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The Role of Splicing Regulators in the Emergence of Treatment-induced Neuroendocrine Prostate Cancer: The Next Generation of Drug Targets?

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Treatment-induced neuroendocrine prostate cancer (t-NEPC) is a highly aggressive, histologically distinct PC subtype consisting of small-cell carcinoma cells or mixed-histology cells that is believed to emerge in response to selection pressure of treatment with potent androgen deprivation therapy (ADT) in the metastatic castration-resistant PC (mCRPC) setting. Transdifferentiation of adenocarcinoma cells results from reactivation of developmental pathways, acquisition of stem-cell-like characteristics, epithelial-to-mesenchymal plasticity, and a proliferative "switch". Because t-NEPCs display diverse morphological and molecular characteristics, differentiation states, and varying proportions of histologically small-cell populations, a consensus definition of t-NEPC remains the subject of much debate. However, molecular features can include low or absent androgen receptor (AR) signalling, *RB1* loss, *TP53* loss, *MYCN* amplification, *AURKA* gain, upregulation of *BRN2*, *SOX2*, or *PEG10*, downregulation of *REST*, altered DNA methylation, and increased Polycomb-mediated gene silencing through EZH2 [1].

In the clinic, t-NEPCs are characterised by high disease burden, frequent incidence of visceral metastases despite low serum prostate-specific antigen (PSA), although not invariably, and short response to ADT. Unfortunately, the aggressive manifestation, complex yet poorly defined molecular aetiology, and greater AR-negative pathology or low AR transcriptional activity compared to prostate adenocarcinoma (AdPC) result in a lack of effective targeted therapies for this disease subtype. Despite an initial response to platinum-based chemotherapy, survival rarely exceeds 2 yr after NEPC diagnosis [2]. While de novo small-cell carcinomas of the prostate are rare, comprising less than 2% of cases [3], evidence of pure t-NEPC or mixed t-NEPC/AdPC was identified in metastatic biopsies for 17% of mCRPC patients [4], representing a considerable clinical burden. In addition, with US Food and Drug Administration approval for abiraterone shifting its use to the earlier, high-risk hormone-sensitive disease stage [5], it is anticipated that the incidence of t-NEPC will increase.

Overall, AdPC and t-NEPC share similar genomic landscapes (with the exception of loss of DNA repair pathway genes, found almost exclusively in AdPC [4]) but have dramatically different transcriptomes and distinct epigenetic profiles [6]. This suggests a common clonal origin of the two PC subtypes. These observations also suggest epigenetic regulation, noncoding DNA and RNA events, and RNA splicing as potential drivers of a highly plastic NEPC transdifferentiation process. Indeed, upregulation of EZH2, the catalytic subunit of PRC2 responsible for H3K27 methylation of lineage specification genes, is one of the key features of NEPC versus primary AdPC and is accompanied by downregulation of EZH2-repressed genes, such as *DKK1* [6]. EZH2 inhibitors can reverse NEPC transdifferentiation in preclinical models [7] and are being evaluated in phase 1 and 2 clinical trials in lymphomas and solid tumours. A second epigenetic regulator, REST, which suppresses neuroendocrine differentiation via recruitment of the co-repressors EZH2 and LSD1 to neuronal gene promoters, is frequently downregulated in NEPC [8]. It can be alternatively spliced by SRRM4 to yield a truncated and

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functionally-distinct protein, lacking the transcriptional repressor domain and with reduced ability to repress neuroendocrine differentiation programmes [9]. Indeed, SRRM4 is highly expressed in 50% of NEPCs versus 3% of AdPCs, and its overexpression in vitro drives NE differentiation through induction of NEPC-specific splicing programmes following AR signalling inhibition [9]. This highlights the key role of alternative splicing events in driving AdPC transdifferentiation and acquisition of t-NEPC characteristics.

Li et al. [9] previously used RNA sequencing data for two independent cohorts to identify a t-NEPC-specific RNA splicing signature and demonstrated dramatic upregulation of an alternatively spliced form of the histone demethylase BHC80 (BHC80-2 vs unspliced BHC80-1) in t-NEPC versus AdPC. BHC80-2 results from replacement of exon 14 with alternative exon 14a, which disrupts a potential nuclear localisation signal. In this issue of *European Urology*, the same authors report a novel, nonepigenetic mechanism of action of BHC80-2 whereby its nuclear exclusion triggers the MyD88-p38-TTP pathway to enhance transcript stability of multiple tumour-promoting cytokines [10].

Using RNA in situ hybridisation (RISH) on a tissue microarray of CRPC tumours, the authors demonstrated strong BHC80-2 positivity in small cell carcinomas and AdPCs with abundant NE histology, with low staining intensity in AdPC. Moreover, BHC80-2 RISH intensity was positively correlated with immunohistochemical scores for the NE markers SYP, CHGA, and CD56, but was inversely associated with AR and PSA, supportive of a role in the biology of the AR-negative subset of NEPCs. Since approximately 75% of t-NEPCs are positive for nuclear AR [4], the role of BHC80-2 in this context remains unclear. Interestingly, splicing of BHC80-2 was found to be mediated in part by SRRM4, which the same authors previously purported to be a master epigenetic regulator of NE transdifferentiations: SRRM4 and BHC80-2 RISH intensity was highly correlated in CRPCs of pure small-cell or mixed NE/AdPC histology, and in cell-line models, BHC80-2 was detected only the presence of SRRM4. Furthermore, SRRM4 depletion led to loss of BHC80-2 but not -1, while its overexpression increased levels of minigene reporter-derived BHC80-2. RNA binding assays confirmed SRRM4 recruitment to the 3′ splice site of BHC80 intron via a UGC motif. Since the roles of U2AF65 and PTB (also found to alter BHC80-2 splicing) were not further explored, it is difficult to ascertain the relative contributions of these versus SRRM4 in the control of BHC80-2 biology.

It is indicative of the complexities of t-NEPC emergence that BHC80-2 overexpression was not sufficient to induce NE markers or transdifferentiation to neuronal morphology, but did promote androgen-independent proliferation, migration, and invasion, including that of the NEPC-prone TRAMP-C1 xenograft model. While this may be explained by the highly heterogeneous nature of t-NEPC, its complex molecular characteristics (not all t-NEPCS express “classical” NEPC markers), and a role for BHC80-2 in induction of a “proliferative switch”, further studies are required to conclusively demonstrate its relevance as a key driver event in t-NEPC development. One example would be the generation of a PC mouse model with prostate-specific BHC80-2 overexpression under castrate conditions.

Microarray analysis performed to further delineate the mechanism of BHC80-2 action in t-NEPC revealed 177 genes as specifically regulated by BHC80-2 but not -1. Ingenuity pathway analysis validated via qualitative real-time polymerase chain reaction identified cytokines including CCL20, CXCL10, CCL2, and TNFα as specific effectors of BHC80-2-activated MyD88 action, since a MyD88 inhibitor reduced BHC80-2-induced cytokine expression and blunted BHC80-2 growth promotion. Since it has been demonstrated that MyD88 activates p38, reducing the affinity of TTP for substrate RNA and leading to reduced turnover of cytoplasmic RNAs [11], the authors hypothesised that disruption of the BHC80-2 NLS through splicing may result in cytoplasmic and nonepigenetic activation of this pathway. Indeed, BHC80-2 enhanced p38 phosphorylation, suppression of p38 abrogated BHC80-2 cytokine induction, and BHC80-2 reduced TTP affinity for its cytokine mRNA targets. While the authors demonstrated that BHC80-2 interacts with MyD88 in the cytoplasm, elucidation of the mechanism by which BHC80-2 activates MyD88 remains unexplored. As an important proof of concept that BHC80-2 acts largely through the MyD88-p38-TTP axis, MyD88 siRNA, a MyD88 inhibitor, and CCL2-neutralising antibodies recapitulated the effects of BHC80-2 silencing in NEPC spheroid models and could be rescued by CCL2 protein. A detailed mechanism of BHC80-2 activation of MyD88-p38-TTP cascade is shown in Fig. 1.

In conclusion, the authors have convincingly demonstrated that alternative splicing of BHC80-2 leads to its noncanonical cytoplasmic activation of the MyD88-p38-TTP pathway and enhanced proliferation in NEPC models. This illustrates the importance of splicing and epigenetic mechanisms in t-NEPC emergence. However, several important questions remain to be answered by the t-NEPC research community:

1. To what extent does splicing of BHC80-2 and its resultant cytoplasmic localisation simply serve to abrogate the canonical nuclear function of BHC80 in demethylating and repressing neuronal genes as part of the BRAF35/HDAC complex?
2. What are the implications of BHC80-2 activation of the MyD88 cytokine cascade for tumour immune cell infiltration? What are the consequences of BHC80-2 and upstream SRRM4 action for immunotherapy response in the t-NEPC setting? Does increased splicing in SRRM4-high t-NEPCs generate neoantigens that could be exploited therapeutically?
3. Is there a role for splicing inhibitors as adjuncts to extend the duration of platinum-based chemotherapy responses? Would BHC80-2 represent an effective predictive biomarker in this context?
4. What is the role of BHC80-2 in the 50% of NEPCs in which SRRM4 is not highly-expressed?

While a “one size fits all” treatment strategy is unlikely to be effective in highly heterogeneous t-NEPC, answers to the

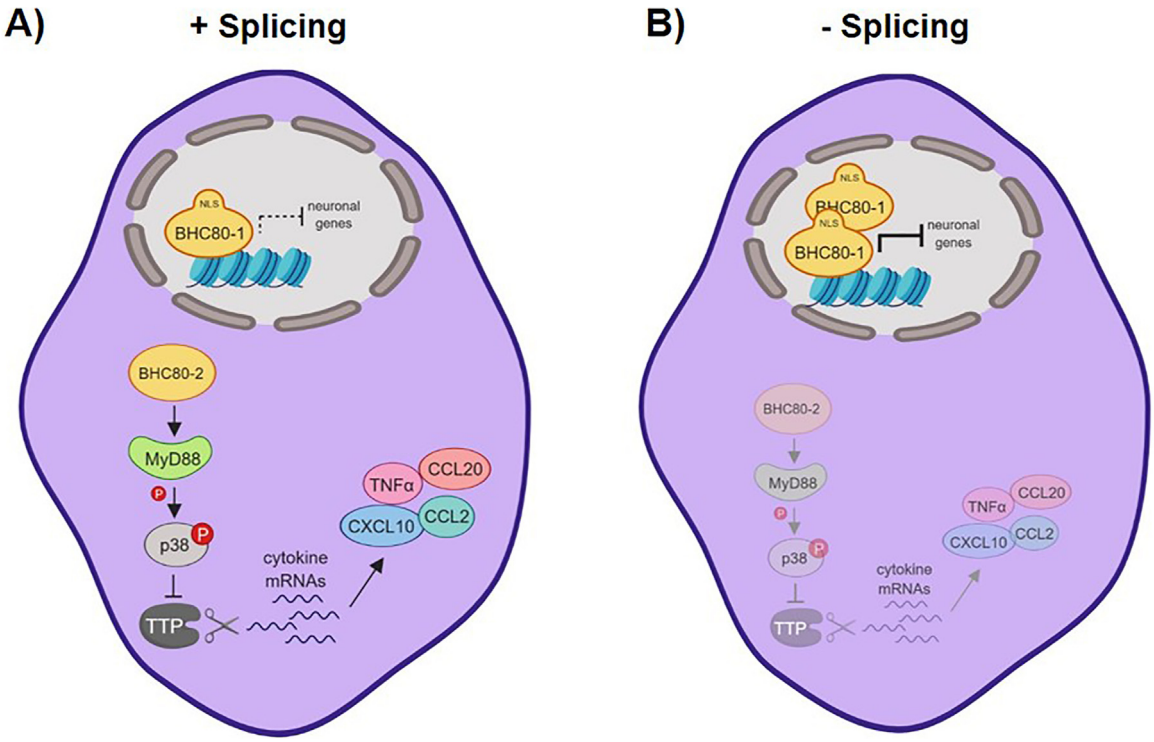

Fig. 1 – SRRM4-mediated splicing of BHC80 promotes proliferation of neuroendocrine prostate cancer models through activation of the MyD88-p38-TTP cascade and loss of BHC80-1 repression of neuronal genes. (A) Splicing of BHC80-2 (by SRRM4, and potentially U2AF65 and/or PTB) disrupts its nuclear localisation sequence (NLS), leading to its cytoplasmic localisation and MyD88 activation. In turn, this results in p38 phosphorylation and repression of TTP activity through loss of affinity for its cytokine mRNA targets and enhanced levels of CCL20, CXCL10, CCL2, and TNFα. In addition, BHC80-2 splicing could lead to a reduction in canonical BHC80-1 demethylase activity in the nucleus, and derepression of neuronal genes. Together, these events promote proliferation of NEPC cells. (B) In the absence of BHC80-2 splicing, BHC80-1 is retained in the nucleus. Activation of the MyD88-p38-TTP axis is reduced and BHC80-1-mediated demethylation and repression of neuronal genes are enhanced, resulting in inhibition of NEPC proliferation. Figure created with BioRender.com.

above questions could pave the way for novel, patient-tailored therapeutic strategies for a lethal mCRPC subset that is expected to represent an increasing clinical burden in the coming years.

Conflicts of interest: The author has nothing to disclose.

References

[1] Davies AH, Beltran H, Zoubeidi A. Cellular plasticity and the neuroendocrine phenotype in prostate cancer. Nat Rev Urol 2018;15:271–86.
[2] Aparicio AM, Harzstark AL, Corn PG, et al. Platinum-based chemotherapy for variant castrate-resistant prostate cancer. Clin Cancer Res 2013;19:3621–30.
[3] Beltran H, Rickman DS, Park K, et al. Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. Cancer Discov 2011;1:487–95.
[4] Aggarwal R, Huang J, Alumkal JJ, et al. Clinical and genomic characterization of treatment-emergent small-cell neuroendocrine prostate cancer: a multi-institutional prospective study. J Clin Oncol 2018;36:2492–503.
[5] Fizazi K, Tran N, Fein L, et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. N Engl J Med 2017;377:352–60.
[6] Beltran H, Prandi D, Mosquera JM, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016;22:298–305.
[7] Dardenne E, Beltran H, Benelli M, et al. N-Myc induces an EZH2-mediated transcriptional program driving neuroendocrine prostate cancer. Cancer Cell 2016;30:563–77.
[8] Bjartell A, Ceder J, Iglesias-Gato D, et al. REST mediates androgen receptor actions on gene repression and predicts early recurrence of prostate cancer. Nucleic Acids Res 2013;42:999–1015.
[9] Li Y, Donmez N, Sahinalp C, et al. SRRM4 drives neuroendocrine transdifferentiation of prostate adenocarcinoma under androgen receptor pathway inhibition. Eur Urol 2017;71:68–78.
[10] Li Y, Xie N, Chen R, et al. RNA splicing of the BHC80 gene contributes to neuroendocrine prostate cancer progression. Eur Urol 2019;76:157–66.
[11] Sun D, Ding A. MyD88-mediated stabilization of interferon-γ-induced cytokine and chemokine mRNA. Nat Immunol 2006;7:375–81.