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Commentary

"Splice" a way towards neuroendocrine prostate cancer

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The prostate is an exocrine gland containing mainly basal and luminal epithelial cells, together with rare neuroendocrine (NE) cells, that are constructed as a pseudo-stratified epithelium surrounded by stromal cells [1,2]. Prostate cancer (PCa) predominantly displays a luminal phenotype and histologically presents as prostate adenocarcinoma (AdPC) largely devoid of basal cells. Due to an androgen-dependency of the organ, androgen deprivation therapy (ADT), which aims to block androgen synthesis in the testis (e.g., Lupron) and adrenal gland (e.g., abiraterone) or by PCa cells (called intracrine androgen biosynthesis), and antiandrogens (e.g., enzalutamide), which inhibit androgen receptor (AR) functions, represent the mainstay therapies for advanced and metastatic PCa. Although initially effective, the majority of patients, unfortunately, will inevitably develop castration-resistant PCa (CRPC), which manifests substantial clinical, pathologic, and molecular heterogeneity. A significant fraction (up to 25%) of CRPC progress into an AR indifferent stage called neuroendocrine prostate cancers (NEPC) or CRPC-NE [3]. Although the *de novo* NEPC is rare (~1%), ADT/antiandrogen-induced NEPC has emerged as a major therapy-resistant clinical entity that kills PCa patients. There is currently no effective treatment for NEPC and the rate of occurrence is predicted to rise with the widespread use of more potent AR inhibitors.

Despite the clinical significance of treatment-induced NEPC, our understanding on its development and evolution at the molecular and cellular levels is scanty due to limited cell and xenograft models available for analysis. In this issue of *EBioMedicine*, Lee and colleagues [4] establish a novel NEPC model called DuNE by overexpressing the splicing factor SRRM4 in DU145 cells. SRRM4 is a master regulator required for neural differentiation from embryonic stem cells [5]. To achieve this goal, the authors tested a panel of non-NE prostate cell lines and found that SRRM4 overexpression induces the expression of NE-related molecular markers and an overall NEPC-specific RNA splicing program in all cell models examined; surprisingly, however, not all cell lines display an overt NEPC cellular phenotype. In fact, only DU145 cells were 'transformed' by SRRM4 into an NEPC phenotype via a pluripotency gene network mediated by SOX2. Previously, the same group has reported that exogenous expression of SRRM4 in AdPC LNCaP cells enables an establishment of NEPC cell and xenograft models (referred as LnNE) through direct transdifferentiation under castration conditions [6,7]. Together, these findings are interesting and timely as the field of PCa research lacks suitable NEPC cell and/or xenograft models.

Cancer cell plasticity and heterogeneity represent huge challenges to effective treatment of aggressive PCa [8]. Generally, NEPC is defined by the expression of mature NE differentiation markers such as SYP and CHGA and a loss or low levels of epithelial makers (e.g., CHD1, PSA, AR, CK8) [5]. Notably, heterogeneity has been reported within NEPC, as NEPC evolves from AdPC with variable levels of AR expression [3] and SYP and CHGA proteins may not always be co-expressed in clinical specimens. This highlights diverse mechanisms of the emergence of NEPC, as supported by the current study showing that SRRM4 transforms AdPC to NEPC through different mechanisms in a cell-type specific manner [4]. Although SRRM4 can induce a NE-like gene expression profile in all cell types tested (e.g., LNCaP, PC-3, DU145, 22Rv1), only DU145 cells exhibit a neuronal-like morphological change *in vitro* and biological characteristic unique to clinical NEPC *in vivo* [4]. Further studies reveal SOX2 as a key factor in augmenting the capacity of SRRM4 in transforming DU145 cells towards a NE phenotype. Notably, the SOX2 protein expression is only observed in DU145 but not in LNCaP cells, suggesting cell type-specific mechanisms. It is unclear why SOX2 protein is induced by SRRM4 only in DU145 but not in LNCaP cells. Perhaps the genetic background of AdPC cells may be a determinant of the effect of SRRM4, as AR^{+} LNCaP cells are wilt-type for both TP53 and RB1 whereas DU145 are null for both. It has recently been shown that loss of TP53 and RB1 functions in LNCaP cells promotes an AR-independent basal- and NE-like phenotype that endows antiandrogen resistance [9]. Consistently, triple knockout of *Pten, Tp53* and *Rb1* in mouse prostate luminal cells generates NEPC after castration of the hosts bearing primary androgen-sensitive tumors [10]. Aside from SRRM4-SOX2 axis, previous studies have implicated several other molecular pathways, such as MYCN, AURKA, REST, EZH2, SOX11, BRN2, and FOXA2, in the development of NEPC [5], again implicating diverse molecular mechanisms. At present, potential crosstalks among these likely interactive pathways remain unknown.

The most important finding of the current study [4] is that the DuNE model recapitulates the molecular and biological properties of a subset of patient NEPC tumors expressing stem-like features, highlighting its clinical relevance and application in advancing our understanding of

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NEPC development and evolution. Further studies are needed to address the following questions: What are the mechanisms underlying the interplay between SRRM4 and SOX2? Can SRRM4 and/or SOX2 be used as biomarkers for precision medicine, given that not all NEPC are $SOX2^+$ and not all high SRRM4-expressing tumors are NEPC? Regardless, the SRRM4-SOX2 axis appears to be promising molecular targets for NEPC. While targeting nuclear transcription-associated factors is difficult, therapeutic modulation of SRRM4-driven alternative splicing events via antisense oligouncleotides (ASO) or splice-switching oligouncleotides (SSO) may represent attractive alternative strategies. Small molecule inhibitors that block SRRM4 functions (e.g., RNA-binding domain, its splicing activity) are also worth developing.

Disclosure

The authors declare no conflicts of interest.

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