

## **Combined gene expression and cell surface proteomics identifies potential therapeutic targets for subtypes of advanced prostate cancer**

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**Background:** Metastatic castration-resistant prostate cancer (mCRPC) is a heterogeneous disease. The primary histologic subtype of mCRPC is adenocarcinoma but mechanisms of resistance to castration are varied including evolution to neuroendocrine prostate cancer (NEPC), a highly aggressive variant with features of neuroendocrine differentiation.

NEPC and prostate adenocarcinoma represent divergent prostate cancer differentiation states based on distinct phenotypes, gene expression, global methylation profiles, and patterns of epigenetic regulator expression. Cell surface phenotypes often reflect differentiation states of cells in normal development and cancer. Defining unique cell surface markers in NEPC and prostate adenocarcinoma would allow for the development of biomarkers and targeted antibody-based therapies.

**Results:** We set out to characterize the cell surface phenotype of NEPC and prostate adenocarcinoma using published gene expression datasets and RNA-Seq gene expression of a panel of newly developed human prostate cancer cell lines (EF1 from the MSKCC PCa4 NEPC organoid line, NB120914 from a patient-derived xenograft of a mCRPC bone metastasis, and LASPC-01 engineered by transforming benign prostate epithelial cells) and established lines (CWR22, LNCaP, NE1.3, DU145, and NCI-H660). We bioinformatically derived a list of 7555 genes comprising putative human cell surfaceome genes using predictions for transmembrane domains, GPI-anchored proteins, and plasma membrane localization. Cell surface genes differentially expressed in NEPC over prostate adenocarcinoma were abundant and enriched for neural processes and ion channels. The prostate cancer cell line panel was further analyzed using cell surface proteomics using a membrane-impermeable biotin labeling agent, protein enrichment with streptavidin, and mass spectrometry. We identified a total of 1080 proteins in the panel of cell lines which were enriched for cell surface annotation by Gene Ontology. Integrative analysis of gene expression and proteomics using rank-rank hypergeometric overlap analysis of the differentially expressed cell surface genes and cell surface proteins demonstrated the highest rank correlation in the NEPC cell lines. High-confidence NEPC and prostate adenocarcinoma cell surface candidates derived from this combined analysis were validated by immunoblot, immunohistochemical, and FACS analyses of human prostate cancer cell lines and xenografts. Further validation is ongoing in archived clinical human prostate cancer tissues.

**Conclusions:** Our findings indicate that the cell surface landscape of mCRPC is distinct between NEPC and prostate adenocarcinoma. Combined RNA-Seq and cell surface proteomics in a unique cell line panel has enabled the prioritization of cell surface markers that could be used as biomarkers or targets for antibody-based therapies for subtypes of advanced prostate cancer in the near future.

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