主要集中在鉴定新的肿瘤标志物，神经内分泌分化以及肿瘤代谢等方面。该文将综述本课题组最新的几项研究成果，以期加深对前列腺癌进展机制的理解并提出新的治疗策略。

［**关键词**］ 前列腺癌； 激素抵抗； 肿瘤标志物； 肿瘤代谢

［收稿日期 2021－05－26］［本文编辑 吕文娟 余 军］

本文引用格式

Xu LF, Butler W, Huang JT. Novel therapeutic avenues for therapy-resistant prostate cancer: a review[J]. Chin J New Clin Med, 2021, 14(7): 642－646.