Molecular correlates of Delta-like-ligand 3 (DLL3) expression in neuroendocrine neoplasms (NENs)

Justin Hwang[1], Julie McGrath[2], John R. Lozada[1,3], Pavel Brodskiy[2], Joanne Xiu[2], Shuanzeng Wei[4], Elisabeth I. Heath[5], Benedito A. Carneiro[6], Emil Lou[1], Helosia P. Soares[7], Rana R. McKay[8], Emmanuel S. Antonarakis[1], Charles Ryan[1], David Spetzler[2], Himisha Beltran[9]

[1]Division of Hematology, Oncology and Transplantation, University of Minnesota, Masonic Cancer Center, Minneapolis, MN. [2]Caris Life Sciences, Irving, TX. [3]Medical Scientist Training Program (MD/PhD), University of Minnesota, Minneapolis, MN. [4]Fox Chase cancer Center, Department of Pathology, Philadelphia, PA. [5]Wayne State University Karmanos Cancer Institute, Department of Oncology, Detroit, MI. [6]Legorreta Cancer Center at Brown University, Providence, RI. [7]University of Utah, Huntsman Cancer Institute, Salt Lake City, UT. [8]Department of Medicine, Division of Hematology/Oncology, University of California San Diego, San Diego, CA. [9]Dana-Farber Cancer Institute, Boston, MA

Abstract

Background: NENs can occur in many locations but have limited precision therapy options. DLL3 is a cell surface protein that is emerging as a promising therapeutic target in NENs including neuroendocrine prostate cancer (NEPC) and small cell lung cancer (SCLC). Our recent study indicated that ~77% of NEPCs expressed DLL3, with expression in circulating tumor cells being highly concordant with matched biopsies (Puca et al Science Transl Med 2019). While there are ongoing clinical trials of drugs targeting DLL3, the repertoire of clinical and genomic features shared across other DLL3-expressing NEN cancers is ill-defined. SCLC tumors will be assessed in future studies.

Methods: We analyzed WES and WTS data from NENs identified across 29 different sites of origin using the Caris Life Sciences platform, excluding SCLC and including neuroendocrine carcinomas and neuroendocrine tumors. We used values above or below median DLL3 expression of all NEN samples to define DDL3-High/Low (H/L). Significance of molecular alterations in DLL3-H vs L was determined using Fisher's-Exact/Mann Whitney/X^2 test with Benjamini-Hochberg correction.

Results: DLL3 expression across all 2672 NEN samples was observed in 27 of 29 NENs after excluding SCLC. NENs of anus, prostate, lung, bladder, and bile duct exhibited the greatest median DLL3 expression, whereas adrenal gland, small bowel, and nervous system displayed the lowest. Certain tissues of origin displayed more robust DLL3 expression, with 71% (50/66) of NEPC, 75% (87/122) of lung, and 77.3% (51/66) of bladder being DLL3-H compared to 14.4% (13/90) of adrenal and 7.9% (12/151) of small bowel NENs. DLL3-H NENs were associated with TMB-high status (>10 muts/Mb; 12.1% vs 4.5%, OR 2.7, q<0.001) and more genomic alterations in several driver genes, including tumor suppressors TP53 (51% vs 23%, OR 2.3, q<0.001) and RB1 (42% vs 10%, OR 4.2, q<0.001), and oncogenes KRAS (14% vs 5.4%, OR 2.5, q<0.001), MYC (5.7% vs 0.9%, OR 6.3, q<0.001) and CCNE1 (5.3% vs 1.3%, OR 4.0, q=0.001). Conversely, DLL3-L NENs exhibited more alterations in CTNNB1 (2.2% vs 5.2%, OR 0.42, q=0.04), MEN1 (3.3% vs 11%, OR 0.30, q<0.001), and BCOR (1.3% vs 4.1%, OR 0.32, q=0.02). DLL3-H NENs also had significantly more alterations in PIK3CA (6.4% vs 3.0%, OR 2.1, q=0.04), chromatin remodeling genes KMT2D (6.7% vs 2.6% OR 2.6, q=0.005) and CREBBP (3.2% vs 0.9%, OR 3.6, q=0.03), and WNT signaling gene APC (9.7% vs 5.2%, OR 1.9, q=0.02).

Conclusion: We confirmed DLL3 expression in NENs across different tissues of origin, with highest expression in poorly differentiated NENs. DLL3-H expression was associated with genomic features considered "undruggable" based on current precision therapy approaches. Therefore, DLL3-targeted therapies may serve as a promising strategy for NEN patients with functional loss of tumor suppressors TP53 and RB1, as well as increased activity of KRAS, WNT and MYC signaling.

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Results

Neuroendocrine Neoplasms (NENs) in the Caris Precision Oncology Alliance

NEN Specimen Site	Number of cases (n)
Unknown Primary	508
Pancreas	424
Lung	270
Small Bowel	268
Colorectal	220
GYN Organ	188
Adrenal gland	132
Prostate	102
Bladder	88
Head and Neck	68
Stomach	58
Esophagus	48
GI Tract, NOS	44
Bile Duct	38
Nervous system	32
unclear/other	30
Appendix	29
Liver	27
Anal	21
Thymus	16
Ileeocecal junction	15
Breast	12
Cartoid Body	7
Peritoneum/Retroperitoneum	7
Kidney	5
Thyroid	4
Bone	4
Abdomen	4
Skin	3
total	2672

	DLL3-high	DLL3-low
Median Age (range) [N]	65.0 (6 - >89) [794]	61.0 (0 - >89) [795]
Male	51.1% (406/794)	53.1% (422/795)
Female	48.9% (388/794)	46.9% (373/795)
P-Value	0.3936	0.016

Differentiation status	Counts
Well differentiated	389
Poorly differentiated	69
Histology	**Counts**
Large cell NE Carcinoma	132

Table 1.
Left. A total of 2672 NEN samples from a total of 29 distinct tissues of origin was collected.
Right. Patient demographics (top) A subset of NENs were classified based on histology or differentiation status (bottom).

DLL3 transcripts are detected across all NEN types

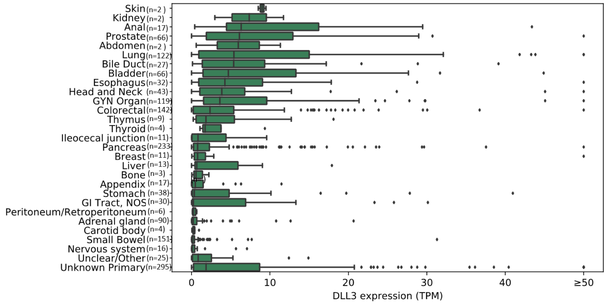
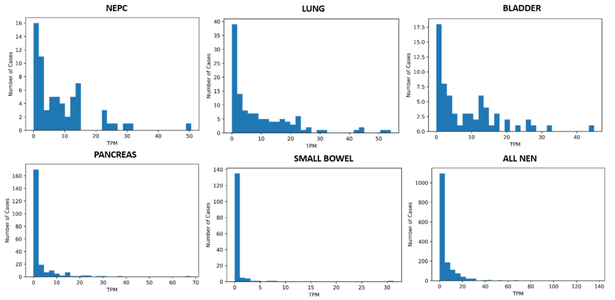


Figure 1. DLL3 expression shown as transcripts per million (TPM) in each sample across all NEN types in which RNA data was available. The median and quartiles are shown.

DLL3 is differentially expressed across NEN types



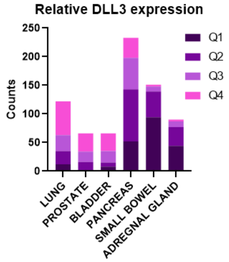


Figure 2.
Top. DLL3 expression (TPM) profiles are distinct across NEN types. NEPC, Lung, Bladder samples exhibit elevated DLL3 expressing NENs as compared to other tissue types.
Left. Expression of DLL3 was categorized based on median and quartiles across all NEN samples. The number of samples in each category is illustrated based on the 4 quartiles. Q1 – 0 to 25th percentile, Q2 – 25th to 50th percentile, Q3 - 50th to 75th percentile, Q4 – 75th to 100th percentile.

DLL3-high NENs are associated with genomic alterations in critical tumor regulatory genes and factors

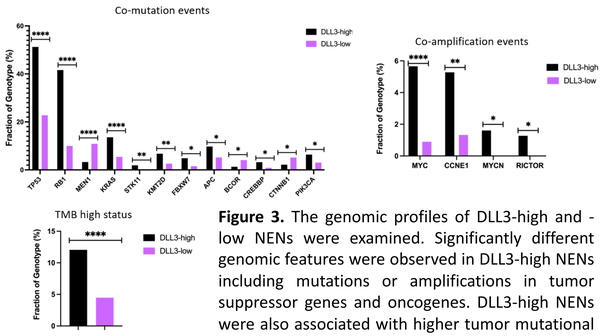


Figure 3. The genomic profiles of DLL3-high and -low NENs were examined. Significantly different genomic features were observed in DLL3-high NENs including mutations or amplifications in tumor suppressor genes and oncogenes. DLL3-high NENs were also associated with higher tumor mutational burden (TMB)

DLL3-high NENs exhibit robust association with ASCL1 expression

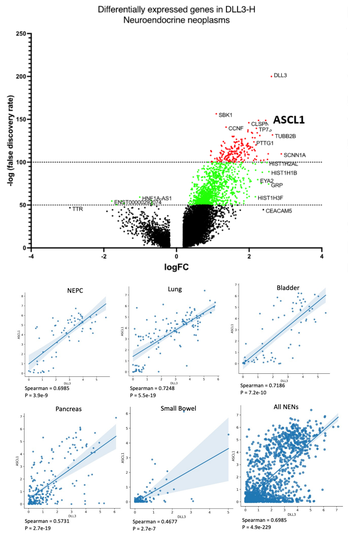


Figure 4.
Top. Whole transcriptome sequencing of NEN samples was analyzed to identify genes with robust association with DLL3 expression. Top gene family correlates (red) include ASCL1 as well as transcription or chromatin regulatory genes (green). Significant levels are shown based on dotted lines.
Bottom. ASCL1 transcripts exhibited robust correlation with DLL3 in all the NEN types based on Pearson correlations conducted across samples. P-values are shown.

Study Highlights

- We aggregated 2,672 NENs across 29 distinct tissue types and analyzed their genomic, transcriptomic, and pathological features.
- DLL3 expression is robustly detected in subsets of NENs including lung, NEPC, and bladder.
- DLL3-high expressing NENs are characterized by additional genomic alterations of tumor regulatory genes.
- Regardless of tissue type, DLL3 expression is robustly associated with transcription factors that regulate differentiation including ASCL1.

Contacts

Justin Hwang, Ph.D. jhwang@umn.edu
Julie McGrath, Ph.D. jmcgrath@carisls.com