SV2A PET Imaging for Noninvasive Assessment of Neuroendocrine Differentiation in Neuroendocrine Tumors

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INTRODUCTION

Neuroendocrine tumor (NET) remains as a considerable diagnostic challenge even with the combination of circulating biomarker test, biopsy, and morphologic/functional imaging. Given the unique feature of NET cells closely related to both nervous and endocrine systems, investigating the contribution of nerves in the initiation and progression of NETs will lead to new strategies for its diagnosis and therapy. Somatostatin receptors (SSTRs) are good targets for specific PET imaging (e.g. ^{68}Ga-DOTATATE) in NET diagnosis and targeted radionuclide therapy (e.g. ^{177}Lu-DOTATATE), but there is a gap of no radiotracers to investigate the tumor innervation and target NE differentiation directly for NETs. Our initial studies have found that synaptic vesicle protein 2 isoform A (SV2A) has the great potential to become a promising biomarker for neuroendocrine differentiation in NETs. Herein, we report our continuous efforts in evaluating and developing NET PET imaging by targeting SV2A.

MATRIALS and METHODS

De-identified human NET (20 lung, 13 pancreas, and 6 intestine) tumor tissues were identified from the Tissue Management Shared Resource at the UTSW Simmons Comprehensive Cancer Center. SV2A, CgA, SYP, GLUT1 (Sigma, 355A-1), and SSTR1–5 (Abcam: ab140945, ab271907, ab227601, ab272677, ab109495) were stained and interpreted based on the histochemical scoring (H-score) assessment incorporating both the staining intensity and a percentage of stained cells at each intensity level. The information we received for each sample include gender, race, ethnicity, histology, prior treatment history, clinicopathologic features including known Gleason grade of the tumor, and site of primary or metastatic biopsy.

Table 1. Human NET tumor tissue sample information

Organs	Grades	Histology
Lung	1 (14)	Atypical carcinoid tumor (5)
	2 (4)	Typical carcinoid tumor (15)
	ND (2)	
Pancreas	1 (8)	Well-differentiated (11)
	2 (4)	Nonfunctional (1)
	4 (1)	High grade (1)
Intestine	2 (6)	Well-differentiated neuroendocrine tumor (carcinoid tumor) (6)

Four new ^{19}F-SV2A binding ligands were synthesized to pre-evaluate their SV2A specific binding affinity through a competition binding assay. The radiolabeling precursor for the -amino analog at the pyridine ring was prepared based on the binding assay, and the ^{18}F-radiolabeling was tested afterwards in an automated synthesizer.

RESULTS

Due to the limit number of human NET tumor tissue samples, all samples were either at grade 1 or 2. Overall, SYP showed the highest H-score across all samples, with 263 ± 24, 263 ± 25, and 225 ± 49 for lung, pancreas, and intestine NETs, respectively. In contrast, SV2A showed the 2nd highest H-score in lung NETs (162 ± 94) and the 3rd highest H-score in pancreas (112 ± 83) and intestine (127 ± 35) NETs. The SSTR2's H-scores were close to SV2A, with 149 ± 120, 227 ± 75, and 112 ± 95 for lung, pancreas, and intestine NETs, respectively ($p > 0.05$). Besides SSTR2, SSTR4 was also found with significant staining in some lung and pancreas NET samples. Due to the relatively low grades, the GLUT1 showed smaller H-scores as expected as 81 ± 43, 103 ± 45, and 93 ± 60 for lung, pancreas, and intestine NETs, respectively. Correlation analyses were tried for SV2A vs other biomarkers using the H-scores, but no conclusion could be made partially because of lack of higher grades NET samples. Additionally, the western blot method is expected to fit better for the correlation analyses than the IHC staining method based on the H-score.

The design of the four candidate ^{19}F-SV2A binding ligands was to address the high background uptake issues in brain, liver, intestine, etc. while maintaining the specific binding affinity to SV2A. Instead of the methyl group on the pyridine ring of ^{18}F-SynVest-1, it is expected to have higher hydrophilicity with amine, methyl hydroxyl, and polyethylene glycol groups. The logP were 1.16, 1.38, 1.11, and 0.63, respectively, which was estimated by Chemdraw 20.0. The competition binding assay showed the -amino analog had the highest binding affinity to SV2A, so it was selected to move forward for radiolabeling. Firstly, we tried to aminate 1-((3-bromopyridin-4-yl)methyl)-4-(3-fluoro-5-iodophenyl)pyrrolidin-2-one, but we obtained two sites substituted (bromide and iodine) product. Then, we adjusted our synthetic route to prepare our radiolabeling precursor from 4-(3-chloro-5-fluorophenyl)pyrrolidin-2-one. A boronic acid pinacolester precursor was obtained. The ^{18}F-labeling went through the Cu(I) mediated process to get 5% radiolabeling yields (n = 2), which made the radiotracer candidate ready for the following PET imaging evaluations.

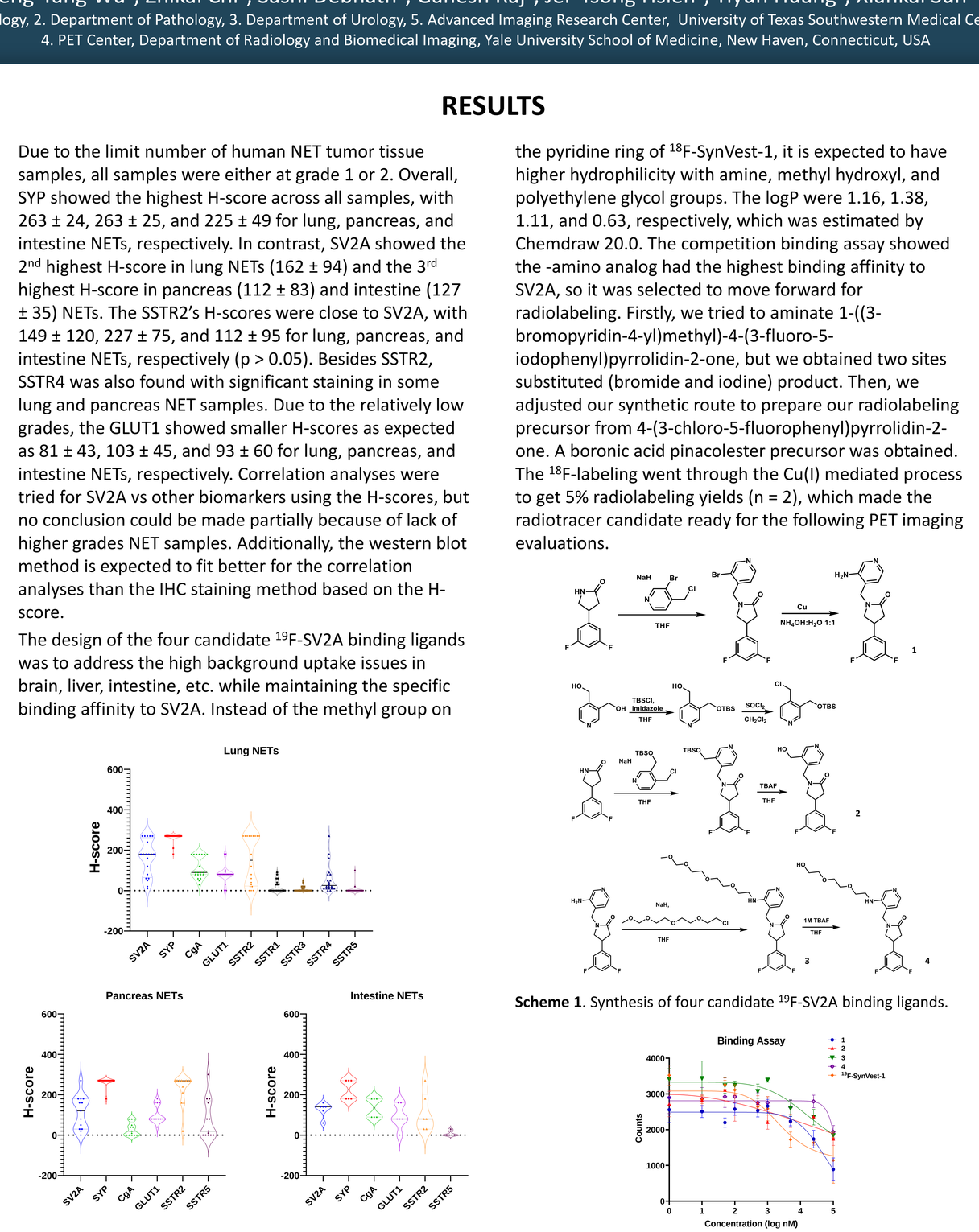

Figure 1. H-score data in lung, pancreas, and intestine NETs for SV2A, CgA, SYP, GLUT1, and SSTR1–5. SV2A: synaptic vesicle protein 2 isoform A; CgA: chromogranin A; SYP: synaptophysin; GLUT1: glucose transporter 1; SSTR: somatostatin receptor .

Scheme 1. Synthesis of four candidate ^{19}F-SV2A binding ligands.

Figure 2. In vitro binding assay of ligands 1-4 using the SV2A+ H720 cells. ^{19}F-SynVest-1 was included as the positive control ligand, and ^{18}F-SynVest-1 was used as the radioligand for this competition assay.

CONCLUSION

SV2A showed strong expressions in low grade lung, pancreas, and intestine NET human tumor tissue samples, which is close to the level of SSTR2. Further correlation analyses are required to include more data in high grade NETs and conduct the experiments using the western blot method. A new ^{18}F-SV2A tracer with higher hydrophilicity was successfully prepared, and it is ready for the following SV2A-PET imaging evaluation in NET xenograft models.

ACKNOWLEDGEMENTS

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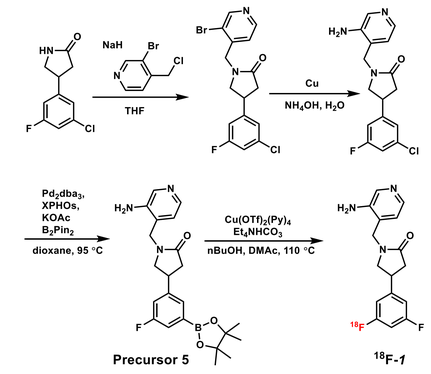

Scheme 2. Synthesis of the precursor ligand 5 and radiolabeling of ^{18}F-1.

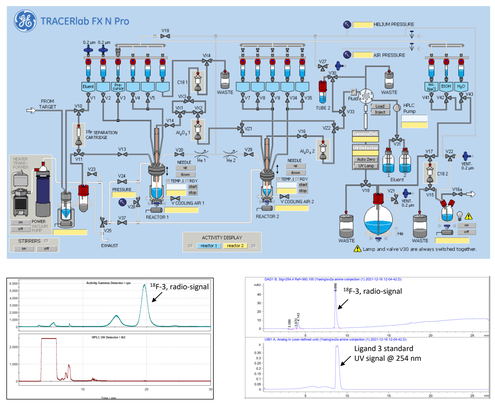

Figure 3. Automated radiolabeling of ^{18}F-3 in a Tracerlab FX N-Pro module.