Neuropeptide Y receptors: a promising target for cancer imaging and therapy

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Juan Li*, Yuchen Tian and Aiguo Wu*

Key Laboratory of Magnetic Materials and Devices & Division of Functional Materials and Nano Devices, Ningbo Institute of Materials Technology & Engineering, Chinese Academy of Sciences, Ningbo 315201, China

*Correspondence address. Key Laboratory of Magnetic Materials and Devices & Division of Functional Materials and Nano Devices, Ningbo Institute of Materials Technology & Engineering, Chinese Academy of Sciences, Ningbo 315201, China. Tel: +86-574-86685039; Fax: +86-574-86685163; E-mail: lij@nimte.ac.cn and aiguo@nimte.ac.cn

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Abstract

Neuropeptide Y (NPY) was first identified from porcine brain in 1982, and plays its biological functions in humans through NPY receptors (Y1, Y2, Y4 and Y5). NPY receptors are known to mediate various physiological functions and involve in a majority of human diseases, such as obesity, hypertension, epilepsy and metabolic disorders. Recently, NPY receptors have been found to be over-expressed in many cancers, so they emerged as promising target in cancer diagnosis and therapy. This review focuses on the latest research about NPY and NPY receptors, and summarizes the current knowledge on NPY receptors expression in cancers, selective ligands for NPY receptors and their application in cancer imaging and therapy.

Keywords: neuropeptide Y receptors; cancers; imaging; therapy

Introduction

Neuropeptide Y (NPY) is a 36 amino acids peptide, which was first identified from porcine brain in 1982 [1]. It belongs to the family of pancreatic polypeptides (PPs), and shows a high sequence similarity to PP and peptide YY (PYY) [2]. NPY is widely distributed in the central nervous system and periphery, involving in a variety of essential physiological key functions, such as feeding, memory, anxiety and energy homeostasis [3, 4]. In human, these functions are mediated by different NPY or the so called Y receptor subtypes: Y1, Y2, Y4, Y5 and Y6, which all belong to the family of G-protein coupled receptors (GPCR). The activation of GPCR leads to the inhibition of adenylyl cyclase and cyclic adenosine monophosphate accumulation, and the modulation of Ca2+ and K+ channels [5, 6].

In the past decades, plenty of basic research has been done to explore the expression and related function of NPY receptors in human (Table 1). Recently, NPY receptors have attracted great attention because of its over-expression in a variety of human cancers, such as breast carcinomas and neuroblastomas [7]. This makes the NPY receptors truly attractive as promising target for cancer imaging and therapy [8]. This review summarizes the current knowledge on the expression incidence and density of NPY receptors in various cancers, the selective ligands for different NPY receptors subtype and their application in cancer diagnosis and treatment.

NPY Receptors Expression in Human Cancers

Recently, more and more evidence suggests that NPY and NPY receptors play an important role in human cancers. The Reubi group has made lots of efforts on the identification of NPY receptors expression in various human tumors by using in vitro receptor autoradiography [7]. It has been found that the two best-investigated Y1 and Y2 receptors were over-expressed in breast, adrenal gland, renal cell, ovarian and brain tumors (Table 2). The in vitro receptor autoradiography study indicated that Y1 receptor was over-expressed in more than 85% primary human breast tumors and 100% of breast cancer derived metastases, whereas normal human breast expressed Y2 receptor preferentially. Interestingly,
neoplastic transformation could switch the NPY receptors expression from Y2 to Y1 subtype [29]. In this case, breast cancer has attracted lots of attention, because of its extremely high expression incidence and density compared with all other NPY receptors positive tumors. Moreover, ovarian malignancies [30], adrenal cortical tumors [31], glioblastomas [32] and nephroblastomas [33] also showed higher Y1 or Y2 receptor incidence and moderate density. In addition, Y1 receptor has also been found in prostate cancer cell lines [34], but was not detected in non-small cell lung, prostate and colorectal cancers.

Besides aforementioned tumors, tumor-associated blood vessels also express Y1 receptor. For instance, Y1 receptor has been found in the blood vessels of renal cell and adrenal cortical tumors, but rare in ovarian tumors [30, 31, 33]. Surprisingly, Y1 receptor has also been identified in the blood vessel of tumors which do not express Y receptor on tumor cells, such as renal cell and gastrointestinal stromal tumors [35]. Hence, the tumoral blood supply might be mediated by the activation of NPY receptors through the endogenous NPY released from intratumoral nerve fibers or tumor cells themselves.

Additionally, it was reported that NPY or its analogs could induce dose-dependent inhibition in the growth of Y1-expressing human SK-N-MC and MCF-7 tumor cells [29, 36], but promote the growth of PC3 tumor cells [34], suggesting that NPY plays its function in cancers via NPY receptors.

### Selective ligands for NPY receptors targeting

As it has been previously described, the expression of NPY receptors subtype varies according to the tumors, which makes the design of subtype selective ligands truly attractive for specific tumor targeting. The Beck-Sickinger group has made many efforts to get the subtype selectivity of NPY receptors [40]. They have presented the first Y1 receptor-preference NPY analogs and investigated their agonistic properties in 2001 [41]. It was indicated that [Phe7, Pro34]pNPY showed the highest Y1 receptor selectivity (\(>1:3000\)-fold) comparing to the Y2 and Y5 subtype (Ki \(\approx 30\) nm), and [Arg6, Pro34]pNPY also showed significant Y1 receptor preference (1:400 \(\approx 1:2000\)) with the variations of position 6 and positions 20–23 of NPY. In contrast, it was found that cyclo S \(\approx S\) [Cys20, Cys24]pNPY was with high Y2 receptor selectivity. However, the variations of positions 31 and 32 of the analogs dramatically reduced its affinity to the Y1 receptor, but with less effect on the affinity of Y2 receptor. This study provided us more information about the ligand–receptor interaction between NPY and Y1, Y2 and Y5 receptors, and contributed to our deep understanding of subtype selectivity. Moreover, Langer et al. and Zwanziger et al. from Beck-Sickinger group have labeled NPY analogs with different radiometal/chelating agent combinations such as \(^{99m}\text{Tc}/2\)-picolylamine-N, N-diabetic acid [42] or \(^{111}\text{In}/\text{DOTA}\) [43] to obtain stable compounds with selective Y1 and Y2 receptor binding. In the end, a Y1 receptor selective ligand [Lys(\(^{111}\text{In}/\text{DOTA})^4, \text{Phe}^7, \text{Pro}^{34}]\text{NPY}\) showed high affinity to Y1 receptor, might be used for breast tumor diagnosis in the future [43].

Recently, smaller peptides have attracted increasing attention, not only because of their higher labeling efficient but also due to the cost of their synthesis is much cheaper than the large peptides, such as NPY and its analogs containing 36 amino acids. Therefore, it is necessary to develop smaller ligands for efficient tumor labeling. Zwanziger et al. [44] designed some small peptides containing nine amino acids of the NPY C-terminus and modified them properly. Three NPY analogs, [Pro30, Nle31, Bpa32, Leu34]NPY(28–36), [Pro30, Nal31, Leu34]NPY(28–36) and [Pro30, Nal31, Leu34]NPY(28–36) were identified with strong Y1 receptor affinity and selectivity. One of the most promising peptide, [Pro30, Nle31, Bpa32, Leu34]NPY(28–36), was found to be with high affinity and

### Table 1. Characterization, expression and function of NPY receptors in human

| Receptor | Amino acids | Native ligand | Expression | Function |
|----------|-------------|---------------|------------|----------|
| Y1       | 384         | NPY, PYY      | Periphery, hypothalamus, hippocampus, neocortex, thalamus | Vasocostriction, anxiolysis, food intake, heart rate, anxiety |
| Y2       | 381         | NPY, PYY      | Brain, hippocampus, thalamus, hypothalamus | Memory, circadian rhythm, angiogenesis, epilepsy, secretion, bone formation |
| Y4       | 375         | PP            | Brain, gastrointestinal tract, pancreas, prostate | Feeding, circadian ingestion, energy homeostasis, colonic transit |
| Y5       | 445, 455    | NPY, PYY      | Hypothalamus, hippocampus | Food intake, epilepsy, circadian rhythm |

### Table 2. NPY receptor expression in human cancers

| Tumor type                  | Receptor expression in tumors                                                                 | Receptor expression in non-neoplastic tissues of origin |
|-----------------------------|---------------------------------------------------------------------------------------------|--------------------------------------------------------|
|                             | Subtype | Incidence (%) | Density | Subtype | Density |
| Breast carcinomas           | Y1      | 79/89 (85)    | High    | Y2      | High    | [29] |
| Ovarian sex cord–stromal tumors | Y1 + Y2 | 10/10 (100)  | Moderate | Y1 + Y2 | Moderate | [30] |
| Adrenal cortical tumors     | Y1      | 14/15 (93)    | High    | Y1      | High    | [31, 37] |
| Glioblastomas               | Y2      | 25/30 (83)    | High    | Y1 + Y2 | High    | [32] |
| Nephroblastomas             | Y1 + Y2 | 8/10 (80)     | Moderate | Y1      | Moderate | [33] |
| Renal cell carcinomas       | Y1      | 14/24 (56)    | Moderate | Y1      | Moderate | [33] |
| Ewing sarcoma tumors        | Y1      | 16/19 (84)    | High    | Not available | Not available | [38] |
| Gastrointestinal stromal tumors | Y1 + Y2 | 5/19 (26)    | High    | Not available | Not available | [35] |
| Testicular tumors           | Y1 + Y2 | 8/24 (33)     | Low     | Y1 + Y2 | High    | [39] |
strong agonistic activity to Y1 receptor, providing a possibility to target the Y1-expressing tumors specifically. Additionally, Leban et al. [45] has described a modified C-terminal decapeptide [Tyr32, Leu34]NPY(27-36), which showed promising antagonistic activity at both Y1 and Y2 receptors. It is well known that BVD15 ([Pro30, Tyr32, Leu34]NPY(28-36)) was a competitive antagonist at human Y1 receptor, but with similar affinity to Y2 receptors [45-47]. More recently, peptides based on this sequence have been described with Y1 selectivity and agonist activity at Y1 receptor transfected HEK293 cells. Guerin et al. [48] reported that a BVD15 analog [Lys6]BVD15 with a single amino acid substitution exhibited greater Y1 receptor affinity (Ki = 7 nM) than BVD15 (Ki = 39 nM). After conjugation with a DOTA chelator group, the produced [Lys(DOTA)4]BVD15 was found to have optimal Y1 affinity over any other DOTA-substituted analogs. Based on the previously reported BVD15, Liu et al. [49] has investigated the synthesis of 15 truncated NPY analogs as models for Y1 receptor-specific radio pharmaceuticals, using competition radioreceptor binding assays from brain tissue homogenates of Y1-Y4-double knockout mice. It was found that lyseine is capable of tolerating various modifications, and the substitution of lysine of the analogs had no effect on its Y1 receptor affinity, while modifications at the N-terminal isoleucine resulted in dramatic reduction of Y1 affinity.

In addition to the peptidic NPY analogs, some non-peptide Y1 receptor ligands have also been developed, such as BIBP3226, BIBO3304 and LY37879. Rudolf et al. [50] designed and synthesized the first non-peptidic Y1 receptor ligand BIBP3226, which showed high affinity to Y1 receptor (Ki = 7 nM). Further literature indicated that BIBO3304 was synthesized with 10-fold higher affinity to Y1 receptor than BIBP3226 [51]. However, most of them are receptor antagonists, which show high affinity but are not able to activate the receptor, because Y1 receptor internalization is only induced by agonists. Due to receptor internalization is important for the improvement of the tumor to back ground ratio in imaging, and necessary for receptor-mediated targeted drug delivery to tumor cells, the agonists are required for either cancer imaging or therapy. Until now, it was reported that Y1 [52-56], Y2 [57], Y4 [58] and Y5 [59] receptors were able to internalize upon agonist exposure, and the agonist-induced receptor internalization of NPY receptor subtypes critically depends on third intracellular loop and C-terminus [53].

**Application in cancer imaging and therapy**

Due to the high expression of NPY receptors in various cancers, their selective ligands could be used as potential targeted molecules for specific cancer imaging and therapy. Until now, most of the studies have mainly focused on the scintimammography and positron emission tomography (PET) imaging for cancers (Table 3). For instance, Khan et al. [60] from Beck-Sickinger group has designed and synthesized two NPY-derived Y1 receptor ligands conjugated with 99mTc in the position of the chelator for tumor labeling. The in vitro and in vivo uptake of developed ligands has also been investigated in breast cancer patients. The results indicated that the over-expressed Y1 receptor in human breast cancer provided a chance for tumor targeting by using selective Y1 receptor ligand, and could be further extended to a tumor-specific targeted therapy by using NPY analogs as a carrier for chemotherapeutics. Hofmann et al. [61] has reported a suitable 18F-labeled high-molecular-weight glycopeptide for imaging of peripheral Y1 receptor positive tumors by preclinical small-animal PET. The corresponding fluoroglycosylated (FGlc) peptide analog [Pra4(FGlc), F7, P34] NPY and its 18F-labeled analog were synthesized by click chemistry-based fluoroglycosylation. It was found that the 18F-labeled NPY glycopeptide was with higher activity and more excellent subtype selectivity to Y1 receptor than Y2 receptor. In vivo PET imaging experiments revealed that 18F-labeled NPY glycopeptide could be partially uptake by Y1 receptor-expressing MCF-7 tumors, while the kidney uptake of DOTA-derivatives of this peptide was decreased. This study suggested that the 18F-labeled NPY glycopeptide might be helpful for the design of small radiopeptides, facilitating the PET imaging for breast cancer.

Guerin et al. [48] substituted a truncated NPY analog BVD15 at various positions with DOTA and evaluated the effect of the coupling position with the binding affinity for Y1 receptor. The synthesized [Lys(DOTA)4]BVD15 was considered as a potent NPY analog, and might be suitable for PET imaging of breast cancer. This work presented the first example of the shortest linear peptide prepared for Y1 receptor targeting. However, there was no in vitro cellular or in vivo experiment to further prove the results. In the future, the PET property of the labeled [Lys(DOTA)4]BVD15 with different metal radioisotopes needs to be evaluated. Moreover, Shrivastava et al. [62] has developed a novel BVD15 analog t-BBN/BVD15-D03A, which was able to target both the GRP-expressing T-47D cells and Y1 receptor-expressing MCF-7 cells. In the next step, t-BBN/BVD15-D03A will be labeled with 68Ga for imaging and 177Lu for radiotherapy, and the in vitro cell binding and in vivo tumor animal studies will also need to be conducted.

Although selective NPY receptor ligands have been widely applied in cancer imaging by scintimammography and PET, only few literatures reported about the NPY receptor-based drug design for cancer therapy. Li et al. from Wu group has developed an Y1 receptor-based nanoparticulate drug delivery system to deliver anticancer drug doxorubicin into the breast cancer cells, and evaluated its potential for breast cancer therapy. In this study, a high selective Y1 receptor ligand [Pro30, Nle31, Bpa32, Leu34]NPY(28-36) (PNBL-NPY) was used as a targeted molecule to conjugate with drug-loaded albumin nanoparticles. In vitro cellular uptake experiment showed that the PNBL-NPY could actively recognize and bind to the Y1 receptor that was significantly overexpressed on the breast cancer cells. Further cell viability test indicated that the PNBL-NPY modified drug-loaded nanoparticles were able to be transferred into the

**Table 3. Application of NPY analogs in cancer imaging and therapy**

| NPY receptor subtype | Cancer type | Imaging or therapy | NPY analogs | Species | Ref. |
|---------------------|------------|--------------------|-------------|---------|-----|
| Y1                  | Breast cancer | Scintimammography | [99mTc(CO)3]N"His-Ac-[Phe5, Pro34]-NPY | Human | [60] |
| Y1                  | Breast cancer | PET | [18F]-[Pra4(FGlc), F7, P34]NPY | Mice | [61] |
| Y1                  | Breast cancer | PET | [Lys(DOTA)4]BVD15 | – | [48] |
| Y1                  | Breast cancer | PET | t-BBN/BVD15-D03A | MCF-7 cells | [62] |
| Y1                  | Breast cancer | Chemotherapy | [Pro30, Nle31, Bpa32, Leu34]NPY(28-36) | MCF-7 cells | [36] |
| Y1                  | Breast cancer | Boron neutron capture therapy | [Phe7, Pro34]-NPY | HEK293-hY,R EYFP cells | [63] |
cancer cells through internalization, inducing a dose-dependent inhibition on the cell growth. Moreover, it was found that the developed drug delivery system was highly selective and able to distinguish the breast cancer cells from the normal breast cells, due to normal breast cells express Y1 receptor only. This study presented the first Y1 receptor-based nanoparticulate drug delivery system, providing a safer and more efficient approach for targeted breast cancer therapy. In addition, Ahrens et al. [63] from Beck-Sickinger group reported novel highly boron-loaded Y1 receptor preferring peptide [Phe2, Pro34]-NPY analogs as smart shuttle systems for carbaboranes as 10B-containing moieties. The results indicated that the developed multi-carbaborane peptide could be uptake by Y1 receptor expressing HEK293-hY1,REYTP cells, which exceed the required amount of 10B boron atoms per cell in boron neutron capture therapy. This system was considered to be a most efficient shuttle system to transport large amounts of boron into Y1 receptor-expressing cells, and could be further extended to other therapeutics such as cytotoxic compounds.

Conclusion and Perspectives

Since NPY was first identified from porcine brain in 1982, NPY receptors have been extensively studied to explore its expression and related functions in human. Surprisingly, the over-expression of NPY receptors has been found in human cancers corresponding to different Y subtype receptors. It makes this multireceptor/multigand system truly attractive for targeted cancer imaging and therapy. This is practically true for breast cancer, which is not only due to its highest incidence and density in breast tumors but also because of the different subtypes expression between breast tumors (Y1 receptor) and normal breast tissues (Y2 receptor). In previous report, most of the NPY receptor-based cancer imaging or therapy is mainly focused on the breast cancer, but it could also be exploited to many other tumors such as prostate, ovary, pancreas and brain tumors. Moreover, the co-expression of Y receptor in tumors and tumor-associated blood vessels makes it a promising target for dual-targeted cancer treatment, because the tumor-associated blood vessels as the main components of tumor microenvironment could provide oxygen and nutrition for the tumor growth [64].

As we know that, successful receptor-mediated tumor imaging and therapy asks not only suitable peptides to target the corresponding receptor but also requires receptor internalization to transfer the receptor bound drug into the tumor cells. For NPY receptors, the receptor subtypes internalization is induced only by agonists rather than antagonists, e.g. Y1, Y2, Y4 and Y5 receptors are able to internalize upon agonist exposure. Hence, developing more potent selective ligands (agonists) will be a focus of interest for specific tumor targeting in the future.

Although selective tumor targeted imaging in breast cancer patients has been achieved successfully by using NPY analogs that specifically binding Y1 subtype receptors, there is still a lack of therapeutics for clinical cancer therapy. Besides the reported Y receptor-based nanomedicine and radiopharmaceuticals, peptide-drug conjugate might be another choice to make Y receptor-based therapeutics as promising candidates for cancer therapy in clinic [65, 66].

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