Differential Expression of Somatostatin Receptor (SSTR) Subtypes Across a Spectrum of Neuroendocrine Neoplasms (NENs)

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Background

- Targeted therapy of NENs based on the presence of SSTRs fills a unique niche in tumor biology and clinical treatment of patients with solid tumors.
- SSTRs have multiple isoforms and are collectively expressed in the majority of NENs.
- However, subtypes are still not routinely tested and thus not assessed for clinical decision-making, especially for patients meriting consideration of targeted radionucleotide therapy.
- Clarifying the landscape of SSTR subtypes using molecular techniques more sensitive than immunohistochemistry (IHC) and identifying associated genomic biomarkers that differ between them, will pave the way for more sophisticated decision-making in the future.
- Additionally, leveraging transcriptomics to better assess mitotic markers such as Ki-67 to assess tumor grade, would increase diagnostic accuracy.

Methods

- 1595 NENs were analyzed using Next Generation Sequencing (NGS, 592 gene panel, NextSeq), Whole Exome and Transcriptome Sequencing (WES, WTS, NovaSeq), and IHC at Caris Life Sciences (Phoenix, AZ).
- In a subset of 492 NENs with accompanying tumor grading information, a median *MKI67* (gene encoding KI-67) TPM value of 2.27 for low grade (LG-) and 38.7 for high grade (HG-) NENs was observed (q<0.05).
- ROC curve analysis was subsequently performed on these tumors to determine the suitability of *MKI67* to infer HG and LG NENs.
- The best threshold of *MKI67* obtained from the training data were used to determine the true positive rate (TPR) and false positive rate (FPR) of the holdout set and then subsequently the remainder of the cohort (lacking grading information).
- Prevalence tables, SSTR subtype expression and correlation analysis were performed from NGS, WES and WTS information.
- Significance was determined using chi-square, Fisher-Exact or Mann-Whitney U and p-adjusted for multiple comparisons (q < 0.05) where applicable.

Results

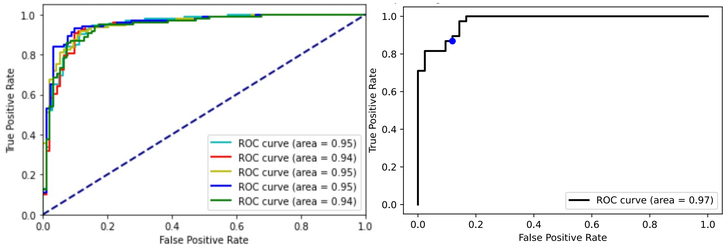

Figure 1 – ROC curve analysis to identify the optimal MKI67 threshold for grade stratification of NENs. A subset of NENs with grade annotations were split into training (left figure) and holdout datasets (right figure). ROC curve analysis from our training data revealed an MKI67 (*TPM*) value of 13.4735, which when applied to the holdout dataset yielded a TPR of 86.84% , an FPR of 11.9% and an AUC of 0.97 .

Prevalence table of molecular alterations in tumors with grade stratification based on pathology

Features	% Prevalence		Δ % Prevalence
	High Grade	Low Grade	
NGS-TP53	64.32	6.10	
NGS-RB1	47.50	0.87	
NGS-KRAS	17.11	2.33	
TMB High	14.52	2.09	
NGS-APC	12.97	1.68	
NGS-PIK3CA	11.83	0.68	
NGS-FBXW7	7.95	0.00	
NGS-PTEN	11.30	3.77	
CNA-CCNE1	6.94	0.00	
CNA-MYC	6.74	0.00	
NGS-CTNNB1	5.91	0.67	
NGS-MEN1	1.60	20.00	

Prevalence table of molecular alterations in tumors with grade stratification based on MKI67 expression

Features	% Prevalence		Δ % Prevalence
	High Grade	Low Grade	
NGS-TP53	56.19	13.36	
NGS-RB1	41.04	5.80	
TMB High	12.91	2.77	
NGS-KRAS	14.13	4.06	
NGS-APC	11.63	2.49	
NGS-PIK3CA	7.80	1.37	
NGS-KMT2D	6.80	1.51	
CNA-CCNE1	5.63	0.60	
NGS-TERT*	7.32	2.29	
NGS-PTEN	7.41	3.00	
NGS-FBXW7	4.94	1.04	
NGS-CTNNB1	5.35	1.69	
CNA-MYC	5.03	1.39	
NGS-KDM6A	3.12	0.48	
NGS-NOTCH1	2.17	0.00	
NGS-MEN1	2.88	13.72	

Table 1 – Differences in the molecular landscape of LG- and HG-NENs stratified by pathology/mRNA expression . All 12 of the statistically significant molecular alterations (q<0.05) observed in the pathologically annotated tumors were also observed in tumors stratified into HG vs LG based on MKI67 mRNA expression (bold font), with concordant differences in prevalence. Green bars indicate that the alterations are more frequent in HG- vs LG- NENs and red bars indicate the opposite.

SSTR Subtype/Grade	High Grade (n=862)	Low Grade (n=733)
	Median (range)	Median (range)
SSTR1	**0.8 (0.0 - 81.3)**	**2.8 (0.0 - 59.2)***
SSTR2	**4.2 (0.1 - 300.8)**	**12.2 (0.0 - 457.4)***
SSTR3	**0.3 (0.0 - 70.4)**	**0.3 (0.0 - 29.4)**
SSTR4	**0.7 (0.0 - 134.9)**	**0.2 (0.0 - 71.4)***
SSTR5	**0.3 (0.0 - 37.4)**	**0.5 (0.0 - 18.0)***

Table 2 – Expression of SSTR subtypes in HG- and LG- NENs. Compared to HG-NENs (n = 862), LG-NENs(n = 733) expressed higher levels of SSTR 1(3.5-fold),2 (2.9-fold) and 5 (1.67-fold); and lower levels of SSTR4 (0.28-fold) (q < 0.05).

Results

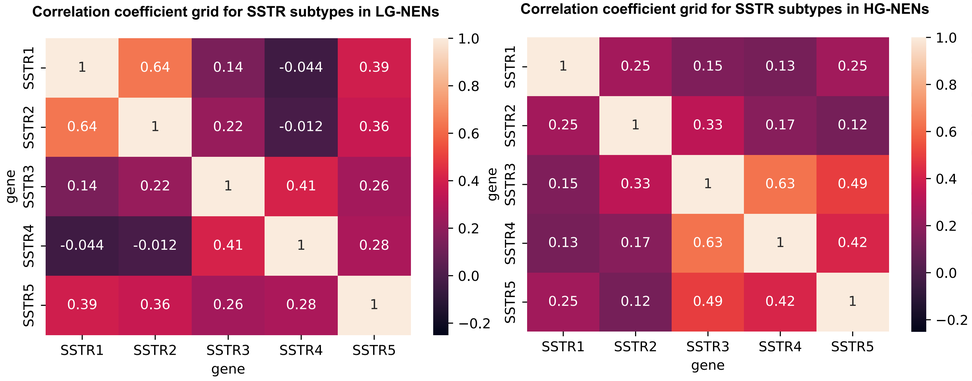

Figure 2 – Correlation of SSTR subtypes in LG- and HG-NENs. mRNA expression was used to analyze the spearman's correlation of the different SSTR subtypes. The expression of SSTRs 1 and 2 in LG-NENs (r_s= 0.64) and SSTRs 3 and 4 in HG-NEN (r_s= 0.63) were positively correlated.

Prevalence table of molecular alterations for specific SSTR subtypes in LG-NENs

Molecular Alterations/ Prevalence in High vs Low SSTR Expressed NENs	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
NGS-TP53	***-14.3733***	***-11.828***	*5.1268*	***11.1111***	-2.1795
NGS-RB1	***-8.7375***	***-8.9636***	*5.9853*	***11.2865***	2.2187
NGS-KRAS	-2.8799	*-4.2076*	-1.0853	2.0655	0.1182
NGS-APC	-1.5216	-2.105	-0.9255	*2.2298*	*-2.657*
NGS-BRAF	*-2.9816*	*-2.4478*	-0.975	1.3713	-0.3197
NGS-CTNNB1	-1.77	***-3.6174***	*-2.0745*	-0.9517	*-2.553*
NGS-MEN1	***10.4223***	***8.5231***	***10.8898***	-1.0652	-2.9691
CNA-MYC	*-2.4524*	-1.4175	0.9686	1.521	-0.5069
TMB-H	***-4.8327***	*-3.3904*	1.3536	*2.4164*	0.1225

Prevalence table of molecular alterations for specific SSTR subtypes in HG-NENs

Molecular Alterations/ Prevalence in High vs Low SSTR Expressed NENs	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
NGS-TP53	*-8.921*	0.8791	-6.5171	1.1894	*-8.0937*
NGS-RB1	*-9.1272*	0.6419	-2.4507	*8.8346*	-7.8099
NGS-KRAS	***9.2393***	*-7.3753*	*-5.5192*	2.0218	0.2164
NGS-APC	***11.52***	-1.3953	*-5.8807*	*6.96*	***-8.8308***
NGS-BRAF	1.1933	*-2.1645*	-1.1195	0.6569	*2.2078*
NGS-CTNNB1	-0.5233	*-3.4238*	0.5213	-1.084	-2.4505
NGS-MEN1	***4.9749***	*2.8282*	1.6794	*-3.9008*	-0.5177
CNA-MYC	-1.6256	3.0441	2.7878	-0.5597	1.0187
TMB-H	*-7.2364*	*-5.3762*	3.489	*5.4584*	1.3359

Table 3 – Molecular landscape of NENs stratified by grade and SSTR subtype expression. For a given grade and SSTR subtype, NENs were categorized as SSTR high or low based on median mRNA expression. The difference in prevalence of a select set of genes is reported above with negative values indicating that the alteration was more prevalent in SSTR low cohort and vice versa. The magnitude of change is shaded from red to green. Italicized numbers depict p<0.05 while those italicized and bolded depict q<0.05. In LG-NENs, alterations in TP53, RB1 and MYC were more frequent in SSTR 1- and 2- and less frequent in SSTR 3- and 4-low groups. In HG-NENs, alterations in TP53 and RB1 were more frequent in SSTR 1, 3 and 5 low groups. Alterations in MEN were more frequent in SSTR 1,2 and 3 high groups regardless of tumor grade

Conclusions

- This study provides evidence that WTS and NGS can be leveraged to predict grade of NENs and define characteristic differences in the genomic landscape across SSTR subtypes in HG and LG NENs.
- Incorporating the molecular profiling of NENs can thus aid in advancing the development of more tailored therapeutic strategies.