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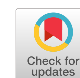
Roles of Alternative RNA Splicing of the Bif-1 Gene by SRRM4 During the Development of Treatment-induced Neuroendocrine Prostate Cancer



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ABSTRACT

Treatment-induced neuroendocrine prostate cancer (t-NEPC) is an aggressive subtype of prostate cancer (PCa) that becomes more prevalent when hormonal therapy, chemotherapy, or radiation therapy is applied to patients with metastatic prostate adenocarcinoma (AdPC). How AdPC cells survive these anti-cancer therapies and progress into t-NEPC remains unclear. By comparing the whole transcriptomes between AdPC and t-NEPC, we identified Bif-1, an apoptosis-associated gene, which undergoes alternative RNA splicing in t-NEPC. We found that while Bif-1a is the predominant variant of the Bif-1 gene in AdPC, two neural-specific variants, Bif-1b and Bif-1c, are highly expressed in t-NEPC patients, patient derived xenografts, and cell models. The neural-specific RNA splicing factor, SRRM4, promotes Bif-1b and Bif-1c splicing, and the expression of SRRM4 in tumors is strongly associated with Bif-1b/-1c levels. Furthermore, we showed that Bif-1a is pro-apoptotic, while Bif-1b and Bif-1c are anti-apoptotic in PCa cells under camptothecin and UV light irritation treatments. Taken together, our data indicate that SRRM4 regulates alternative RNA splicing of the Bif-1 gene that enables PCa cells resistant to apoptotic stimuli under anti-cancer therapies, and may contribute to AdPC progression into t-NEPC.

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1. Introduction

While primary (de novo) neuroendocrine prostate cancer (NEPC) is extremely rare, accounting for ~1% of all prostate cancers (PCa) [9], treatment-induced NEPC (t-NEPC) is more prevalent in patients who have a history of adenocarcinoma (AdPC), and have received single or multiple rounds of hormonal therapy, radiation therapy, or chemotherapy [24]. T-NEPC is responsible for approximately 25% of PCa-related deaths [2,22,32]. Because t-NEPC is highly aggressive and metastatic, once a diagnosis is confirmed patient survival is only ~7 months [34]. Beyond systematic chemotherapy, no targeted therapy is currently available due to our limited knowledge on the molecular underpinning of t-NEPC development.

At this time, a consensus on how AdPC is transformed into t-NEPC has not been reached. However, multiple hypotheses have been proposed including that t-NEPC originates from: i) PCa stem-like cells that retain traits of resistance to apoptosis under anti-cancer therapy, self-renewal, and invasion [29]; ii) AdPC cells that undergo NE differentiation followed by t-NEPC tumorigenesis [5,20,39]; or iii) benign prostatic neuroendocrine cells that acquire tumorigenesis capacity [26]. Whole-exome sequencing had revealed that t-NEPC and AdPC cells in patients have similar genetic mutation landscapes, including the distribution of non-silent point mutations, polyploidy, and somatic copy-number genomic burden [5,20]. These findings support that t-NEPC is likely derived from AdPC.

Because AdPC and t-NEPC share similar genomic features but have dramatically different transcriptomes, we hypothesized that alternative RNA splicing may play a key role in AdPC progression to t-NEPC. Through analyzing published whole-transcriptome sequencing data sets from two patient cohorts [4,13] and paying particular interests in identifying RNA splicing events that are unique to t-NEPC, we have identified a t-NEPC specific RNA splicing signature that is predominantly controlled by the RNA splicing factor, SRRM4 [19]. We have

Abbreviations: PCa, prostate cancer; t-NEPC, treatment-induced neuroendocrine prostate cancer; Bif-1, Bax-interacting factor 1; Bax, BCL-2 Associated X Protein; AdPC, prostate adenocarcinoma; SRRM4, Serine/Arginine Repetitive Matrix 4; NE, neuroendocrine; RISH, RNA in situ hybridization; SCNC, small cell neuroendocrine carcinoma; CPT, camptothecin; N-BAR, N-terminal Bin-amphiphysin-Rvs.

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further demonstrated that SRRM4 not only induces a NEPC transcriptome and neuroendocrine (NE)-like morphology to AdPC cells, but more importantly transforms AdPC cells into NEPC xenografts [19]. We have subcutaneously inoculated LNCaP cells that overexpress SRRM4 into castrated nude mice continuously for five passages over 18 months, and generated a series of t-NEPC xenograft models, called LnNE [18]. These LnNE tumors express strong NE markers and present with NEPC morphology. Tumors at later passages grow more aggressively and become androgen-insensitive and PSA negative [18]. These features mimic the characteristics of AdPC progression to t-NEPC in patients. Together, these findings highlight that SRRM4 is a clinically relevant driver gene of t-NEPC by regulating alternative RNA splicing of multiple genes. Therefore, further characterization of these SRRM4 target genes would help understand how AdPC progresses into t-NEPC in patients.

Since t-NEPC develops after patients are given anti-cancer therapies for AdPC, we speculate that the AdPC cells that give rise to t-NEPC should have phenotypes that render them resistant to therapy-induced cell death. Through investigating the SRRM4 transcriptome, we have found that an apoptotic-associated gene, Bax interacting factor-1 (Bif-1), undergoes alternative RNA splicing in t-NEPC. There are three major splice variants of the Bif-1 gene in human cells [6,23,25]. Inclusions of exons 6s and 7, or exons 6l and 7, into Bif-1a (NM_016009.4) mRNA give rise to Bif-1b (NP_001193581) and Bif-1c (XP_006710735), respectively. Most studies on the Bif-1 gene have been focused on Bif-1a, since it is the predominant variant in non-neuronal cells [6,30]. Bif-1a and Bax are pro-apoptotic proteins that are localized in the cytosol in their inactive forms. When activated by apoptotic stimuli, they are translocated to the mitochondrial membrane for mitochondria-mediated apoptosis [6]. Bif-1a was reported to either activate Bax to promote mitochondrial outer membrane permeabilization [8,30], or self-oligomerize independent of Bax to form pores on the mitochondrial membrane and cause apoptosis [28]. In neuronal cells, Bif-1b and Bif-1c are the predominant forms of the Bif-1 gene, and their cellular functions are relatively unclear. However, Morrison et al. reported that Bif-1 in neuronal cells promotes cell viability and maintains mitochondrial morphology [33]. Together, these findings implied that Bif-1 splice variants may have opposite effects to cell apoptosis. Therefore, this study aimed to characterize the function of alternative RNA splicing of the Bif-1 gene in PCa cells during t-NEPC development.

2. Materials and Methods

2.1. Ethics Approval and Consent to Participate

Studies involving human data or human tissues were approved by the Research Ethics Committee of the University of British Columbia (H09-01628) and performed according to relevant guidelines and regulations expressed in the Declaration of Helsiniki. Informed consent was obtained from all participating individuals.

2.2. RNA-seq Data from PCa Patients

Whole transcriptome sequencing data from two Vancouver Prostate Centre (VPC) and Beltran patient cohorts as well as the LnNE tumor models are from our previous publications [4,13,18].

2.3. Tissue Microarrays (TMAs)

Prostate tumor samples were extracted from the VPC tissue bank and used to build a castration-resistant PCa TMA, as previously reported [17,36]. This TMA contains 64 tissue cores from 32 patients who had received hormonal therapy, chemotherapy or radiotherapy. The recurred tumors were removed by transurethral resection prostatectomy to relieve lower urinary tract symptoms. This TMA also contains two brain tissue cores from donors.

2.4. RNA In Situ Hybridization (RISH) and Immunohistochemistry (IHC)

The RISH probes targeting the 952–1003 bp of NM_001206652.1 for both Bif-1b and -1c variants, and the 496–835 bp of NM_194286.3 for SRRM4 were designed by Advanced Cell Diagnostic (Hayward, USA). A probe targeting the dapB gene of bacteria was used as a negative control probe. RISH assays were performed using the BaseScope™ assay kit following manufacture's instruction. IHC was performed by Ventana Discovery XT (Ventana) using a DAB MAP kit, as previously reported [17,36]. A Leica SCN400 scanner to form digital images scanned all stained slides.

Positive RISH signals were presented as red dots under 40 × magnification. RISH signals were scored as 0 if no positive signal; 1 if RISH signals were positive in ≤ 20% of all cells within a core; and 2 if RISH signals were positive in > 20% of the cells in the entire tissue core. RISH positive cells with a score of 1 usually have ≤ 2 RISH dots/cell, and RISH positive cells with a score of 2 usually have multiple dots that can merge into dot clusters. IHC scores of CHGA, SYP, CD56, AR, and PSA were calculated by IHC signal intensity (no, low, medium, and high as 0–3) multiplied by the percentage of positive cells (0–100%). IHC scores ≥ 0.3 were considered to be positive.

The histology of castration-resistant tumors is classified either as AdPC, small cell neuroendocrine carcinoma (SCNC), or AdPC with abundant neuroendocrine cells (AdNC). AdPC contains tumor cells that form glandular structures. Comparing to benign prostate glands, AdPC glands are smaller, more compacted, and homogeneous. AdPC cells are large, with vesicular nuclei and prominent nucleoli. AdPC have rare NE cells. SCNC contain only NE tumor cell populations that grow as solid sheets, cords, or individual cells without glandular formation. The tumor cells exhibit NE features including hyperchromatic nuclei, finely granular and homogenous chromatin pattern, and no nucleoli. Cells have scant cytoplasm and high nucleus:cytoplasm ratios. SCNC often contain areas of necrosis and crush artifact. Mitotic and apoptotic figures are frequent. AdNC are more histologically similar to AdPC than to SCNC, but cannot be classified as typical AdPC or SCNC. These tumors contain mixed cell populations with a large number (> 10%) of NE cells.

2.5. PCa Cell Models and Patient Derived Xenografts

LNCaP, 22RV-1, PC-3, DU145, NCI-H660 PCa cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Dr. Rennie from the Vancouver Prostate Centre generously provided C4-2 and 293 T cell lines. LNCaP95 cells were a kind gift from Dr. Alan Meeker of Johns Hopkins University. Cell culture conditions were described before [14–17,19,21]. AdPC and NEPC patient derived xenografts (PDXs) were previously reported [20] and shared by Dr. Yuzhuo Wang from Vancouver Prostate Centre.

2.6. PCR and Immunoblotting Assays

Real-time qPCR and immunoblotting arrays were performed as previously reported [21]. Information on primers and antibodies is listed in Tables S1 and 2, respectively. Experiments were repeated at least three times.

2.7. siRNA and DNA Transient Transfection

Cells were transfected with control siRNA (Dharmacon) and siRNA SMARTpool targeting total Bif-1 (#L-017086-00-0005, Dharmacon) and SRRM4 (#L-019322-02-0005, Dharmacon) using Lipofectamine RNAiMAX (Invitrogen). Lipofectamine 3000 (Invitrogen) and SuperFect Transfection Reagent (QIAGEN) were used for plasmid transfection.

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