Identification of Genes Related to ^{68}Ga -DOTATATE Organ Distribution in Homo Sapiens

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Research Article

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Abstract

Purpose ^{68}Ga -DOTATATE is a somatostatin analogue that has been used for imaging neuroendocrine tumours. However, there is nonspecific uptake in some organs, and the reasons for that are not clear. The aim of the study is to outline the dynamic distribution pattern of ^{68}Ga -DOTATATE in human body, and identify the genes responsible for ^{68}Ga -DOTATATE uptake by bioinformatics analysis.

Methods ^{68}Ga -DOTATATE PET/CT was performed in 32 patients, and dynamic total-body PET scanning was performed with uEXPLORER. The gene expression datasets of human organs were downloaded from the Human Protein Atlas. WGCNA analysis was performed to screen the potential genes related to ^{68}Ga -DOTATATE. BindingDB, SEA and SwissTargetPrediction databases were used to predict the potential binding proteins of DOTATATE based on molecular structure.

Results Dynamic total-body PET scanning showed that ^{68}Ga -DOTATATE uptake was not consistent with expression of SSTR2 in human organs. WGCNA analysis revealed 800 genes whose expression level was positively correlated to ^{68}Ga -DOTATATE uptake. According to the molecular structure of DOTATATE, 135 proteins with potential binding ability to DOTATATE were screened by analysis based on drug database. Based on the results of the above two parts, 8 proteins were finally obtained, respectively AVPR1A, EPHA8, EPHB4, OPRL1, SSTR2, SSTR5, ST3GAL1 and NPY1R, which had potential binding possibility with DOTATATE, and their expression was positively correlated with ^{68}Ga -DOTATATE uptake.

Conclusion WGCNA analysis was combined with the drug database to obtain new potential binding targets of DOTATATE. The bioinformatics approach developed in this study could potentially be used to discover new potential binding targets for molecular imaging agents.

Introduction

Neuroendocrine tumors are tumors of neuroendocrine cells, usually associated with high expression of somatostatin receptor subtype 2 (SSTR2)[1]. ^{68}Ga -DOTA- D Phe¹, Tyr³-octreotate (^{68}Ga -DOTATATE), a somatostatin analogue with high affinity for SSTR2, has been used in the imaging of neuroendocrine tumors[2]. Once SSTR2 expression is confirmed by ^{68}Ga -DOTATATE based imaging, the same SSTR2 analog, DOTATATE, can be attached to the therapeutic radioisotope (^{177}Lu , typically) and be used for molecular targeted therapy, which is called peptide receptor radionuclide therapy (PRRT)[3]. The uptake of DOTATATE can be observed not only in tumor tissues, but also in normal organs, such as liver and spleen. This will cause damage to normal organs during PRRT treatment[4]. However, it is still needs further research which targets DOTATATE could binds to besides SSTRs[5]. If we can identify which molecules DOTATATE can combine with and block these molecules during PRRT treatment, it will be possible to reduce the damage of PRRT treatment to normal organs.

Positron emission tomography / computed tomography (PET/CT) permits the measurement of physiological parameters through a dynamic study of tracer dynamics, which is a great advantage over

other imaging modalities[6]. In 2019, United Imaging Healthcare released the world's first total-body PET / CT scanner, named uEXPLORER[7]. With this device, 4D high-definition dynamic imaging of multi-tissues and organs in the total-body was realized, and it is the only instrument capable of total-body, continuous and dynamic PET scanning at present[8]. The improved sensitivity allows more accurate dynamic imaging of the total-body and dynamic analysis of the physiology of all organs at the same time[9]. Another advantage is that it can obtain high-quality tracer input function from PET images for pharmacokinetic studies, avoiding arterial intubation during blood collection[10]. In addition, the introduction of total-body PET has created an innovative "multisystem biology" framework for the study of human body[11].

The objectives of this study were to map the distribution pattern of ^{68}Ga -DOTATATE among human organs. Using dynamic total-body PET/CT to calculate standardized uptake values (SUVs) for various organs in the body. Comparing the data with the current knowledge on SSTR2 expression. Moreover, bioinformatics methods were used to compare the correlation between gene sequencing of each organ and DOTATATE uptake, and analyze the proteins that could potentially bind to DOTATATE in addition to SSTR2.

Materials And Methods

Patient selection

From our cohort of patients, we selected 32 patients (18 men, 14 women, age 18–81 years, median 54 years) who underwent ^{68}Ga -DOTATATE PET/CT. The patients who were included in this study had no evidence of recurrent disease on conventional imaging performed before the ^{68}Ga -DOTATATE PET scan. No tracer-avid lesions were found on the PET scan. All 32 patients met these criteria and informed consent was waived for the study, which was approved by the Institutional Review Board of Renji Hospital and was in accordance with the principles of the 2013 revision of the Declaration of Helsinki.

Imaging Protocol

^{68}Ga -DOTATATE (2MBq/kg) was administered intravenously, and after a distribution time of 45 minutes, PET/CT images were acquired from the head to mid-thighs with the arms positioned above the head. Transmission images were acquired to correct for attenuation using a low-mAs CT scan without contrast.

Data Analysis

Two experienced nuclear medicine physicians reviewed the map together, and SUVmax and SUVmean were measured by ROI method. The ROI of different organs was outlined as follows: the ROI of brain, cerebellum, salivary gland, mediastinal blood pool, lung, liver, spleen, prostate and uterus were outlined in the center of the maximum cross section of transverse tomography of each organ, with a diameter of 2 cm. Muscle ROI was delineated in the maximum cross-section of transverse pectoralis major, with a

diameter of 1 cm. Skeletal SUV was used to delineate the entire L4 vertebral body at the maximum coronal section. The ROI diameter of kidney, intestinal tract and pancreas (each segment) was 1 cm, and the RENAL ROI was outlined in the renal cortex to avoid urine interference. Intestinal ROI was delineated by small intestine and ascending colon respectively. Pancreas ROI was outlined by head, neck, body and tail segments, and the average was taken as pancreatic SUV. Due to the uneven distribution of radioactivity in intestinal tract and pancreas, the region with the highest concentration of radioactivity should be selected according to visual analysis when sketting ROI, and the interference of surrounding organs such as spleen and kidney to measurement should also be avoided.

Dynamic data acquisition and reconstruction

Considering the high sensitivity of the total-body PET scanner, which allowed for low-dose imaging, the injected dose was a half reduction of the full dose used for routine PET imaging at our department. Low-dose CT was performed for attenuation correction (AC). Then, 60-min dynamic PET scanning was started simultaneously with a bolus injection of ^{68}Ga -DOTATATE into a vein near the ankle. The images were corrected for radioactive decay, attenuation, scatter, and randoms, and were reconstructed into a $239 \times 239 \times 679$ matrix with voxels of 2.85 mm^3 by the list-mode ordered subsets expectation maximization algorithm incorporating TOF and point spread function modeling (OSEMTOF-PSF). For dynamic analysis, the images were divided into 72 frames.

Weighted correlation network analysis (WGCNA)

The co-expression gene and enrichment analysis were applied to WGCNA package of R software, which revealed the correlation between genes [12]. Because the genes with little expression variation usually represent noise, we filter the most variable genes ($\text{SD} > 1.2$) and construct a network. The power of β was set to 5 to ensure a scale-free network. The minimum number of module genes was set to 30. The hierarchical cluster tree summarizes the gene modules of different colors. GO analysis was performed using KOBAS [13].

Gene expression analysis

High-resolution images corresponding to immunohistochemically stained tissue microarrays of different tissue types corresponding to all antibodies analyzed in the present investigation are obtained from the Human Protein Atlas (HPA). The normalized consensus transcript expression levels based on transcriptomics data from the HPA as well as the annotated protein expression levels based on IHC can also be accessed as previously described[14].

PPI network construction

Using STING database to analyze the interaction among the indicated proteins. The tree diagram of PD-L1 related protein network was constructed with the Cytoscape software. The confidence score cut-off was set to 0.8.

Statistical Analysis

SPSS 22.0 statistical software and R (<https://www.r-project.org/>) were used for data analysis. The measurement data conforming to normal distribution was expressed as mean ±SD. Two-sample T test was used for comparison between the two groups, and univariate analysis of variance was performed for comparison between multiple groups. P<0.05 was considered statistically significant.

Results

Distribution pattern of ⁶⁸Ga-DOTATATE

As shown in the maximum intensity projection images, the ⁶⁸Ga-DOTATATE was enriched in the bladder, adrenal gland, spleen, kidney, pituitary gland, liver, stomach, intestine, pancreas, salivary gland and thyroid gland (Figure 1). ⁶⁸Ga-DOTATATE is excreted through the urinary system, resulting in a large amount of radioactive urine retention in the bladder. In addition, significant uptake of ⁶⁸Ga-DOTATATE was observed in adrenal gland, spleen, kidney and pituitary gland, while mild uptake was found in liver, stomach, intestinal tract, pancreas, salivary gland and thyroid gland. The average and range of the SUVmax as well as SUVmean for all the organs measured are shown in Table 1.

Table 1. Maximum and mean of SUVs in different organs.

Organ	SUVmax		SUVmean	
	mean±s.d	range	mean±s.d	range
cerebral cortex	0.10±0.06	0.02~0.17	0.07±0.06	0.00~0.13
basal ganglia	0.13±0.12	0.00~0.35	0.11±0.11	0.00~0.31
pituitary gland	12.23±6.27	6.00~21.49	7.12±4.81	0.12~12.66
pons and medulla	0.07±0.04	0.03~0.12	3.11±7.48	0.02~18.38
cerebellum	0.07±0.06	0.02~0.17	0.06±0.06	0.01~0.16
tongue	1.02±0.48	0.18~1.49	0.87±0.43	0.15~1.33
salivary gland	1.73±0.79	0.85~3.12	1.44±0.66	0.71~2.59
tonsil	2.24±1.00	0.77~3.43	1.85±0.83	0.61~2.79
spinal cord	0.59±0.18	0.35~0.76	0.51±0.14	0.34~0.68
thyroid gland	2.55±2.22	0.97~6.77	2.26±1.97	0.83~5.96
lung	0.38±0.17	0.17~0.67	0.30±0.15	0.09~0.55
breast	0.67±0.47	0.40~1.22	0.54±0.36	0.33~0.95
heart muscle	1.34±0.45	0.84~1.95	1.09±0.31	0.70~1.48
esophagus	1.32±0.45	0.72~1.72	1.11±0.34	0.61~1.41
stomach	6.22±3.34	1.98~9.38	5.36±3.03	1.68~8.58
liver	6.30±2.54	2.70~9.91	5.25±2.32	2.18~8.79
spleen	20.13±10.92	7.73~36.55	16.61±8.73	6.19~27.66
adrenal gland	20.68±7.81	8.97~30.69	17.39±6.66	7.17~25.86
kidney	12.75±5.68	6.32~21.7	10.90±4.39	5.11~17.40
pancreas	3.46±0.94	2.74~5.17	2.89±0.81	2.19~4.36
gallbladder	3.00±2.50	1.63~8.06	2.23±1.31	1.31~4.85
duodenum	3.95±2.00	2.28~7.85	3.30±1.76	1.81~6.71
small intestine	4.77±1.79	2.53~6.92	4.08±1.82	2.01~6.64
colon	1.82±0.67	1.23~2.84	1.54±0.57	1.08~2.47
rectum	2.98±1.09	1.37~4.01	2.29±0.73	1.37~3.14
ovary	1.83±0.14	1.71~1.99	1.56±0.15	1.46~1.73
endometrium	1.88±0.24	1.72~2.16	1.64±0.17	1.47~1.80

cervix, uterine	1.45±0.36	1.03~1.67	1.28±0.38	0.87~1.61
vagina	2.52±0.05	2.47~2.57	2.05±0.05	2.01~2.10
seminal vesicle	1.38±0.36	1.02~1.73	1.28±0.42	0.86~1.70
prostate	4.74±2.71	3.12~7.87	3.98±2.25	2.57~6.57
testis	1.17±0.38	0.78~1.53	1.01±0.36	0.64~1.36
skeletal muscle	0.86±0.39	0.56~1.63	0.66±0.30	0.46~1.24
adipose tissue	0.39±0.10	0.24~0.51	0.35±0.10	0.23~0.48
skin	0.53±0.16	0.33~0.81	0.47±0.14	0.30~0.73
bone marrow	0.73±0.13	0.59~0.94	0.64±0.10	0.52~0.79

Dynamic total-body ⁶⁸Ga-DOTATATE PET

To determine the dynamic process of DOTATATE uptake in each organ, the total-body PET/CT dynamic images were acquired with uEXPLORER (Figure 2A). And the dynamic uptake curve of multiple organs of the whole body was drawn. The process of dynamic uptake curve is different among organs. In some organs, the uptake of DOTATATE increases gradually, such as pituitary gland, adrenal gland, spleen (Figure 2A). Some organs, such as the thyroid and pancreas, have an early peak of DOTATATE uptake followed by a gradual decrease in concentration (Figure 2B). In some other organs, DOTATATE uptake fluctuates greatly, and the uptake of DOTATATE is not obvious at the early stage. After a period of rapid uptake, radioactivity reaches the platform. Such organs include stomach, duodenum and small intestine (Figure 2C).

Somatostatin receptor SSTR2 expression in human organs

⁶⁸Ga-DOTATATE is known to be a radiopharmaceutical acting through specific binding to somatostatin receptor 2 (SSTR2). To explore the reasons for the difference in dynamic uptake of DOTATATE between organs, we analyzed the distribution of SSTR2 expression in human organs with datasets from the Human Protein Atlas [14]. The mRNA expression of SSTR2 in various organs of the human body is showed in figure 3A. SSTR2 expression was found in most tissues of the nervous system, especially cerebral cortex and cerebellum. In endocrine organs, adrenal gland and pituitary gland had a high expression level. In addition, high levels of SSTR2 expression are also found in kidney, Spleen and Stomach. Immunohistochemistry also confirmed that the distribution of SSTR2 protein in organs was basically the same as that of RNA.

In silico screening of potential DOTATATE binding proteins

Although DOTATATE is an imaging agent targeting SSTR2, the distribution of DOTATATE in human organs was not completely consistent with the expression of SSTR2. Therefore, there may be other reasons for the concentration of DOTATATE in some organs. It is not clear whether other genes in the

human body can bind to DOTATATE. By screening BindingDB, SEA and SwissTargetPrediction databases, and 134 potential binding proteins of DOTATATE were obtained after merging and removing the repeated items (figure 4A).

To find the genes related to DOTATATE uptake, we used WGCNA to analyze the genes most closely related to DOTATATE uptake in human organs. After reading and preprocessing data with R software, a total of 19670 expression profile genes were obtained. In this study, 6542 genes encoding membrane proteins were screened as research objects. Then, with $SD > 1.2$ as the screening criteria, 5755 genes were obtained. When the soft threshold β value is set at 5, the connections between genes are distributed in a scale-free network (Figure 4B). A total of 36 modules were obtained by WGCNA analysis (Figure 4C and 4D). With $GS > 0.25$ and $P_{GS} < 0.05$ as the limiting criteria, a total of 800 genes were screened. The targets obtained from the above drug database were intermingled with DOTATATE uptake related genes, and finally eight potential DOTATATE targets were obtained, respectively AVPR1A, EPHA8, EPHB4, OPRL1, SSTR2, SSTR5, ST3GAL1 and NPY1R (Figure 4E and Table 2).

Next, the protein-protein interaction (PPI) network of these proteins was established with STRING. The PPI network showed that OPRL1 and NPY1R had close relationship with SSTR2 and SSTR5. Whereas AVPR1A, EPHA8, EPHB4 and ST3GAL1 did not interact with SSTR2 or SSTR5 (Figure 4F).

Table 2.

Target	Description	GS	p.GS	Module	Target Class
AVPR1A	Vasopressin V1a receptor	0.253755	0.04846	brown	G protein-coupled receptor
EPHA8	Ephrin type-A receptor 8	0.665851	4.76E-09	turquoise	Kinase
EPHB4	Ephrin type-B receptor 4	0.45657	0.000217	darkgreen	Kinase
OPRL1	Nociceptin receptor	0.334693	0.008377	turquoise	G protein-coupled receptor
SSTR2	Somatostatin receptor 2	0.778737	1.49E-13	turquoise	G protein-coupled receptor
SSTR5	Somatostatin receptor 5	0.285126	0.025928	darkolivegreen	G protein-coupled receptor
ST3GAL1	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1	0.26774	0.036969	tan	Transferase
NPY1R	Neuropeptide Y receptor type 1	0.672818	2.86E-09	darkgreen	G protein-coupled receptor

Discussion

The uptake and distribution of DOTATATE in human organs have been reported in the past[15]. On this basis, this study further explored and more comprehensively described DOTATATE uptake in various organs of the human body. In this study, the uEXPLORER panoramic dynamic PET/CT scanner was used to monitor the dynamic uptake of DOTATATE in all organs of the whole body. By comparing the characteristics of the temporal activity curve of each organ, we found that the dynamic changes of DOTATATE uptake in human organs showed three patterns: one was that the uptake gradually increased and reached a plateau, such as the adrenal gland, pituitary gland and spleen. The uptake of these organs may be due to the presence of DOTATATE target protein expression. The second type of DOTATATE concentration decreases after early rapid uptake, such as in kidney, pancreas and thyroid. The early cause of DOTATATE concentration may be related to blood transport. The third type has a large fluctuation range, which fluctuates from high to low, such as the stomach, duodenum and small intestine. These organs are all cavity organs, and peristalsis leads to the fluctuation of radioactive count.

At present, DOTATATE is known to be an imaging agent targeting somatostatin receptors, among which the binding force with SSTR2 is the strongest[16]. By analyzing the expression distribution of SSTR2 in human organs. We found that the expression of SSTR2 in various organs of the human body was not completely consistent with the uptake degree of DOTATATE. Despite high uptake of DOTATATE in some

organs, the expression degree of SSTR2 was not high, such as liver. This suggests that there may be other reasons for DOTATATE uptake in these organs. However, it is not fully described in previous studies whether DOTATATE has binding targets other than somatostatin receptors.

Weighted correlation Network Analysis (WGCNA) is a systems biology method used to describe gene association patterns among different samples[17]. It can be used to identify highly covaried gene sets and to identify candidate biomarker genes or therapeutic targets based on the interconnectivity of the gene set and the association between the gene set and phenotype[18]. By using WGCNA, we identified the gene set correlated with DOTATATE uptake in human organs, and analyzed the co-expression relationship between these genes. A total of 800 genes correlated with DOTATATE uptake were screened.

In addition, we also predicted and analyzed the proteins that DOTATATE might bind to, by analyzing the molecular structure of DOTATATE. Using BindingDB, SEA and SwissTargetPrediction databases, we obtained 728 potential DOTATATE binding proteins. Among them, 8 genes were found to be positively correlated with DOTATATE uptake by WGCNA, respectively are AVPR1A, EPHA8, EPHB4, OPRL1, SSTR2, SSTR5, ST3GAL1 and NPY1R.

SSTR2 and SSTR5 are known DOTATATE targets. EPH Receptor A8 (EPHA8) encodes a member of the ephrin receptor subfamily of the protein-tyrosine kinase family[19]. EPH and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system[20]. Opioid Related Nociceptin Receptor 1 (OPRL1) is a member of the 7 transmembrane-spanning G protein-coupled receptor family, and functions as a receptor for the endogenous, opioid-related neuropeptide, nociceptin/orphanin FQ[21]. Neuropeptide Y Receptor Y1 (NPY1R) belongs to the G-protein-coupled receptor superfamily, and mediates the function of neuropeptide Y, a neurotransmitter, and peptide YY, a gastrointestinal hormone[22]. WGCNA indicates that EPHA8, OPRL1 and SSTR2 all belong to Turquoise module, indicating that there is a co-expression relationship between them. And the PPI network shows that OPRL1 and NPY1R had close relationship with SSTR2 and SSTR5, which may be the reason why they are related to DOTATATE uptake.

Arginine vasopressin receptor 1A (AVPR1A) acts as receptor for arginine vasopressin, which belongs to the subfamily of G-protein coupled receptors[23]. EPH Receptor B4 (EPHB4) mediate numerous developmental processes, particularly in the nervous system[24]. ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 1 (ST3GAL1) is a type II membrane protein that catalyzes the transfer of sialic acid from CMP-sialic acid to galactose-containing substrates[25]. These proteins are structurally possible to bind to DOTATATE, and their distribution in human organs is positively correlated with DOTATATE, and they do not have a co-expression relationship with SSTRs, so there is a greater probability that they may be potential binding targets of DOTATATE.

However, there are still some shortcomings in this paper. Firstly, due to the lack of an appropriate mathematical model, the dynamic changes of DOTATATE uptake could not be further quantitatively analyzed. Second, the genes related to DOTATATE uptake obtained by WGCNA analysis, except for

SSTR2, need further experimental verification. Thirdly, whether WGCNA's method of finding and targeting is suitable for other imaging agents needs further study.

Declarations

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Conflicts of interest/Competing interests

The authors have declared that no competing interest exists.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

Not applicable

Ethics approval (include appropriate approvals or waivers)

All procedures performed in studies involving human participants were approved by the Institutional Review Board of Shanghai Jiao Tong University-affiliated Ren Ji Hospital and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This article does not describe any studies with animals performed by any of the authors.

Consent to participate

Not applicable

Consent for publication

Not applicable

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Figures

Figure 1

Maximum intensity projection (MIP) image showing the distribution of ^{68}Ga -DOTATATE. The important organs are highlighted with numbered arrows: 1, pituitary gland; 2, salivary gland; 3, thyroid; 4, spleen; 5, liver; 6, adrenal; 7, kidney; 8, bladder.

Figure 2

Part of dynamic images and time-activity curve (TAC) of ^{68}Ga -DOTATATE uptake. A. Dynamic images of ^{68}Ga -DOTATATE total-body PET/CT; B. TAC of pituitary gland, adrenal gland and spleen; C. TAC of thyroid, kidney and pancreas; D. TAC of stomach, duodenum and small intestine.

Figure 3

SSTR2 expression in human organs. A. The mRNA levels of SSTR2; B. Immunohistochemistry of SSTR2 in indicated organs.

Figure 4

In silico screening of potential DOTATATE binding proteins. A, Potential binding proteins of DOTATATE screened by BindingDB, SEA and SwissTargetPrediction databases; B, Soft threshold selection in the WGCNA network analysis. B, Gene distribution in the WGCNA network analysis; D, Relationships between modules and SUVmax; E, The intersection of WGCNA analysis results and data from the drug databases. F, PPI network of the potential DOTATATE binding proteins.