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Review

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

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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 ($Y_1$, $Y_2$, $Y_4$ and $Y_5$). 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 overexpressed 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: $Y_1$, $Y_2$, $Y_4$, $Y_5$ and $y_6$, 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 $Ca^{2+}$ 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 $Y_1$ and $Y_2$ receptors were over-expressed in breast, adrenal gland, renal cell, ovarian and brain tumors (Table 2). The *in vitro* receptor autoradiography study indicated that $Y_1$ receptor was over-expressed in more than 85% primary human breast tumors and 100% of breast cancer derived metastases, whereas normal human breast expressed $Y_2$ receptor preferentially. Interestingly,

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

| Receptor | Amino acids | Native ligand | Expression | Function | Ref. |
|---|---|---|---|---|---|
| $Y_1$ | 384 | NPY, PYY | Periphery, hypothalamus, hippocampus, neocortex, thalamus | Vasoconstriction, anxiolysis, food intake, heart rate, anxiety | [6, 9–11] |
| $Y_2$ | 381 | NPY, PYY | Brain, hippocampus, thalamus, hypothalamus | Memory, circadian rhythm, angiogenesis, epilepsy, secretion, bone formation | [6, 12–17] |
| $Y_4$ | 375 | PP | Brain, gastrointestinal tract, pancreas, prostate | Feeding, circadian ingestion, energy homeostasis, colonic transit | [18–24] |
| $Y_5$ | 445, 455 | NPY, PYY | Hypothalamus, hippocampus | Food intake, epilepsy, circadian rhythm | [15, 25, 26] |
| $y_6$ | 371 | NPY, PYY | Not in human | – | [27, 28] |

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

| Tumor type | Receptor expression in tumors | | | Receptor expression in non-neoplastic tissues of origin | | Ref. |
|---|---|---|---|---|---|---|
| | Subtype | Incidence (%) | Density | Subtype | Density | |
| Breast carcinomas | $Y_1$ | 79/89 (85) | High | $Y_2$ | High | [29] |
| Ovarian sex cord–stromal tumors | $Y_1+Y_2$ | 10/10 (100) | Moderate | $Y_1+Y_2$ | Moderate | [30] |
| Adrenal cortical tumors | $Y_1$ | 14/15 (93) | High | $Y_1$ | High | [31, 37] |
| Glioblastomas | $Y_2$ | 25/30 (83) | High | $Y_1+Y_2$ | High | [32] |
| Nephroblastomas | $Y_1+Y_2$ | 8/10 (80) | Moderate | $Y_1$ | Moderate | [33] |
| Renal cell carcinomas | $Y_1$ | 14/24 (56) | Moderate | $Y_1$ | Moderate | [33] |
| Ewing sarcoma tumors | $Y_1$ | 16/19(84) | High | Not available | Not available | [38] |
| Gastrointestinal stromal tumors | $Y_1+Y_2$ | 5/19 (26) | High | Not available | Not available | [35] |
| Testicular tumors | $Y_1+Y_2$ | 8/24 (33) | Low | $Y_1+Y_2$ | High | [39] |

neoplastic transformation could switch the NPY receptors expression from $Y_2$ to $Y_1$ 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 $Y_1$ or $Y_2$ receptor incidence and moderate density. In addition, $Y_1$ 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 $Y_1$ receptor. For instance, $Y_1$ receptor has been found in the blood vessels of renal cell and adrenal cortical tumors, but rare in ovarian tumors [30, 31, 33]. Surprisingly, $Y_1$ 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 $Y_1$-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 $Y_1$ receptor-preference NPY analogs and investigated their agonistic properties in 2001 [41]. It was indicated that [Phe$^7$, Pro$^{34}$]pNPY showed the highest $Y_1$ receptor selectivity (>1:3000-fold) comparing to the $Y_2$ and $Y_5$ subtype ($K_i \approx 30$ nm), and [Arg$^6$, Pro$^{34}$]pNPY also showed significant $Y_1$ receptor preference (1:400 ± 1:2000) with the variations of position 6 and positions 20–23 of NPY. In contrast, it was found that cyclo S ± S [Cys$^{20}$, Cys$^{24}$]pNPY was with high $Y_2$ receptor selectivity. However, the variations of positions 31 and 32 of the analogs dramatically reduced its affinity to the $Y_1$ receptor, but with less effect on the affinity of $Y_5$ receptor. This study provided us more information about the ligand–receptor interaction between NPY and $Y_1$, $Y_2$ and $Y_5$ 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 $^{99m}$Tc/2-picolylamine-*N*, *N*-diacetic acid [42] or $^{111}$In/DOTA [43] to obtain stable compounds with selective $Y_1$ and $Y_2$ receptor binding. In the end, a $Y_1$ receptor selective ligand [Lys($^{111}$In/DOTA)$^4$, Phe$^7$, Pro$^{34}$]NPY showed high affinity to $Y_1$ 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, [Pro$^{30}$, Nle$^{31}$, Bpa$^{32}$, Leu$^{34}$]NPY(28–36), [Pro$^{30}$, Nal$^{32}$, Leu$^{34}$]NPY(28–36) and [Pro$^{30}$, Nle$^{31}$, Nal$^{32}$, Leu$^{34}$]NPY(28–36) were identified with strong $Y_1$ receptor affinity and selectivity. One of the most promising peptide, [Pro$^{30}$, Nle$^{31}$, Bpa$^{32}$, Leu$^{34}$]NPY(28–36), was found to be with high affinity and

strong agonistic activity to $Y_1$ receptor, providing a possibility to target the $Y_1$-expressing tumors specifically. Additionally, Leban *et al.* [45] has described a modified C-terminal decapeptide [Tyr$^{32}$, Leu$^{34}$]NPY(27–36), which showed promising antagonistic activity at both $Y_1$ and $Y_2$ receptors. It is well known that BVD15 ([Pro$^{30}$, Tyr$^{32}$, Leu$^{34}$]NPY(28–36)) was a competitive antagonist at human $Y_1$ receptor, but with similar affinity to $Y_4$ receptors [45–47]. More recently, peptides based on this sequence have been described with $Y_1$ selectivity and agonist activity at $Y_1$ receptor transfected HEK293 cells. Guerin *et al.* [48] reported that a BVD15 analog [Lys$^4$]BVD15 with a single amino acid substitution exhibited greater $Y_1$ receptor affinity ($K_i \approx 7$ nM) than BVD15 ($K_i \approx 39$ nM). After conjugation with a DOTA chelator group, the produced [Lys(DOTA)$^4$]BVD15 was found to have optimal $Y_1$ 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 $Y_1$ receptor-specific radiopharmaceuticals, using competition radioreceptor binding assays from brain tissue homogenates of $Y_2Y_4$-double knockout mice. It was found that lysine$^4$ is capable of tolerating various modifications, and the substitution of lysine$^4$ of the analogs had no effect on its $Y_1$ receptor affinity, while modifications at the N-terminal isoleucine resulted in dramatic reduction of $Y_1$ affinity.

In addition to the peptidic NPY analogs, some non-peptide $Y_1$ receptor ligands have also been developed, such as BIBP3226, BIBO3304 and LY35789. Rudolf *et al.* [50] designed and synthesized the first non-peptidic $Y_1$ receptor ligand BIBP3226, which showed high affinity to $Y_1$ receptor ($K_i = 7$ nM). Further literature indicated that BIBO3304 was with 10-fold higher affinity to $Y_1$ receptor than BIBP3226 [51]. However, most of them are receptor antagonists, which show high affinity but are not able to activate the receptor, because $Y_1$ 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 $Y_1$ [52–56], $Y_2$ [57], $Y_4$ [58] and $Y_5$ [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 $Y_1$ receptor ligands conjugated with $^{99m}$Tc 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 $Y_1$ receptor in human breast cancer provided a chance for tumor targeting by using selective $Y_1$ 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 $^{18}$F-labeled high-molecular-weight glycopeptide for imaging of peripheral $Y_1$ receptor positive tumors by preclinical small-animal PET. The corresponding fluoroglycosylated (FGlc) peptide analog [Pra$^4$ (FGlc), F$^7$, P$^{34}$] NPY and its $^{18}$F-labeled analog were synthesized by click chemistry-based fluoroglycosylation. It was found that the $^{18}$F-labeled NPY glycopeptide was with higher activity and more excellent subtype selectivity to $Y_1$ receptor than $Y_2$ receptor. *In vivo* PET imaging experiments revealed that $^{18}$F-labeled NPY glycopeptide could be partially uptake by $Y_1$ receptor-expressing MCF-7 tumors, while the kidney uptake of DOTA-derivatives of this peptide was decreased. This study suggested that the $^{18}$F-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 $Y_1$ 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 $Y_1$ 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-DO3A, which was able to target both the GRP-expressing T-47D cells and $Y_1$ receptor-expressing MCF-7 cells. In the next step, t-BBN/BVD15-DO3A will be labeled with $^{68}$Ga for imaging and $^{177}$Lu 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 $Y_1$ 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 $Y_1$ receptor ligand [Pro$^{30}$, Nle$^{31}$, Bpa$^{32}$, Leu$^{34}$]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 $Y_1$ 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. |
|---|---|---|---|---|---|
| $Y_1$ | Breast cancer | Scintimammography | $^{99m}$Tc(CO)$^3$-N$^{\alpha}$His-Ac-[Phe$^7$, Pro$^{34}$]-NPY | Human | [60] |
| $Y_1$ | Breast cancer | PET | $^{18}$F-[Pra$^4$(FGlc), F$^7$, P$^{34}$]NPY | Mice | [61] |
| $Y_1$ | Breast cancer | PET | [Lys(DOTA)$^4$]BVD15 | – | [48] |
| $Y_1$ | Breast cancer | PET | t-BBN/BVD15-DO3A | MCF-7 cells | [62] |
| $Y_1$ | Breast cancer | Chemotherapy | [Pro$^{30}$, Nle$^{31}$, Bpa$^{32}$, Leu$^{34}$]NPY (28-36) | MCF-7 cells | [36] |
| $Y_1$ | Breast cancer | Boron neutron capture therapy | [Phe$^7$, Pro$^{34}$]-NPY | HEK293-h$Y_1$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 $Y_2$ receptors only. This study presented the first $Y_1$ 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 $Y_1$ receptor preferring peptide [$Phe^7$, $Pro^{34}$]-NPY analogs as smart shuttle systems for carbaboranes as $^{10}B$-containing moieties. The results indicated that the developed multi-carbaborane peptide could be uptake by $Y_1$ receptor expressing HEK293-h$Y_1$REYFP cells, which exceed the required amount of $10^9$ 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 $Y_1$ 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/multiligand 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 ($Y_1$ receptor) and normal breast tissues ($Y_2$ 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 explored 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. $Y_1$, $Y_2$, $Y_4$ and $Y_5$ 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 $Y_1$ 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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