Prostate Neuroendocrine Carcinoma - An Emerging Neuroendocrine Cancer with Unmet Need

Tito Fojo[1], Prabhjot Mundi[1], Thomas Litman[2], Jia Li[1], Michael Shen[1], W. Doug Figg[3], Olufemi John-Fatinikun[1], Susan Bates[1]
[1]Columbia University, [2]Copenhagen University, [3]National Cancer Institute

Background: In its later stages prostate cancer (PC) is often characterized by neuroendocrine features, an evolution that has led to use of the term neuroendocrine prostate cancer (NEPC) – a variant commonly treated with platinum containing regimens, that is nearly always fatal, allowing one to characterize NEPC as a disease with unmet therapy needs. Although clinically and biologically heterogenous, NEPC are histologically similar to other neuroendocrine carcinomas (NECs) and express neuroendocrine markers including chromogranin and the somatostatin receptor (SSTR), suggesting SSTR-targeting agents such as lanreotide, octreotide, or Lutathera (^{177}Lutetium 177-DOTATATE) may offer therapeutic benefit. While we lack a consensus on how NEPC emerges, animal models and clinical data are gradually providing a greater understanding including evidence of frequent inactivation of TP53 and PTEN. Increasing evidence points to NEPC as a treatment emergent histologic subtype following treatment with an androgen receptor-targeting agent, such as abiraterone, enzalutamide or darolutamide. While the fraction of men who develop this invariably aggressive and lethal complication is uncertain, some studies suggest as many as 17% of all men whose tumors develop resistance to an androgen receptor–targeting agent will acquire this phenotype. Differences in estimates likely reflect both gradual evolution of the tumor neuroendocrine phenotype and our ability to detect it molecularly. Those confirmed histologically clearly represent a more distinct phenotype, while detection in DNA via a molecular signature may be more sensitive and detect the phenotype earlier in its evolution. Our analysis of the data suggests this is the case.

Materials and Methods / Experimental Approach: Standard RNA expression analysis and Virtual Inference of Protein-activity by Enriched Regulon Analysis (VIPER) analysis.

Results: NEPC express very high levels of the SSTR – most notably SSTR2. Using a pan-cancer (mostly TCGA) output as the reference point, SSTR2 is shown to be universally over-expressed in NEPC, with very high levels in small cell tumors. The levels of expression in NEPC are exceeded only by that in tumors we recognize as neuroendocrine, including common neuroendocrine tumors (NETs) arising from diverse tissues, meningiomas and pheochromocytomas / paragangliomas. Importantly, the levels are much higher than those in *de novo* PCs. Additionally, using a VIPER analysis strategy to infer functional protein levels, VIPER-inferred SSTR2 activity levels were comparable to those of mRNA expression, with only a few outliers. As observed with mRNA expression, VIPER analysis also confirms NPEC as amongst the few cohorts that demonstrate functional SSTR2 at very high levels and similarly at much higher levels than *de novo* PCs. While high SSTR levels can be detected in nearly all NEPC characterized molecularly, our analysis also finds increased SSTR2 expression in many PC samples not classified as neuroendocrine by either histology or gene expression signatures. Since this is not seen in treatment-naive PC samples in TCGA, it suggests SSTR2 expression in more advanced PC may precede overt morphological features of neuroendocrine differentiation. Finally, in preliminary experiments we examined the activity of peptide-derived drug conjugates targeting the somatostatin receptor in four NEPC models: PC3, LASCPC-01, NCI-H660, and murine NEPC organoids derived from tumors arising in NPp53 mice, a genetically engineered mouse (GEM) model with combined inactivation of TP53 and PTEN. In these assays we found marked sensitivity to our peptide drug conjugates.

Conclusions: The totality of the data suggests SSTR is likely to be broadly expressed in NEPC, implicating it as an important therapeutic target. Note here an important contrast with what occurs in the context of more traditional NECs, where SSTR expression is lower or even absent in the more advanced, aggressive and lethal high-grade tumors. The data further suggest NEPC gradually emerges with SSTR expression appearing with development of resistance to an androgen receptor (AR)-targeting agent as AR expression disappears. Because enzalutamide, apalutamide and darolutamide are increasingly being used clinically in earlier settings, one can be concerned this neuroendocrine proclivity may occur earlier in the history of treated PC. This possibility adds some urgency to addressing this unmet need. Finally, the marked sensitivity to our peptide drug conjugates suggests the SSTR can be exploited as a target in NEPC.

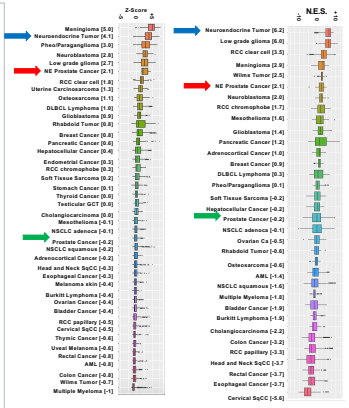


Figure – [Above] SSTR2 Expression and inferred activity. [Left] Boxplots showing Z-score distributions of SSTR2 mRNA expression across 42 different tumors. NPEC is amongst few cohorts that expresses SSTR2 at very high levels. Higher levels are found in NETs. Sources: TCGA, TARGET pediatric cohorts, MMRF for multiple myeloma, NCI for DLBCL and Burkitt, and the Beltran data set for NEPC. **[Right]** VIPER inferred SSTR2 activity is similar to **[Left]** Abbreviations: NES = normalized enrichment/VIPER score for SSRT2. **Red, NEPC; Green, Prostate adenocarcinoma; Blue, Neuroendocrine tumors**

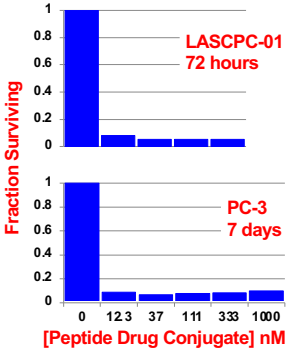
Binding of DM1 conjugates to tubulin does not require hydrolysis and occurs in a cell free reaction

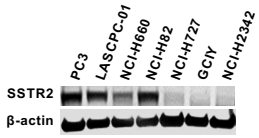
Impact of drugs and conjugates on polymerization of purified tubulin

Compound	Concentration μM	MT pellet, % Total protein	Pellet % Inhibition of control polymerization
DMSO control	4	100	-
	8	100	-
Ansamytocin P3	4	60	40
	8	20	80
P182-1-DM1	4	68	32
	8	28	72
P182-2-DM1	4	12	88
	8	12	88
Paclitaxel	4	100	-
	8	100	-

[Above] Rat brain tubulin was 12.5 μM in 0.85 M glutamate / 0.1 M Pipes, 1 mM MgCl2 pH 6.9, + 1 mM GTP. Compounds added to indicated concentrations, incubated at 37˚C x 30 min. Centrifuged 8' x 1000x g. Polymerization was done at 37˚C rather than 30˚C previously, and tubulin concentration was higher, accounting for the ~100% polymerization in the control and, or course, also paclitaxel. The assay could not distinguish 95% from 100%.

Figure –Cytotoxicity of peptide drug conjugate (PDC) in two NEPC cell lines. [Right] Both the LASCPC-01 and the PC3 NEPC cell lines were found to be very sensitive to the PDC with IC50 values in the nanomolar range





[Left] Baseline SSTR2 expression in selected cell lines. 25 μg protein / lane; SSTR2 (A-8) from Santa Cruz. NEPC: PC3, LASCPC-01, NCI-H660
Neuroendocrine: NCI-H82, NCI-H727
Gastric adenocarcinoma: GCIY Lung adenocarcinoma: NCI-H2342

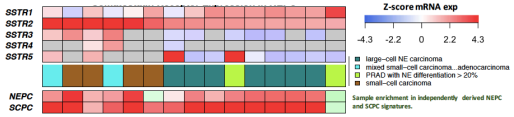


Figure –[Above] Expression of somatostatin receptors in NEPC. The top five rows of the heatmap depict Z-scores for expression of the five somatostatin receptors in 15 NEPCs. This analysis used a pan-cancer (mostly TCGA) reference point. SSTR2 is universally over-expressed in NEPC, with very high levels in the small cell tumors. The bottom two rows based on signatures published by Tsai et al [REF] using an independent data set, help annotate signature enrichment. The top row of the bottom two is a general high grade NEPC signature, while the other is specific for small cell prostate cancer. All samples here except the rightmost are strongly enriched in these gene expression signatures, even though only 4 samples were classified histologically as small cell.

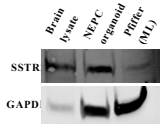


Figure – Expression of SSTR2 and activity of PDCs in murine NEPC organoids. [Above] Immunoblot using an anti-SSTR2 antibody demonstrates robust SSTR2 expression in murine NEPC model in the experiments shown here – high levels in brain lysates as (+) control. **[Right]** NEPC organoids were treated for three days with DM1, Lan-MCC-DM1 and P182-2-DM1 at concentrations of 1 μM, 0.33 μM, 0.11 μM, 0.037 μM, 0.012 μM, 0.0041 μM, 0.00137 μM and 0.00046 μM. CellTiter-Glo Luminescent Cell Viability assay measured viable cells. The percentage of viable cells after drug treatment compared to vehicle control (DMSO) was used to generate a dose–response curve. Note IC50 value for P182-2-DM1 is similar to DM1.

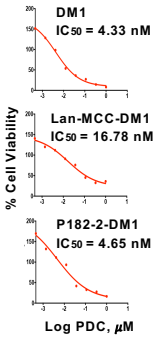
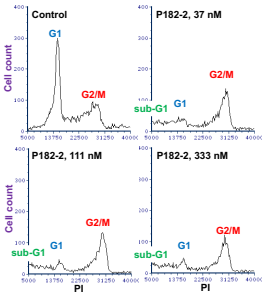


Figure – [Right] Cell cycle effects of PDC. Cell cycle analysis of cells incubated 24 hours with a PDC at concentrations noted. Note marked **G2/M** arrest even at lowest concentrations of the PDC, comparable to that observed with free DM1



CONCLUSIONS:

1. Neuroendocrine prostate cancers (NEPCs) express high levels of the somatostatin receptor (SSTR), and the levels increase as transformation to a NEPC phenotype occurs.
2. The high levels of the SSTR make this a very attractive candidate for a precision therapy strategy of NEPC with both existing options and those under development.

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