Turning on neuronal gene programs in prostate cancer cells

Cancer of the prostate gland is a common malignancy afflicting males worldwide. This cancer is the second deadliest amongst male in United States, with most of the mortality resulting from aggressive, advanced stage, metastatic disease. Prostate cancer thrives on male sex hormones androgens that act primarily via a signaling axis mediated by a receptor called ‘Androgen receptor (AR)’. Therefore, starving prostate cancer cells of androgens or inhibiting AR via employing its specific inhibitors is a common form of treatment for prostate cancer. These treatments are initially beneficial. However, cancer cells learn to trick and make these treatments ineffective via various mechanisms. One such mechanism involves a lineage shift in prostate cancer cells wherein prostate cancer cells acquire characteristics of neuronal cells, express neuronal markers and learn to grow in absence of androgens and in presence of AR inhibition.

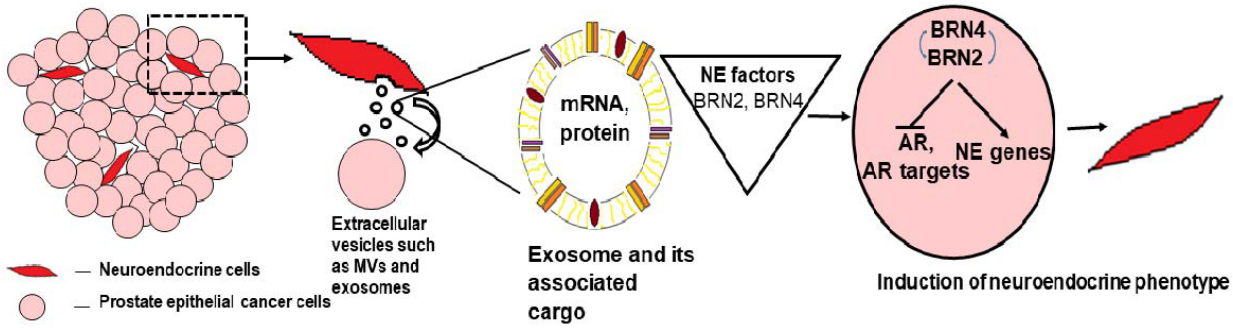

Fig. 1. Schematic representation showing a possible mechanism leading to conversion of prostate epithelial cells to neuroendocrine cells. We propose that in response to androgen deprivation/ treatment with AR pathway inhibitors, prostate cancer cells express and secrete BRN4 and BRN2 (RNA and protein) in exosomes/extracellular vesicles. These vesicles are taken up by prostate epithelial cells wherein these factors activate neuronal genes concomitant with repression of AR and AR target genes. These alterations drive neuroendocrine phenotype in prostate cancer cells. Also depicted is that BRN2 and BRN4 are in a regulatory loop.
Adapted from: Bhagirath et al., CCR, 2019

This process is referred to as ‘neuroendocrine differentiation’ that leads to a highly aggressive variant of prostate cancer – neuroendocrine prostate cancer (NEPC)- with enormous soft tissue metastatic burden and survival rates of less than 1 year. How prostate cancer evolves to NEPC via NED is still an enigma. Several genetic studies have focused on understanding how these states evolve and have identified that characteristic genomic alterations such as double loss of tumor suppressor genes *TP53* and *RB1* promote these states along with other concomitant alterations such as amplification of N-Myc oncoprotein. In our study, we showed that a novel transcription

factor (TF) Brain 4 (BRN4) is a key player in driving this transition in conjunction with a previously implicated transcription factor Brain 2 (BRN2). *BRN4* is located on X chromosome and is involved in the patterning of the neural tube, paraventricular, and supraoptic nuclei of the hypothalamus in the developing embryo. Mutations in *BRN4* have been linked to X-linked nonsyndromic deafness. However, its involvement in prostate cancer has not been studied before. Our data shows that BRN4 is induced in prostate cancer cells upon treatment with AR pathway inhibitor drug enzalutamide or by dual knockdown of *TP53* and *RB1* or by N-Myc overexpression. Further, BRN4 could be co-immunoprecipitated with BRN2 and BRN4 levels changed in response to alterations in BRN2 levels suggestive of a regulatory interplay between these factors. Our data suggest that these two TFs together drive the expression of another pluripotency factor called SOX2 that in turn, drives neuronal differentiation in advanced prostate cancer.

Importantly, we found that BRN4 and BRN2 are released from prostate cancer cells in tiny vesicles called ‘exosomes’ upon treatment with androgen pathway inhibitors or in response to other stimuli that drive NED. Exosomes are small membranous extracellular vesicles (EVs), typically 30-150 nm in size, that are released by all cells in our body. Exosomes carry a cargo of protein and RNA molecules that are selectively packaged into these vesicles by parental cells. Cells use exosomes to communicate with each other as these vesicles mediate intercellular communication by transferring their cargo such as mRNA and proteins to recipient cells that can lead to modulation of target cell functions. We hypothesized that in addition to genetic determinants of NED in prostate cancer, tumor exosomes/EVs are important determinants of this trans-differentiation. In agreement, we found that exosomes mediate NED via horizontal transfer of functional BRN4 and BRN2 between cancer cells. We could detect both mRNA and protein corresponding to these two TFs in exosomes. Employing clinical serum samples from advanced prostate cancer patients, we found that EV-associated BRN4 and BRN2 are potential, non-invasive molecular biomarkers for diagnosing NED induction in these patients. These findings have important translational implications as currently, specific biomarkers for diagnosing NEPC are largely lacking.

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Publication

BRN4 Is a Novel Driver of Neuroendocrine Differentiation in Castration-Resistant Prostate Cancer and Is Selectively Released in Extracellular Vesicles with BRN2
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Clin Cancer Res. 2019 Nov 1