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Review

Neuropeptide Y receptors 1 and 2 as molecular targets in prostate and breast cancer therapy

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ABSTRACT

Recent advances have revealed the overexpression of Neuropeptide Y (NPY) receptors in multiple cancers, positioning them as attractive molecular targets for cancer diagnostics and therapeutics. Despite this, a comprehensive roadmap for the rational development of anticancer agents targeting NPY receptors remains lacking. Therefore, we present the characteristics of NPY receptor subtypes, their abundance, and the correlation of their expression in different cancer types. It was found that NPY receptor subtypes 1 and 2 were extensively studied, especially in connection with breast and prostate cancer. Many tumors express NPYR, but only breast cancer tissue shows a significant difference in NPYR subtype expression levels between tumor and normal tissues, and, therefore, can represent a promising target. In the context of anticancer therapy, this review provides key findings from the use of wild-type and synthetic NPY analogs. We highlight the critical residues in the NPY sequence that play a critical role in interactions with receptors and provide the recent literature findings on NPY analogues as efficient and specific cancer-targeting agents. Potential solutions to improve NPY analogs' stability are provided, such as sequence modifications of linear peptides, peptide stapling, and conjugation for drug delivery systems. In general, NPY treatment can not be used efficiently as a single therapy but as a combinatorial therapy with anticancer drugs to improve the specificity of the treatment via high-affinity binding to the cancer cells and sensitizing them to chemotherapy.

1. Introduction

Disrupting pro-survival protein-protein interactions in cancer cells is emerging as an innovative strategy for the development of more efficient targeted therapeutics in precision medicine. G protein-coupled receptors (GPCRs) play a critical role in the development and progression of numerous cancers, making them the most abundant type of cell surface receptor implicated in these processes. Given their tissue distribution and since GPCRs' structures are characterized by a high number of druggable sites, these receptors comprise the largest class of pharmaceutical targets. Currently, over 30 % of FDA-approved drugs target GPCRs or their related [1]. Therefore, the development of anticancer drugs or sophisticated delivery systems that specifically target GPCRs holds great promise to improve such treatments.

In recent years, neuropeptide Y receptors (NPYR), which belong to the vast GPCR superfamily, have drawn the attention of researchers due to their potential use as molecular and druggable targets in cancer imaging and treatment. These transmembrane proteins play, in fact, a pivotal role in signal transduction across cell membranes, allowing communication between the extracellular environment and the intracellular machinery. Among their endogenous ligands/modulators, the neuropeptide Y (NPY), a 36 amino acid polypeptide, interacts with the 1 – 6 subtypes of NPYRs to regulate a variety of key physiological functions [2],[3], has also grown attention as a suitable template to develop peptide-based pharmacological therapies targeting NPYRs. These nervous system-located peptides, in fact, NPYs, not only play a pivotal role as growth factors in normal cells but are also responsible for the autocrine/paracrine regulation of tumor cell proliferation and migration. The primary structure of NPY consists of a chain of 36 amino acids, whose sequence is Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2 (Fig. 1). This sequence is similar to peptide YY (PYY) by 70 % and

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pancreatic polypeptide (PP) by 50 % [4]. PP, PYY, and NPY are each composed of 36 amino acids and, while structurally similar, they are distributed in distinct regions of the gastrointestinal tract and nervous system, exhibiting unique biological functions. In addition to their physiological roles, both PYY and PP are associated with tumorigenesis. For example, PYY has been shown to have an antitumor effect against pancreatic and breast cancers, while PP levels are indicative of the growth of neuroendocrine and pancreatic tumors [5].

Based on the key role of the NPYR subfamilies in the pathophysiology of several diseases (including cancer), the targeting of these receptors has been exploited for the development of selective imaging and diagnostic tools [6]. A comprehensive review paper about this topic has been recently published by Fonseca et al. [7], highlighting how new radiotracers, selected for the different NPY receptor subtypes, have been successfully applied in PET studies, leading to significant insights into the molecular mechanisms involving the modulation of NPYRs. Also, interesting studies have been made highlighting the potentialities of the NPY template for the development of shortened, truncated peptide analogs able to bind to the selected reporters for imaging purposes without triggering undesired receptor-mediated responses [7]. Moreover, applying a similar strategy, Park et al. explored the use of the endogenous modulator NPY to develop peptide-based drug delivery systems for the central nervous system, showcasing its potential as a carrier molecule for targeted drug delivery [8]. This approach exploits the high affinity of NPY (and related analogs) for specific NPYR subtypes to build tissue-selective drugs, which are of utmost importance in cancer therapy.

In light of all these considerations, we previously developed innovative theranostic systems for cancer therapy (tools that combine diagnosis and treatment) based on the use of gold nanocages decorated with NPY analogs to detect and target overexpressed NPYR subtypes in cancer cells [9].

However, the downstream events of the molecular targeting of NPYR using NPY analogs are still far from being rationalized when it comes to cancer, as the expression patterns of different NPYR subtypes can vary from normal and cancer tissues (see sections below). As an example, the inhibition of the NPYR subtype 1 (NPYR1) decreased proliferation and increased apoptosis of mouse CT26 colon cancer cells. In contrast, NPY treatment decreased the proliferation of LNCaP and DU145 prostate cancer cells but increased it in PC3 cells and [10] in the breast cancer cell line 4T1 [11].

Since a concise but comprehensive "literature guide" for the rational development of anticancer drug candidates targeting NPYRs is still missing, the aim of this review paper is to delineate the profile of NPYRs as druggable targets in cancer therapy and to critically summarize the most important findings about the use of wild-type and/or synthetic NPY analogs providing guidelines for future research. A particular emphasis will be dedicated to the potential role of NPYR as a target for the development of novel and efficient chemotherapies in which the NPYR-NPY system is exploited as a molecular target for sophisticated delivery systems to release specific cytotoxic cargos selectively to tumor tissues.

Accordingly, the first part of the paper will present the nature and role of NPYR subfamilies, followed by a review of their function, their connections to the diagnosis and/or treatment of specific cancers, and the description of the role and modulatory effects of their endogenous ligand, NPY.

Afterward, the narration will be shifted toward the development of synthetic NPY analogs (NPYs) as NPYR modulators with enhanced affinity and specificity for cancer cells, sparing healthy tissue. Importantly, since peptide-based biotechnological drugs are often affected by poor stability and pharmacokinetic profiles, efficient and convenient strategies for structural optimization will be presented and discussed in detail. Among them, peptide stapling, a technique that preserves the bioactive conformation of the peptide, potentially leading to high-affinity binders with enhanced cellular uptake and stability, will be one of the main focuses.

A review of the current state-of-the-art covering both inorganic and organic drug carriers decorated with NPYs to build highly selective, active-targeted therapeutics will then be furnished. Finally, following the identification of the most critical challenges related to the use of NPYs in cancer treatment, critical parameters for future development will also be suggested.

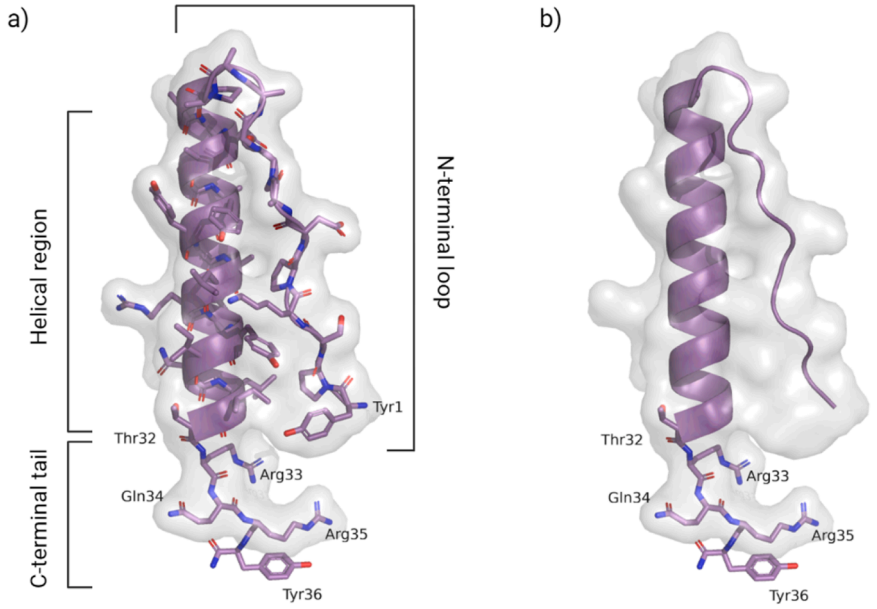

Fig. 1. The structure of NPY is shown (PDB: 7X9A). a) The complete structure of Neuropeptide Y (NPY) is shown, with all residues visible and key regions labeled for clarity. The N-terminal loop, spanning residues Thr1 to Arg19, is distinctly identified. This loop comes before the helical region, which includes residues Tyr20 to Ile31. The helical region is crucial for maintaining the overall shape of NPY. Following the helical region is the C-terminal tail, composed of residues Thr32 to Tyr36. b) The focus is on the C-terminal tail of NPY, which includes residues Thr32 to Tyr36. This segment is crucial for interaction with the NPYR1. In this image, the C-terminal residues are shown in detail, while the rest of the structure is presented in a simplified cartoon representation. Created with PyMOL (Schrödinger, LLC).

2. Subfamilies of NPY receptors and their roles

Within the GPCR superfamily, NPYRs fall under the class A (rhodopsin-like) family due to their shared structural and functional characteristics [3]. Historically, NPYRs were labeled as Y1, Y2, Y4, Y5, and Y6 based on their distinct pharmacological and binding properties. However, recent advancements in receptor nomenclature advocate for a standardized system. This system classifies them under the NPYR family, with individual subtypes denoted as NPYR1, NPYR2, and so on, with the NPYR6 receptor being active in rabbits, mice, and chickens [12]. NPYR subfamilies exhibit distinct tissue distribution patterns (Fig. 2), highlighting their unique contributions to diverse physiological processes. For example, whole-sequence PYY has an affinity for all human NPYRs, while PYY3–36 exhibits high specificity for NPYR2 [13]. On the other hand, PP exhibits a selective affinity for NPYR4 [13]. In this review, we will focus only on NPY activity towards NPYRs. The role and distribution of PYY and PP are reviewed elsewhere [14], [15].

Let's embark on a journey through the primary NPYR subfamilies, exploring their distribution and known functions in physiological conditions and normal tissues:

The NPYR1 receptor (Fig. 3a) is made up of 384 amino acids, and it reacts to NPY, peptide YY (PYY), and pancreatic polypeptides (PP) with different levels of affinity. NPYR1 has a high affinity for NPY, PYY, and similar peptides having a proline residue in their N-terminus. Thus, NPYR1 has a low affinity for analogs that are truncated at the N-terminus, as well as for PP. This subfamily is widely expressed throughout the central nervous system (CNS), including key areas like the hypothalamus, hippocampus, and cortex. Additionally, NPYR1 is found in peripheral tissues like the gut and blood vessels, highlighting its key role in various regulatory processes [16], [17]. This subfamily plays a crucial role in regulating appetite, anxiety, and cardiovascular function [12].

NPYR2 is made of 381 amino acids (Fig. 3b) with an affinity for neuropeptides in the following rank order: PYY > NPY > PP [13]. It is predominantly expressed in hippocampal neurons, in the thalamus, hypothalamus, and parts of the peripheral nervous system, with a significant presence in the gastrointestinal tract, urogenital system, and cardiovascular system [12], [16]. Activation of this receptor subfamily is associated with enhanced memory retention, regulation of the circadian rhythm, angiogenesis, and bone formation. It also regulates neurotransmitter release and influences memory, circadian rhythm, angiogenesis, and bone formation. Additionally, it is involved in regulating smooth muscle contractility, vasodilation, and inflammation and may contribute to wound healing processes [12].

The existence of a third neuropeptide Y receptor subtype, NPYR3, has been proposed based on pharmacological studies in various preparations, including cardiac myocytes, brainstem tissue, and bovine chromaffin cells [18]. NPYR3 was initially distinguished by its insensitivity to peptide YY, which contrasts with the known profiles of other NPY receptors [19]. Functional assays have suggested that NPYR3 may couple to G-proteins, as indicated by its ability to inhibit cAMP production, but no gene encoding a distinct NPYR3 receptor has been identified to date [19]. While six NPY receptor subtypes have been described in the literature, only five have been cloned in humans. NPYR3 remains uncloned, lacks selective ligands, and is not included among the functionally established members of the NPY receptor family [20].

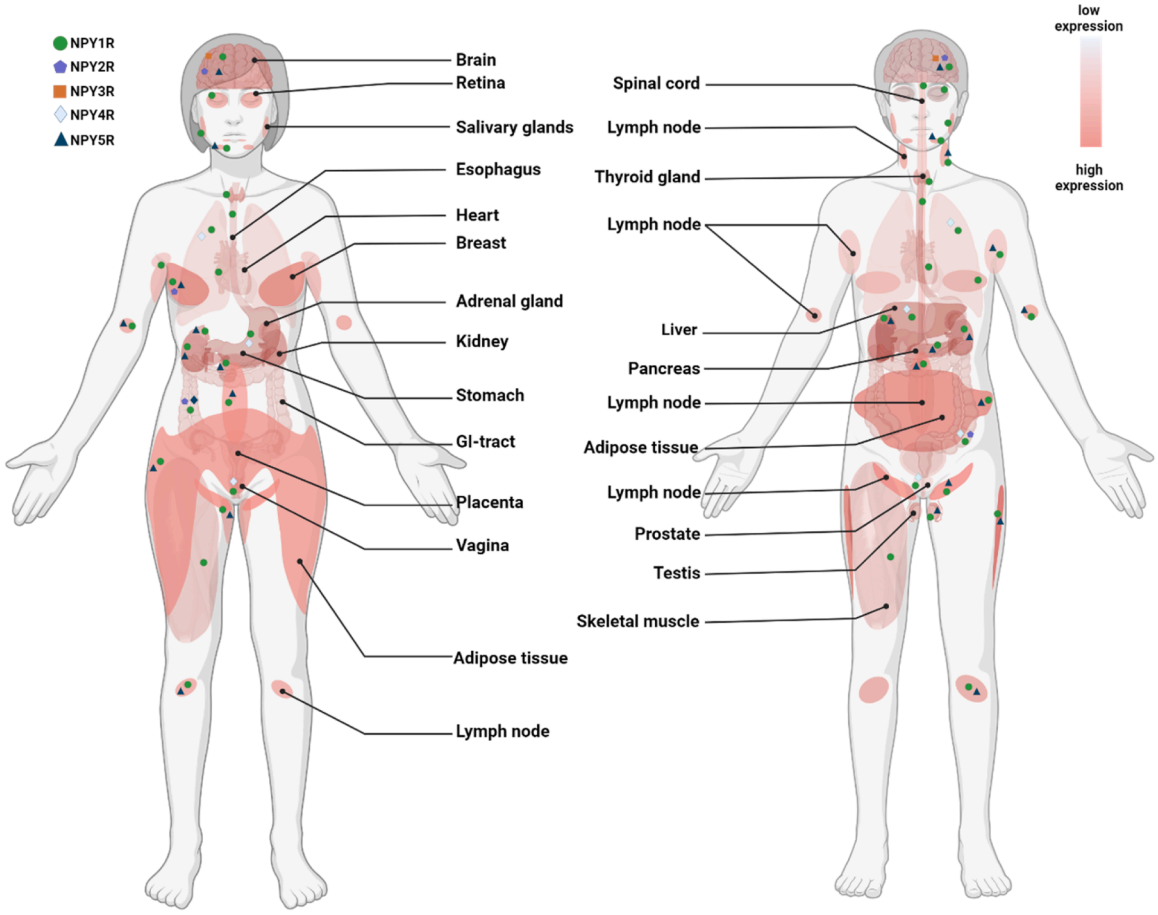

Fig. 2. This anatomogram illustrates the expression levels of NPY receptors in various human tissues. Each receptor subtype is marked with specific locations on the anatomogram and is represented by different symbols for easy identification. The color intensity on the diagram signifies the qualitative level of receptor expression within the tissue, with darker colors indicating higher expression levels and lighter colors indicating lower levels. Created with BioRender.com.

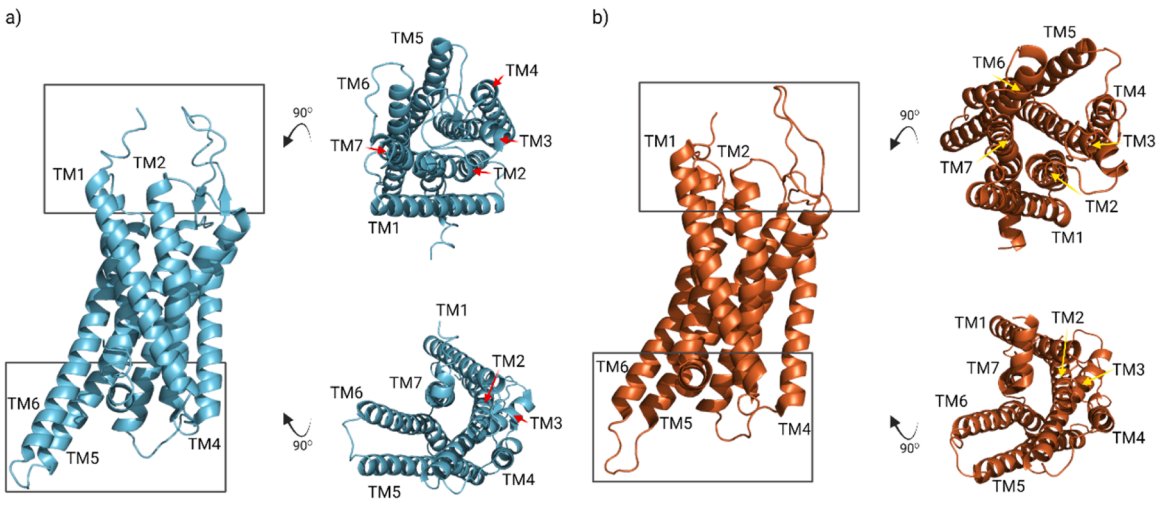

Fig. 3. The structural organization of the NPYR1 and NPYR2 is illustrated using PyMOL. a) The NPYR1, with views from the extracellular (top-down) and intracellular (bottom-up) perspectives. The transmembrane (TM) regions are explicitly labeled, providing a clear representation of the receptor's orientation and its potential interaction sites for ligand binding and G-protein coupling. b) The NPYR2, with equivalent views from both the extracellular and intracellular perspectives. The TM regions are also labeled here, allowing for a direct comparison between NPYR1 and NPYR2. Created with BioRender.com and PyMOL (Schrödinger, LLC).

The NPY4 is composed of 375 amino acids and responds to PP. While PYY and NPY can also activate this receptor, they do so with minor potency. It is primarily found in the gastrointestinal tract, brain, pancreas, and prostate. This receptor is associated with regulating feeding, circadian ingestion, energy balance, colonic transit, and luteinizing hormone release [12].

The NPYR5 has 445 amino acids and two splice variants. Both receptor isoforms bind NPY and PYY with similar affinities. The affinity for PP is slightly lower but still in the nanomolar range [12]. It is predominantly expressed in the CNS, with specific localization in areas like the hypothalamus, hippocampus, and amygdala, suggesting its involvement in regulating higher-order functions [16]. NPYR5 plays a role in regulating food intake, energy expenditure, and anxiety-like behaviors. Activation of this subfamily can stimulate food intake and decrease energy expenditure, potentially contributing to weight gain. Additionally, it might influence anxiety-like behaviors, although the exact mechanisms remain under investigation [12].

The NPYR6 receptor encodes a 371-amino-acid protein that has been cloned from rabbit, mouse, and chicken, among other species. However, in humans and monkeys, the sequence contains a frameshift mutation in the third intracellular loop, which results in a non-functional truncated receptor protein [12].

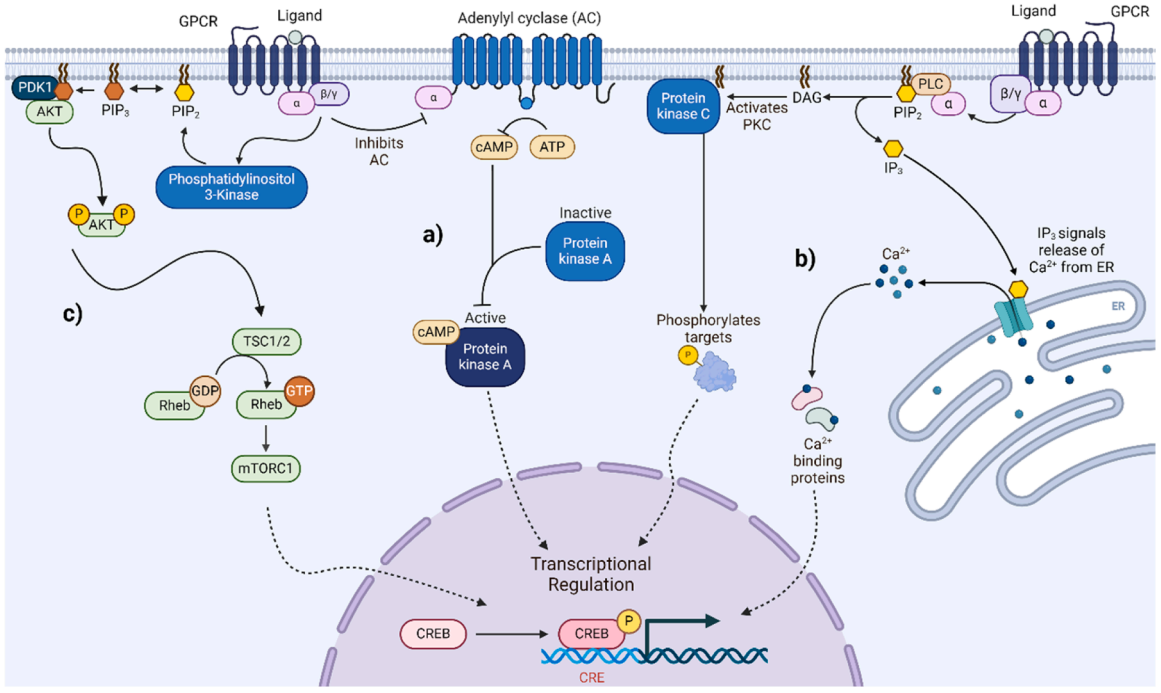

Fig. 4. This illustration shows Neuropeptide Y binding to its receptor, which activates heterotrimeric G-proteins (Gα, Gβ, Gγ). a) Upon NPY binding, the receptor activates the Gα subunit, which inhibits adenylyl cyclase, reducing cAMP levels. b) NPYR modulates calcium signaling via PLC activation and IP3 production, influencing intracellular calcium levels. c) NPYR1 and NPYR2 activate MAPK pathways (ERK, JNK, p38), while distinct pathways involve PI3K for NPYR1 and PLC/PKC for NPYR2. NPYR4 and NPYR5 also activate PLC, leading to increased intracellular calcium and PKC activation, contributing to varied physiological functions. Created with BioRender.com.

Moving to the biochemical mechanism of the physiological functions of NPYRs, it is generally accepted that all the NPYR subfamilies share some common signaling pathways. These pathways include the inhibition of 1) adenylyl cyclase, 2) modulation of calcium signaling, and 3) activation of MAPK pathways [21]. The scheme for the general NPYR signaling pathways is shown in Fig. 4. NPYRs are coupled to heterotrimeric G-proteins, which consist of three subunits: alpha (Gα), beta (Gβ), and gamma (Gγ). Firstly, all NPYRs inhibit adenylyl cyclase. When NPY binds to the receptor, it undergoes a conformational change, leading to the activation of the Gα subunit. The activated Gα subunit dissociates from the Gβγ dimer and interacts with adenylyl cyclase, inhibiting its activity. By reducing cAMP levels, NPY can modulate cellular responses that are regulated by cAMP-dependent pathways [21], [22], [23], for example, downregulation of PKA activity and altered gene expression [24]. A decrease in cAMP has profound implications for cellular signaling pathways, influencing processes such as cell growth, survival, and death. Decreased cAMP levels can also weaken the signaling pathways downstream of NPYRs, leading to reduced cellular responses to NPY. Lower cAMP levels may change how NPY interacts with other signaling pathways, leading to less activation of NPYRs and impacting downstream signaling events.

Secondly, NPYR1 and NPYR2 can modulate calcium signaling through various mechanisms, such as the inhibition of voltage-gated calcium channels, the release of calcium from intracellular stores through the activation of phospholipase C (PLC), and the production of inositol trisphosphate (IP3), and interactions with other signaling pathways such as protein kinase C (PKC) [21]. Changes in cAMP levels can influence these calcium signaling pathways, potentially affecting NPY's ability to regulate calcium levels. This pathway is connected to cancer invasion and drug resistance [25].

Thirdly, NPYRs, especially the NPYR1 and NPYR2 subtypes, can activate mitogen-activated protein kinase (MAPK) pathways through the activation of intracellular signaling molecules. This involves the activation of G-proteins, phospholipase C (PLC), protein kinase C (PKC), and various downstream kinases such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK. The specific MAPK pathway activated by NPY receptors can vary depending on the receptor subtype and the cell type involved [21]. The MAPK pathway is known as the main proliferative pathway.

However, each subtype also has distinct pathways associated with it. The NPYR1 has a potential involvement in the phosphatidylinositol 3-kinase (PI3K) pathway, while the NPYR2 has a potential involvement in phospholipase C (PLC) and protein kinase C (PKC) pathways. The NPYR4 and NPYR5 both activate PLC, leading to the production of inositol trisphosphate (IP3) and diacylglycerol (DAG), resulting in an increase in intracellular calcium levels and activation of PKC. These distinct pathways contribute to the diverse functions of NPYRs in various tissues and systems [21]. In addition, more signaling pathways may coexist and cross-talk with other ligand-receptor pathways, which leads to adaptive effects in cellular mechanisms [26].

3. NPY receptors in cancer tissues

Ongoing research is exploring the link between NPY and cancer progression, and different literature reports have shown that specific subfamilies of NPYR have been found to be overexpressed in several tumor tissues [5], [27], [28], [29]. For example, Li *et al.* [30] have shown that the subfamilies NPYR1 (and in some cases NPYR2) are overexpressed in various types of tumors, including ovarian, renal cell, brain tumors, and breast [30]. Sanchez *et al.* [5] reported that NPYR1 is highly expressed in brain tumors and gliomas, while both NPYR1 and NPYR2 are present in ovarian cancer, as well as in pancreatic cancer. Also, NPYR1 receptors have been detected in adrenal cortical tumors, blood vessel tumors, and renal cells by Silva *et al.* [31]. These studies suggest that these specific NPY receptor subtypes may play distinct roles in different types of cancer [32]. NPY can have a direct influence on cancer cells, such as proliferation, cell death, cell metabolism, invasion, and metastasis. The stimulation or inhibiting effect depends on the cell type and the potential interaction between heterotypic receptors [29]. However, breast and prostate cancer were most widely investigated in connection with NPYRs and show high therapeutic potential; therefore, we dedicate two specific sections to review the state-of-the-art for breast and prostate cancers. In the third section, we summarize research activities on other cancer types.

While this review focuses on NPY and its therapeutic implications, it is important to recognize the antitumor effects of related peptides such as PYY and PP. These peptides provide additional insights into the role of NPY-related systems in cancer biology, potentially broadening therapeutic strategies [5]. PYY and PP interact with various NPY receptor subtypes, which can influence cancer progression. Additionally, PYY has been shown to inhibit proliferation and promote apoptosis in breast cancers [33]. Fragments of PYY, such as PYY 14–36, demonstrate antitumor activity against pancreatic cancer cells by engaging in specific receptor interactions. In contrast, elevated levels of PP have been observed in pancreatic neuroendocrine tumors, indicating its potential role as a biomarker in cancer progression [5]. For further reading on this topic, please consult [13].

3.1. Breast cancer

Breast cancer is the most frequently diagnosed cancer (23.8 %) and the leading cause of cancer death in females worldwide, with an estimated 2.3 million new cancer cases (1 in 4 new cancer cases) and 666,000 cancer deaths (1 in 6 deaths) in 2022 [34]. It was noticed that breast cancer cells express different types of peptide receptors, such as vasoactive intestinal peptide receptor (VIP), somatostatin, and gastrin-releasing peptide (GRP) receptor [35].

Interestingly, it was discovered that 85 % of the patients with breast cancer have a high expression of NPYRs [27,28]. Among the NPYR-positive breast cancer tissues, NPYR1 was found in 100 % of patient samples, while NPYR2 was found only in 24 % of cases, always together with NPYR1. In normal breast tissues, the situation is vice versa: NPYR1 and NPYR2 are expressed together in 58 % of the study samples, while NPYR2 was found alone in 42 % of the samples [35]. Moreover, it was reported that normal tissues become cancerous when the highest abundant NPYR in normal mammary glands, i.e., the NPYR2, is converted to the NPYR1 subtype [27]. Expression of NPYR1 varies among the different breast cancer types. The highest NPYR1 expression was found in luminal A breast cancer tumors, and the lowest in the human epidermal growth factor receptor 2 (HER2)-positive subtype of breast cancer [36]. In addition, in estrogen receptor-positive breast cancer cells, the expression of the NPYR1 gene increased in response to estrogen and was reduced with endocrine therapy. On the other hand, in estrogen receptor-positive breast cancer cells that are resistant to endocrine therapies, the NPYR1 expression is reduced [36].

In addition to tissue-level analyses, a clinical study by Liu et al. quantitatively assessed NPY1R mRNA expression in peripheral blood samples from 142 breast cancer patients and 60 healthy controls. Using nested qPCR, the authors found that NPY1R expression was significantly elevated in patients compared to controls ($-\Delta$Ct: -3.93 ± 2.5 vs. -8.21 ± 2.9; $P < 0.01$), with a calculated expression ratio (Q) of 55.54 ± 27.3. NPY1R positivity—defined conservatively based on ROC analysis—was observed in 44.4 % of patients, with no expression detected in healthy controls. High NPY1R levels significantly correlated with clinical stage, lymph node metastasis, and ER, PgR, and HER2 status ($P < 0.05$). Notably, NPY1R-positive patients had reduced tumor-specific survival over 38 months compared to NPY1R-negative individuals (43.3 % vs. 76.1 %). These findings highlight NPY1R not only as a highly expressed receptor subtype in breast cancer but also as a potential circulating biomarker for disease progression and prognosis [37].

Sheriff et al. demonstrated that the pharmacological modulation of NPYR1 could represent a potential strategy for cancer chemotherapy

[38]. Specifically, they showed that NPYR1 activation inhibited cell proliferation in estrogen receptor-positive human breast carcinoma cell line, MCF-7, and that its expression is upregulated in response to estrogen [39]. Also, in a follow-up study [38], the authors identified that NPYR5 is expressed in several human breast carcinoma cell lines, with high expression in MCF-7, modest in triple-negative BT-549, and low in triple-negative MDA MB-231 cell line. In addition, BT-549 cells express mainly NPYR5, whereas MCF-7 cells express both NPYR1 and NPYR5. In none of these cell lines, NPYR2, the receptor subtype usually associated with normal mammary tissues, was detected.

Moreover, it was reported that triple-negative breast cancer cell line 4T1 expresses NPYR1, NPYR2, and NPYR5 and that NPY treatment promoted a concentration-dependent increase in proliferation through increased phosphorylation of ERK 1/2 [11]. Recently, Pascetta et al. explored how NPY affects angiogenesis in 4T1 breast cancer cells by modulating the Vascular Endothelial Growth Factor (VEGF) [32]. NPY-treated 4T1 cells showed a significant rise in VEGF expression and secretion, mainly through NPYR5 activation, indicating a pro-angiogenic effect. Also, they found that NPYR1 and NPYR5 antagonists can inhibit breast cancer cell migration and proliferation under hypoxic conditions.

Additionally, Wang et al. explored the use of an NPYR1 targeting in combination with a drug resistance inhibitor for breast cancer, suggesting a potential strategy to overcome resistance mechanisms [40]. They incorporated [Asn6, Pro34]-NPY into nano micelles as a nano drug delivery system and loaded them with the chemotherapeutic drug doxorubicin and the P-glycoprotein (P-GP) inhibitor tariquidar. Doxorubicin is a commonly used chemotherapy drug, while P-GP inhibitors are used to overcome drug resistance by inhibiting drug efflux from cancer cells. Their findings suggest that targeting NPYR1 with NPY, in combination with P-GP inhibitors, would be a precise and effective strategy for breast cancer treatment [40]. The summary of the most important findings discussed in this section are shown in Table 1.

3.2. Prostate cancer

Prostate cancer (PCa) is a common cancer among men, with 1.5 million new cases and 400.000 deaths in 2022 [34]. Growth factors and neurohormones are believed to play a role in promoting the androgen-independent stage of PCa. In the human prostate, particularly in the smooth muscle layer, NPY is mainly localized in nerve fibers and neuroendocrine cells [41]. NPY can serve as a biomarker for the progression and prognosis of PCa. A high concentration of NPY-positive PCa cells is very likely linked to higher aggressiveness of cancer and poorer prognosis [42]. In addition, prostate cancer patients were found to have elevated concentrations of NPY in plasma [43].

Further, 75 % of patient samples were positive for the presence of NPYR, which indicates the potential role of NPY in the disease progression [44]. In a pioneer study by Magni and Motta from 2001, the presence of NPYR1 and NPYR2 receptors was confirmed in an androgen-independent PCa cell line (PC3) and not in DU145 (androgen-independent) or LNCaP (androgen-dependent). The activation of NPYR in PC3 by NPY stimulated the proliferation of PC3 cells [45].

Later, Ruscica et al. evaluated the effect of NPY on the growth of all three PCa cell lines (LNCaP, DU145, and PC3). Importantly, they proved that all tested PCa cell lines expressed NPYR1. Strikingly, NPY treatment reduced the proliferation of LNCaP and DU145 cells and increased that of PC3 cells, indicating activation of different pathways upon exposure to NPY antagonists [10], [46]. On the contrary, the study from Nagakawa et al. from 2001 evaluated ten different neuropeptides, among them also NPY, and concluded that only GRP, CGRP, and PTH-rP increased the invasive capacity of DI-145 prostate tumor cells, while others not [47].

Recently, it was shown that PCa induces NPYR-positive nerve growth in PCa tissue, which correlates with aggressive tumor behavior, meaning that NPY is a critical regulator of cancer–nerve interaction [48]. For example, inhibition of NPYR1 resulted in increased cancer apoptosis in LNCaP and DU145 cell lines, decreased motility in DU145 and PC3 cells, and energetic metabolic pathway changes (tested on LNCaP cells). Overall, 13 common pathways were identified during the comparison of transcriptomic changes in human tissues with metabolomic changes of NPY inhibition [48].

Table 2 summarizes the crucial findings of this section. All these studies show the importance of paracrine NPY in PCa biology and progression. However, a more detailed and systematic study is warranted to undoubtedly define the role of NPY in PCa due to the still contradictory findings from the early studies.

3.3. Other cancer types

NPY-NPYR interactions are also important in many other cancer types, as will be demonstrated in this section. At least 22 different cancer types have been studied so far in this respect [13]. The presence of NPYR was evaluated in a group of ovarian tumors [30]. Remarkably, all sex

Table 1
Summary of NPY receptor expression and functional outcomes in breast cancer cell lines. ↑ Indicates an increase; ↓ indicates a decrease in cellular function.

Cell line	NPY receptor	Effect Observed	Main Finding	Reference
Breast MCF−7	NPYR1	↓ proliferation; ↑ expression with estrogen	NPYR1 inhibits estrogen-induced proliferation; estrogen increases NPYR1 expression	[39], [40]
Breast BT−549 (TNBC)	NPYR5	↑ NPYR5 expression	BT−549 mainly expresses NPYR5; no functional effect described	[38]
Breast MDA-MB−231 (TNBC)	NPYR5	↓ NPYR5 expression	NPYR5 expression is low; not responsive to estrogen	[38]
Breast 4T1 (TNBC)	NPYR1, NPYR2, NPYR5	↑ proliferation, migration, VEGF secretion	NPY stimulates ERK1/2; NPYR5 drives VEGF-mediated angiogenesis	[11], [35]
Breast MCF−7 xenograft (mouse)	NPY1	Targeting via PET and nanotherapy	NPYR1 used for imaging and nano-delivery strategies in vivo	[40]

Table 2
Effects of NPY and NPY receptor expression in prostate cancer cell lines. Arrows indicate direction of observed cellular response: ↑ = increase, ↓ = decrease. All observed effects were reported upon exogenous NPY treatment or NPY receptor inhibition.

Cell line	NPY receptor	Effect Observed	Main Finding	Reference
Prostate PC3 (androgen-independent)	NPYR1, NPYR2	↑ proliferation (with NPY)	NPY stimulates proliferation through NPYR1/NPYR2	[45]
Prostate LNCaP (androgen-dependent)	NPYR1	↓ proliferation; ↑ apoptosis (NPYR1 inhibited)	NPYR1 inhibition increases apoptosis, alters metabolism	[46,48]
Prostate DU145 (androgen-independent)	NPYR1	↓ proliferation; ↑ apoptosis; ↓ motility	NPYR1 inhibition reduces motility and increases apoptosis	[46,48]
Prostate DI−145	-	No effect on invasion	NPY has no effect on invasion; GRP and CGRP do	[47]

cord-stromal tumors (granulosa cell tumors, Leydig cell tumors, and Sertoli–Leydig cell tumors) expressed NPYR (both NPYR1 and NPYR2, heterogeneous distribution within the tumor, and density varied significantly among them), but at a lower density than, for example, in breast carcinomas. In contrast, NPYRs (total, not subtype specific) were found in only 32 % of adenocarcinomas [49]. Moreover, receptors were not detected in any of the investigated adenomas, borderline tumors, pure stromal neoplasms, or dysgerminoma. NPYRs were also detected in 67 % of investigated cases in the blood vessels within tumors or the surroundings of the tumor tissues (predominantly NPYR1 subtype) [49]. These findings indicate that NPYR targeting might be beneficial only in selected ovarian tumors and in combination with other therapeutic approaches [30].

NPYR was detected in 93 % of adrenal cortical tumors, and 90 % of neuroblastomas express NPYRs. Also, non-neoplastic tissues such as chromaffin cells and nerve fibers in the adrenal cortex were positive for NPYRs [50]. In glioblastomas, 83 % of the samples express NPYR2 with high density, but also non-neoplastic tissues contain NPYR1 and NPYR2 [51]. 56 % of renal cell carcinomas expressed the NPYR1 subtype in moderate density, and 80 % of nephroblastomas expressed NPYR1 and NPYR2 subtypes in moderate to high density. Importantly, NPYR1 was also highly expressed in intratumoral blood vessels. Furthermore, NPYR was observed in the vicinity of tumor cells, while no PYY or PP was detected in these sites [52]. In neuroblastoma, NPY and NPYR2 are expressed, which are key to neuroblastoma cell proliferation and tumor vascularization. Therefore, NPYR2 antagonists suppress tumor growth. In addition, some neuroblastoma cells also express NPYR5 [53]. In the study of Czarnecka et al. [54], it was demonstrated that activation of NPY/NPYR5 enhances neuroblastoma growth, which leads to chemoresistance. On the other hand, blocking NPYR5 sensitizes the cells to chemotherapy. Therefore, the NPY/NPYR5 pathway may be a promising therapeutic target for patients with neuroblastoma [54], [55].

In Ewing sarcoma tumors, 84 % of samples expressed NPYR1 in high density, with positive expression also in tumor blood vessels. Unfortunately, no data about normal tissues was provided. Reversely, in testicular tumors and non-neoplastic tissues, both NPYR1 and NPYR2 are expressed, but the density of receptors is lower in tumor cells, so the specificity of the targeting is limited [56]. From these studies, it can be concluded that many tumors express NPYR, but only breast cancer tissue shows a significant difference in NPYR subtype expression levels between tumor and normal tissues. Therefore, as discussed in the previous section, this could be a promising target.

In summary, the contrasting roles of NPY in promoting or inhibiting tumor growth can be attributed to the differential expression of NPY receptors across various tumor types, as can be seen from the Table 3. This duality underscores the necessity for a nuanced understanding of NPY receptor signaling in cancer treatment, as the therapeutic effects of NPY analogs may vary significantly depending on the tumor microenvironment and receptor expression profiles.

Table 3
NPY receptor expression and functional effects in other solid tumors. Arrows indicate direction of the effect: ↑ = increase, ↓ = decrease. Effects were observed upon endogenous or exogenous NPY activity or inferred from expression and functional studies.

Cell line	NPY receptor	Effect Observed	Main Finding	Reference
Ovarian tumors (sex cord-stromal)	NPYR1, NPYR2	Expression only	Heterogeneous NPYR expression in stromal tumors	[30,49]
Adrenal cortical tumors	NPYR1	93 % NPYR expression	NPYRs are highly expressed in the tumor and nerve tissue	[56]
Neuroblastoma	NPYR2, NPYR5	↑ proliferation; ↑ chemoresistance	NPYR2 promotes growth; NPYR5 linked to resistance	[54,55]
Glioblastoma	NPYR2	83 % express NPYR2	NPYR2 is highly expressed; also present in non-neoplastic brain tissue	[51]
Renal cell carcinoma	NPYR1	56 % expression	Moderate density of NPYR1 in tumor samples	[52]
Nephroblastoma	NPYR1, NPYR2	80 % expression	Moderate-to-high density of NPYR1 and NPYR2	[52]
Ewing sarcoma	NPYR1	84 % express NPYR1	High NPYR1 in tumors and tumor vasculature	[56]
Testicular tumors	NPYR1, NPYR2	Lower expression in tumors vs. normal	Low targeting specificity due to receptor distribution	[56]

4. The NPYR1-NPY system as a molecular target

4.1. NPY receptors in cancer tissues

Cancer involves the abnormal and uncontrolled growth of cells, leading to aggressive malignancies that cause millions of deaths annually. Over the past few decades, a variety of therapeutic combinations have been developed and are currently used to treat different types of cancer. These treatments have traditionally relied on cytotoxic agents that target rapidly dividing cells. However, the long-term side effects of anticancer chemotherapy continue to be a significant concern for both patients and healthcare providers, as modern drugs and approaches to mitigate these adverse effects often fall short [57]. With advancing knowledge of the molecular mechanisms driving cancer progression, the understanding of the disease is rapidly expanding, leading to the development and trial of many new therapeutic strategies. Emerging treatments include targeted drug therapy, immunotherapy, nanoparticle-based delivery systems, ablation therapies, and personalized medicine. As a result, cancer-specific biomarkers are increasingly being identified and studied. Systematic and targeted therapies hold promise for the future development of more potent and efficient cancer treatments, also considering patients' quality of life after the disease [58], [59].

Among the NPY receptor subtypes, NPYR1 and NPYR2 have appeared as the most biologically and clinically relevant in cancer research due to their expression patterns and functional roles. NPYR1 is frequently overexpressed in a range of tumors, including breast, prostate, and neuroblastoma, where its activation is associated with increased cell proliferation, angiogenesis, and migration [30,32,48]. In contrast, NPYR2 is more frequently expressed in normal tissues, such as the mammary gland, and is detected in a much smaller proportion of tumor samples, suggesting a shift in receptor dominance during malignant transformation [35]. This switch from NPYR2 to NPYR1 dominance during malignant transformation has been described in breast cancer and may reflect a shift from regulatory to proliferative signaling [27,35]. These opposing expression patterns and functions make NPYR1 and NPYR2 especially valuable targets for understanding tumor biology and developing subtype-selective therapies.

The development of subtype-selective ligands for the different NPYR subtypes, acting either as agonists or antagonists depending on the desired therapeutic effect, is pivotal in this context. The following paragraph will thus be dedicated to a brief description of the molecular features at the basis of NPY binding events to the NPYR-1 and −2 receptor subtypes. The former is generally overexpressed in breast cancer cell lines, while the latter is the constitutive receptor form of normal

mammary tissues.

4.2. NPY vs NPYRs: the binding modes

NPYR subtypes have different profiles of ligand recognition, indicating that the ligand-binding pocket displays subtype-specific differences [60]. For example, NPYR1 and NPYR4 require the complete N terminus of the NPY ligand, NPYR5 can bind peptides with the deletion of the first amino acid, and NPYR2 can accept even shorter peptides with minimal changes in affinities. Nonetheless, the C-terminal region of all-natural ligands is critical for binding to all NPYRs, particularly the Arg residues at positions 33 and 35, which must be preserved [60]. In this discussion, we will focus on the interactions between NPY and the two most extensively studied NPYRs: NPYR1 and NPYR2.

4.2.1. NPY vs NPYR1

NPY interacts with its receptor(s) through specific amino acids in the active site, and the structural basis of its signaling through the NPYR1, as well as the features of ligand recognition with the NPRY2 subfamily, were recently established at a molecular level [8,61,62].

Two approaches are usually applied to investigate the NPY subtype selectivity: a) truncation of certain fragments and b) the alanine-scanning method, where each residue in the sequence is individually substituted with Ala and naturally occurring Ala is replaced by Gly. All synthesized analogs are then tested for affinity binding or activation of the receptor [12].

Both the N- and C-terminal regions of NPY are important for NPYR1 binding. The N-terminal region is necessary for receptor activation and the initiation of downstream signaling pathways (Fig. 5a). It contributes to the stability, proper folding of the ligand structure, and specificity of the ligand-receptor complex. Post-translational modifications, such as proteolytic cleavage or amidation, can occur in the N-terminal region and affect the binding affinity, stability, and activity of NPY [8]. The complex NPY-NPYR1 crystal structure, combined with mutagenesis studies and molecular dynamic simulations, has revealed the interaction fingerprints of this receptor-ligand system (Fig. 5b). Fig. 5c and d show a close-up view of the interactions within the binding interface. Deleting the entire N-terminus does not interfere with receptor signaling but

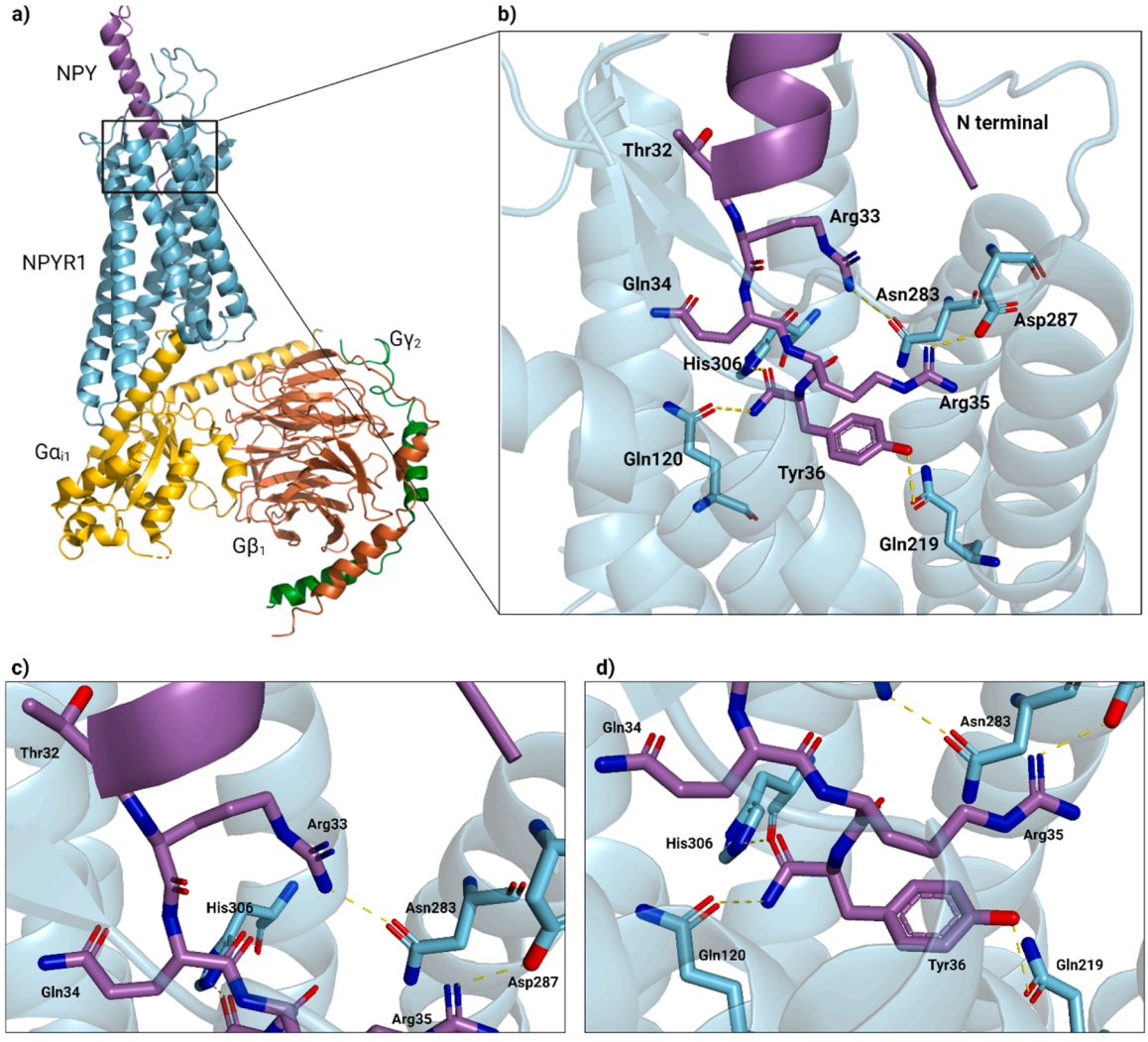

Fig. 5. a) The crystal structure of the Neuropeptide Y receptor 1 (PDB: 7X9A) in combination with Neuropeptide Y and the related G-protein heterotrimer (Gα1, Gβ1, Gγ2). NPYR1 is shown in cyan, while NPY is depicted in purple. The Gα1 subunit is displayed in yellow, the Gβ1 subunit in orange, and the Gγ2 subunit in green. b) This enlarged view focuses on the binding interface between NPY (purple) and NPYR1 (cyan). Key interacting residues are labeled, including Thr32, Arg33, Gln34, Arg35, and Tyr36 from NPY, and Asn283, Asp287, His306, Gln120, and Q219 from NPYR1. Hydrogen bonds are represented as yellow dashed lines. These interactions are crucial for the stabilization of the NPY-NPYR1 complex. c) Close-up view of the interactions within the binding interface, particularly focusing on the residues Thr32, Arg33, and Gln34 from NPY and Asn283 from NPYR1. d) Detailed interactions within the binding site are shown, depicting the orientation of additional residues such as Gln34, Arg35, and Tyr36 from NPY, and His306, Gln120, and Gln219 from NPYR1. Created with BioRender.com and PyMOL (Schrödinger, LLC).

reduces NPY binding by about 95 % compared to the full-length receptor [63]. Truncating the first two residues of NPY (resulting in NPY(3−36)) reduces peptide potency by more than 50-fold, highlighting the importance of the N-terminal region in receptor binding [8]. Mutating the first two residues of NPY to alanine ([A1, A2]NPY) does not significantly affect peptide potency, suggesting that the peptide backbone also contributes to receptor binding.

The N-terminal region of NPY makes close contact with a fragment in the second extracellular loop (ECL2) of NPYR1 and is in proximity to the receptor N-terminus. Photo-crosslinking studies involving NPY analogs and NPYR1 have identified crosslinked fragments in two receptor regions: the N terminus (K21-D32) and ECL2 (A191-D205) [62]. This indicates that the N-terminus of NPYR1 is involved in recognizing and positioning the NPY ligand. Mutations in ECL2 of NPYR1 (F184A/N and V197N) significantly reduce NPY potency, further supporting the role of ECL2 in recognizing NPY's N-terminus and facilitating receptor activation [8], [31], [62]. The N-terminus of NPY is thus critical at a molecular level for establishing NPY physiological regulatory effects, which, as stated, include food intake, stress response, anxiety, and memory retention.

The interaction between NPY and NPYR1 also highlighted the importance of the C-terminal Tyr36, Arg33, Arg35, Gln34, and Thr32 residues as critical for this ligand binding as they establish hydrogen bonding and p-p interactions with receptor's Asn283, Phe286 and Phe302. Specifically, Arg35 forms an electrostatic interaction with Asp287 and a van der Waals interaction with Phe173. Additionally, Gln34 and Thr32 are noted for their interaction with Thr97 and Tyr100 in NPYR1, respectively, both of which are vital for the downstream signaling of NPY. Ala at positions 26 and 30 is well tolerated, whereas substitution at position 27 led to a reduced affinity [64].

The structural integrity and functional capability of NPY are further emphasized through the C-terminal amphipathic end that facilitates adsorption at lipid surfaces and the α-helix, which is key for receptor binding selectivity and biological impacts. Moreover, the polyproline type II helix's role in sustaining the stability of the peptide's secondary and tertiary structures is highlighted. In this context, alanine scanning experiments further highlighted the NPY residues pivotal for ligand affinity to different NPYRs. Specifically, Pro2, Pro5, Arg19, and Tyr20, as well as the amino acids between positions 27 and 36, especially position 27, were found to be essential for the peptide's affinity towards NPYR1. Positions 33 and 35 were also found to be extremely important for all NPYRs [60].

For example, analogs with Ala at these positions showed a significant loss in binding affinity to NPYR1 of over 5000-fold compared to the wild type [12]. Interestingly, alanine scanning revealed that Tyr36 contributes to ligand binding, and it cannot be replaced with other amino acids (Ala, His) without affecting its function. However, the substitution of Tyr36 with a similar Phe is relatively well tolerated. The results of the complete alanine scanning on NPY and the affinities towards NPYR1, NPYR2, NPYR4, and NPYR5 are well described and summarized in the review paper by Cabrele and Beck-Sickinger [65].

Although the alanine scanning showed that the central positions of NPY are not essential for binding to the NPYR1 [64], a study performed by Kirby et al. [66] proved that a centrally truncated and conformationally constrained analogs showed only moderate affinity towards NPYR1. In order to maintain high receptor affinity, the maximum deletion of residues is eight (positions 10—17). Furthermore, the location of the disulfide bridge and the chirality of the Cys residues influence the NPYR1 receptor affinity as well. NPY cyclo-analogue with Cys7-Cys21 disulfide bridge proved to be optimal with similar affinity to natural NPY. These findings underscore the significance of structural modifications in tailoring NPY analogs for specific receptor subtypes. A schematic representation of NPY hot-spot residues to guarantee binding affinity to the NPYR1 subtype is reported in Fig. 6.

4.2.2. NPY vs NPYR2

Also, NPYR2 has attracted considerable interest as a drug target due to its role in cancer treatment, food intake, and bone formation. [45]. Authors have performed functional studies to elucidate the structural importance of NPYR2-ligand binding mode and NPY receptor subtype specificity. The NPYR2 structure exhibits seven transmembrane helices I–VII, typical architecture of GPCRs (Fig. 3b).

NPYR2 is less sensitive towards amino acid substitutions in NPY than NPYR1. Alanine scanning showed that replacement of single amino acids reduced affinity 2–20-fold, with exceptions for positions Pro 5, Arg19, Tyr20, Tyr27, Asn29, Thr32, and positions 33–36. The substitution of Pro5 for Ala led to a 600-fold loss of affinity [10]. Arg35 seems to be the most susceptible residue in the NPY; its substitution with Ala reduces NPYR2 affinity 75000-fold [49]. Less dramatic loss is observed for other Ala substitutions: Tyr32 (1000-fold), Arg33 (1350-fold), Gln34 (150-fold), and Arg36 (19500-fold). Thus, the C-terminal part of the NPY analog is crucial for high-affinity binding to the NPYR2.

In order to selectively target NPYR2, a series of analogs were prepared and tested [61], [67]. The most promising is a high-affinity, brain-penetrant small-molecule antagonist of NPYR2 (called JNJ-31020028) that binds with phenyl ethyl and diethyl amide groups in the binding pocket formed by residues from the first extracellular loop (ECL1) and helices II-VII of NPYR2 via hydrophobic contacts (Fig. 7). High binding affinity proved that the hydrophobicity of the ligand is beneficial in this region. After applying different mutations to the receptor, it was shown that the polar network between JNJ-31020028 and the receptor helix VI plays a crucial role in mediating receptor-ligand recognition. Further, the residue $W^{6.48}$ has been found to be highly important for the NPY-induced G protein activation. The interaction of this residue with the antagonist can stabilize the receptor in an inactive conformation and suppress the receptor activity. In addition, the fluorine atom in the central phenyl core of JNJ-31020028 was recognized as critical to obtaining high binding affinity to NPYR2 because it forms a hydrogen bond with the side chain of $Q130^{3.32}$. They also showed the difference in the receptor-ligand interaction modes on the extracellular side of the binding pocket in NPYR1 and NPYR2, which is the key to the development of selective ligands.

4.3. Dual role of NPY in cancer progression

Neuropeptide Y exhibits a dualistic role in cancer progression, acting as both a tumor promoter and, in select contexts, a suppressor of tumorigenic processes. This functional versatility is largely shaped by cancer type, tumor microenvironment, receptor subtype expression, and external factors such as hypoxia.

On the pro-tumorigenic side, NPY is well recognized for enhancing proliferation, migration, invasion, angiogenesis, and therapy resistance across multiple malignancies. In breast, prostate, neuroblastoma, and Ewing sarcoma models, NPY promotes tumor cell proliferation primarily via Y5R and Y2R signaling, especially under hypoxic conditions, which induce receptor upregulation and convert NPY into selective agonists [5, 32,68]. NPY also enhances angiogenesis, often matching VEGF in potency, and contributes to metastasis and therapy resistance through modulation of the tumor microenvironment and immune evasion mechanisms [13,25]. Notably, NPY supports the expansion of cancer stem-like cells in hypoxic Ewing sarcoma, contributing to aggressive behavior and relapse [68].

Contrastingly, NPY can exert anti-tumorigenic effects in specific contexts. For example, in Ewing sarcoma cells under normoxic conditions, simultaneous activation of Y1R and Y5R induces cell death [13, 68]. In prostate cancer, differential responses to NPY are observed: NPY stimulates proliferation in androgen-independent PC3 cells but inhibits growth in LNCaP and DU145 lines, an effect mediated via Y1R and associated with sustained ERK1/2 activation [10]. Similarly, in ER-positive breast cancer, Y1R activation suppresses estrogen-driven proliferation, suggesting a protective role in hormone-responsive

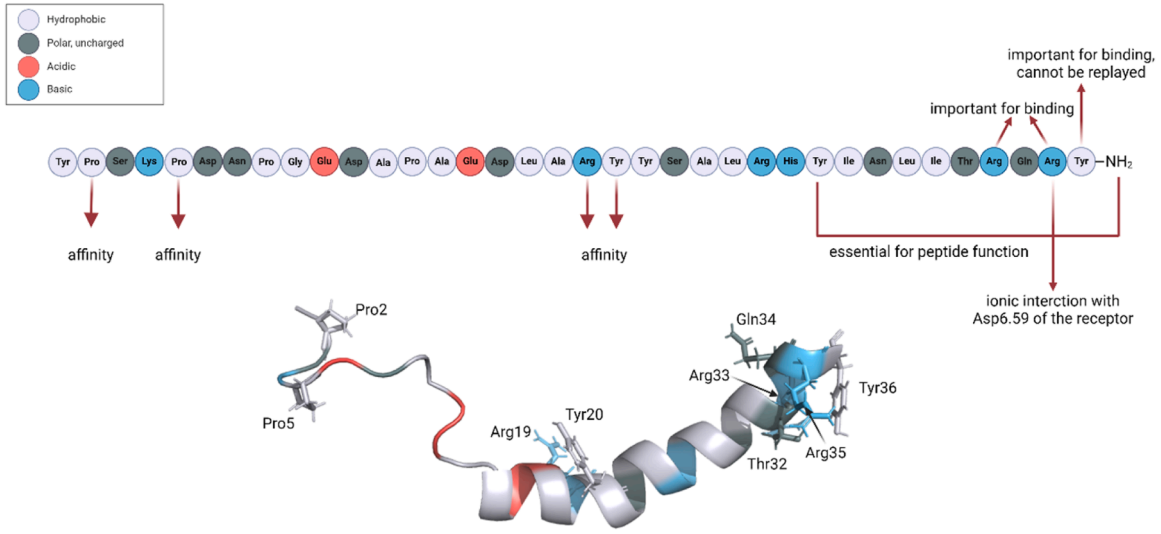

Fig. 6. This image displays a schematic of NPY (PDB: 7X9A), highlighting the critical residues for its interaction with the NPYR1. The peptide is represented in a cartoon form, with specific residues shown as sticks to emphasize their importance in maintaining receptor affinity and function. Residues are color-coded based on their properties: light gray for hydrophobic residues, dark gray for polar uncharged residues, red for acidic residues, and blue for basic residues. The image demonstrates that while the central residues (positions 10–17) can be deleted without drastically impacting receptor affinity, other regions, particularly those on the N-terminal (positions 32–36), are essential for peptide function. Created with BioRender.com and PyMOL (Schrödinger, LLC).

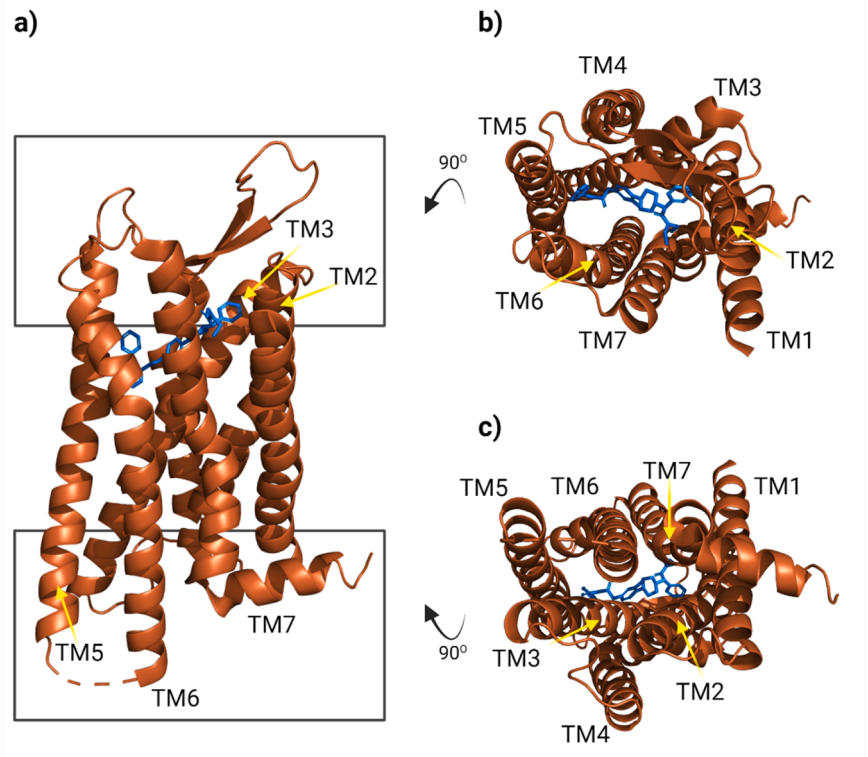

Fig. 7. a) The side view of the NPYR2–JNJ-31020028 structure (PDB: 7DDZ). NPYR2 is depicted in an orange cartoon representation. JNJ-31020028 is shown in blue stick representation. b) The NPYR2, with views from the extracellular (top-down) perspective. The transmembrane (TM) regions are explicitly labeled, providing a clear representation of the receptor's orientation and its potential interaction sites for ligand binding and G-protein coupling. JNJ-31020028 is shown as sticks. c) The NPYR2, with views from the intracellular (bottom-up) perspectives. Created with BioRender.com and PyMOL (Schrödinger, LLC).

tumors [68]. Additional evidence highlights the role of PYY, an NPY-family peptide, in blocking proliferation and inducing apoptosis in colorectal, pancreatic, and hepatic cancers, further illustrating receptor- and tissue-specific inhibitory effects [13].

5. Synthetic NPY analogs as potential anticancer agents

The previous section furnished the molecular basis of NPY interaction with two important NPYR subtypes derived from structural analysis. These data can be used for the rational design of NPY analogs, potentially targeting one or the other receptor isoform that can be

applied in sophisticated cancer therapies. The following report notably includes examples of the design and development of NPYR1-targeting NPY analogs. It was shown that NPYR1 is highly expressed in breast cancer tissues [27,28]. Therefore, the development of highly NPYR1-selective NPY analogs would be of great interest.

Importantly, positions 7, 25, 26, 31, 34, and 35 in NPY were identified as essential for receptor subtype selectivity [12]. Modifications in positions 25 and 26 showed that [D-Arg25]NPY and [D-His26]NPY bind selectively to the NPYR1 [69]. Another specific interaction was noticed between receptor subtypes. Asp6.59 of NPYR1 and NPYR4 interact with Arg35 of NPY, while Asp6.59 of NPYR2 and NPYR5 connect with Arg33 of NPY. This indicates variations in how NPY ligands dock with NPYR2/5 compared to NPYR1/4 [60]. Further, it was demonstrated that substituting position Ile31 with Leu and Gln34 with Pro brings a specific high-affinity ligand for NPYR1 ([Leu31, Pro34]NPY) [70]. These findings emphasize the significance of both the N- and C-terminal fragments of NPY, particularly for binding to the NPYR1 subtype [12].

Modification of NPY ortho-carbaborane leads to improved selectivity for either NPYR1 or NPYR2 [71]. With this, NPYR1-selective [F7, P34]-NPY and NPYR2-selective [Ahx(5−24)]-NPY sequences were developed [72].

Similarly, a study on neuropeptide Y analogs with a preference for the NPYR1 revealed the importance of distinct amino acid positions for binding to different receptor subtypes, aiding in the development of highly selective ligands [73]. For example, [Phe7, Pro34]pNPY (pNPY = porcine neuropeptide Y) showed the highest preference against NPYR1 (3000-fold more than NPYR2 and NPYR5) [73]. Similarly, [Arg6, Pro34] pNPY showed a significant preference for the NPYR1. On the contrary, cyclo analog [Cys20, Cys24]pNPY showed high selectivity towards the NPYR2, maintained similar efficiency to NPY, and had reduced affinity for the NPYR1 [30]. On the other hand, C-terminal decapeptides, such as [Tyr32, Leu34]NPY(27–36), were created as antagonists for both NPYR1 and NPYR2 [74]. [Pro30, Tyr32, Leu34]NPY(28–36), named BDV15, acted as a competitive antagonist at the NPYR1 receptor but also showed affinity for NPYR4 [75]. Smaller peptides such as [Pro30, Nle31, Bpa32, Leu34]NPY(28–36) were found to have a strong affinity and agonistic activity for NPYR1, making them potential candidates for targeting NPYR1-expressing tumors [74,76]. In general, it is believed that positions 31 and 32 are relevant for agonistic properties [76]. Newer analogs, primarily [Lys4]BVD15, showed improved NPYR1 selectivity with higher affinity than BVD15 when conjugated with a DOTA chelator group [30].

Hild et al. [77] developed three different NPY analogs to target the NPYR1 subtype to develop potential anticancer agents: [1][Lys4 (αN-Acetyl-Cys)]-pNPY, [2][Lys4(αN-Acetyl-Cys), Arg6, Pro34]-pNPY and [3][Lys4(αN-Acetyl-Cys), Ahx9–17]-pNPY. Saturation of binding sites and low nonspecific binding showed that all three analogs bind specifically to the receptor of the MCF-7 human breast cancer cell line. Fluorescence microscopy has demonstrated that fluorescence was strictly limited to the cell membrane, indicating that particles were only on the cell surface. The MCF-7 human breast cancer cell line showed around 300,000 NPYR1 per cell. Importantly, no binding was observed to MDA cells lacking the receptor [77].

Furthermore, Silva et al. created a peptide called MAP, with a unique *in-silico-guided* design that has an amphipathic structure and intense interaction with cell membranes [31]. This peptide features an α-helical conformation and contains both hydrophilic and hydrophobic residues on opposite sides of the helix. Its sequence consists of a combination of Ala, Leu, and Lys residues in the order of KLALKLALKALKAALKLA [78]. Their results have shown that MAP has a higher affinity for the NPYR1 receptor compared to NPY. In addition, molecular docking revealed that MAP interacts with the N-terminal amino acid residues, penetrating deep into the NPYR1 cavity [31].

Finally, the development of hetero-bivalent peptidic ligands targeting multiple receptors, such as NPYR1 and gastrin-releasing peptide receptor (GRPR), showcases the potential for designing multifunctional ligands with applications in breast cancer imaging [79]. This approach emphasizes the versatility of NPY-based ligands in targeting specific receptors for diagnostic and therapeutic purposes and underscores the importance of receptor selectivity in drug design. To summarize the key findings, Table 4 shows the list of linear NPY analogs with high NPYR1 affinities.

In conclusion, research on NPY modifications and their effects on receptor affinity and selectivity has provided valuable insights into the complex interactions between NPY and its receptor subtypes. Structural studies, receptor binding assays, and ligand design strategies have enhanced our understanding of NPY signaling pathways and opened up possibilities for developing novel therapeutics targeting specific receptor subtypes. By unraveling the intricacies of NPY-receptor interactions, researchers are paving the way for the development of more selective and effective treatments for a range of physiological and pathological conditions.

6. Targeting the NPRY-NPY system in cancer therapy

From the point of view of a medicinal chemist, the selection of a proper molecular target sets the basis for the development of target-oriented novel (and potential) anticancer agents endowed with improved pharmacokinetic and pharmacodynamic profiles, *i.e.,* potent but selective drugs. Targeted therapy possesses, in fact, breakthrough potential, inhibiting the growth and spread of specific cancer cells, causing less damage to healthy cells with respect to traditional cancer chemotherapies. Moreover, targeting cancer-specific biomarkers offers the possibility of developing sophisticated nano-delivery systems that are able to combine the potent anticancer effects of a highly cytotoxic cargo with the selectivity features of targeted therapies.

In this context, the NPYR1-NPY system is emerging as a promising molecular target in cancer therapy. Several studies highlighted the potential of targeting specific NPY receptor subtypes for therapeutic development [10,32,46]. However, NPY and its analogs, like many peptide-based molecules, face challenges of pharmacokinetics and bioavailability during drug development processes. Peptides are, in fact, traditionally considered inadequate druglike molecules due to factors like poor cellular uptake, susceptibility to chemical and enzymatic degradation, and loss of secondary structure *in vivo* [81]. In recent times, two effective solutions have been developed to address these issues: a) peptide stapling and b) advanced delivery methods (Fig. 8). Peptide stapling entails making structural modifications to improve stability, providing an alternative approach to enhance the effectiveness of NPY [81]. At the same time, delivery systems not only optimize the pharmacokinetics but also offer multifunctionality for therapeutic and diagnostic applications. Both approaches will be discussed in the following sections.

6.1. Addressing NPY analogs' drug-likeness issues

Synthetic peptides offer many benefits in drug development due to their ability to mimic natural biological molecules, providing specificity and lower toxicity profiles. Also, their adaptable design and ease of synthesis make them helpful in addressing complex diseases such as cancer. However, peptides face challenges, mainly due to their susceptibility to proteolytic degradation, which limits their effectiveness and oral bioavailability as drugs (Fig. 9). Peptides' poor pharmacokinetic profiles thus represent an obstacle in the development of peptide-based drugs, but the rational design of smart retroinverso, cyclic and/or stapled peptides, the introduction of nonproteinogenic residues as well as

[1] H-YPSK(Ac-N-C)PDNPGEDAPAEDLARYYSALRHYINLITRQRY-NH2
[2] H-YPSK(Ac-N-C)PRNPGEDAPAEDLARYYSALRHYINLITRPRY-NH2
[3] H-YPSK(Ac-N-C)PDNP-Ahx-ARYYSALRHYINLITRQRY-NH2 where Ahx is 6-aminohexanoyl

Table 4
The list of linear NPYR1-selective NPY analogues with defined agonistic/antagonistic effects in selected cell lines.

NPY analog sequence	Agonist/antagonistic effect + Notes	Cell line:[a]EC_{50} (nM)	Ref
Wild type (NPY)	Agonist in COS−7 cells	[b]Cos−7: 2.6	[63]
Wild type (NPY)	Agonist in SK-N-MC neuroblastoma cells	[c]SK-N-MC: 2.1	[70]
[d]pNPY (2−36)	Agonist, based on negative coupling to cAMP concentration. N-terminal truncated form of porcine NPY (pNPY); retains high affinity for NPYR1.	[e]LMTK rat cells: 3.4 (wild type: 0.15)	[80]
pNPY (3−36)	Agonist, based on negative coupling to cAMP concentration. N-terminal truncated form of pNPY; significant loss of affinity for NPYR1	LMTK rat cells: 110 (wild type: 0.15)	[80]
[D-Arg25]NPY	Agonist, based on negative coupling to cAMP concentration. Modification at position 25 increases the selectivity for NPYR1. 12-, 82-, and 48-fold higher affinity than the Y2, Y4, and Y5 receptor subtypes, respectively.	SK-N-MC: 0.2	[69]
[D-His26]NPY	Agonist, based on negative coupling to cAMP concentration. Modification at position 26 increases the selectivity for NPYR1. 14-, 10-, and 17-fold greater than for the Y2, Y4, and Y5 receptor subtypes, respectively.	SK-N-MC: 0.5	[12], [69], [70]
[Leu31,Pro34]NPY	Agonist and high-affinity ligand selective for NPYR1. Increase in the intracellular Ca^{2+} concentration, similar to wild-type NPY.	SK-N-MC: 5.1	[70]
[Phe7,Pro34]-NPY	Selective agonist for NPYR1, which leads to Y-receptor internalization. Selective uptake of the ^{99m}Tc-labelled sequence in human breast cancer patients	SK-N-MC: 11.8	[72]
[Phe7,Pro34]pNPY	The highest selectivity among the tested sequences towards NPYR1. The agonistic effect was shown with the cAMP-inhibition assay.	SK-N-MC: 10.9 (wild type: 11.2)	[73]
[Arg6, Pro34]pNPY	Highly selective agonist for NPYR1. 7x decrease in affinity at the NPYR5.	SK-N-MC: 31	[73]
[Tyr32, Leu34]NPY (27–36)	3700x higher affinity towards NPYR2 than native NPY(27−26) and agonist for NPYR1 with potency compatable to native NPY (1−36). Ca^{2+} assay.	HEL cells Y1: 8.8 Wild type: 3.9	[74]
[Tyr32, Pro34]NPY (27–36)	Antagonist at NPYR1.	HEL cells: 100	[74]
[Pro30, Tyr32, Leu34]NPY(28–36)	Antagonist at the NPYR1 receptor but also showed affinity for NPYR4.	HEK 293 cells: 39 (wild type: 3.1)	[75]
[Lys(DOTA)4] [Pro30, Tyr32, Leu34]NPY (28–36))	Antagonist at NPYR1; no binding to NPYR2 or NPYR4. Radiolabelling for PET imaging of breast cancer.	HEK 293 cells: 63 (wild type: 3.1)	[75]
[Pro30, Nal32, Leu34]NPY(28–36)	significant Y1-receptor affinity, weak binding to NPYR4, but loss in affitiny to NPYR2 and NPYR5.	MCF−7 cells: 94.3 (wild type: 2)	[76]
[Pro30, Nle31,[f]Bpa32, Leu34]NPY(28–36)	Agonist; selectively binds NPYR1 (Bpa = p-benzoyl-L-phenylalanine) with negligible binding to NPYR2, NPYR4 and NPYR5.	MCF−7 cells: 29.7 (wild type: 2)	[76]
Lys4(αN-Acetyl-Cys)]-pNPY bound to[g]QDs	Agonist: highly specific for NPYR1. Receptor-mediated endocytosis of NPs.	MCF−7 cells: 6.9	[77]
[Lys4(αN-Acetyl-Cys),Arg6,Pro34]-pNPY bound to QDs	Agonist: highly specific for NPYR1. Receptor-mediated endocytosis of NPs.	MCF−7 cells: 12.3	[77]

[a] Half maximal effective concentration
[b] Cos-7 fibroblasts
[c] A neuroblastoma cell line
[d] Porcine Neuropeptide Y
[e] A type of fibroblast-like cell line
[f] p-benzoyl-L-phenylalanine
[g] Quantum dots

peptidomimetic chemistry offers a plethora of solution to medicinal chemists [82–84].

Krieger et al. [85] discussed a series of NPY analogs designed for enhanced receptor affinity, subtype selectivity, and improved stability against proteolytic degradation. They tested two approaches to prevent the degradation of the peptides. First, the peptides were chemically modified to enhance their stability. One modification involved adding a lauroyl group to the N-terminal lysine residue of the peptide, which improved stability against peptidase degradation. Another modification substituted a tyrosine residue with an artificial biphenylalanine (Bip) amino acid, contributing to increased stability. Secondly, they used enzyme inhibitors to protect the radioligands against proteolytic degradation. The NPY analog that showed high stability against peptidase degradation is [Lys (lauroyl)27, Pro30, Lys(DOTA)31, Bip32, Leu34]NPY27–36. It demonstrated high stability in human serum and liver microsomal stability assays, with the longest half-life in human serum (144 min) among the tested analogs. This indicates its resistance to degradation by peptidases and suggests its potential for prolonged circulation in the bloodstream, making it a promising candidate for in vivo applications.

Another method to improve peptide stability while preserving the bioactive conformation is peptide stapling. A stapled peptide is a modified peptide that is constrained by a synthetic brace, a "staple". The staple is formed by a covalent linkage between two amino acid side chains, forming a peptide macrocycle via one-component or two-component strategies (see examples in Fig. 10a). Stapled peptides are usually characterized by improved stability and pharmacokinetic profiles, as, with respect to short and un-ordered peptides, they are less flexible and thus less accessible to proteolytic enzymes, making them more resistant in vivo and bioavailable [68–71]. Additionally, protease resistance could enable the oral administration of stapled peptides without being rapidly degraded in the gastrointestinal tract or in other biological barriers. The restriction in the degrees of conformational freedom of the carbonaceous backbone of stapled peptides also reduces their dynamic steric radii in water solution, thus enhancing their membrane permeability and cellular uptake. Interestingly, in some cases, rationally designed stapled peptides may also assume α-helical conformations, enhancing their potential activity as protein binders or as disruptors of protein-protein interactions, which usually occur via defined secondary structure motives [86]. In this scenario, peptide stapling provides a potent strategy to develop improved NPY analogs as this technique generally preserves a peptide bioactive conformation, potentially leading to high-affinity binders with enhanced cellular

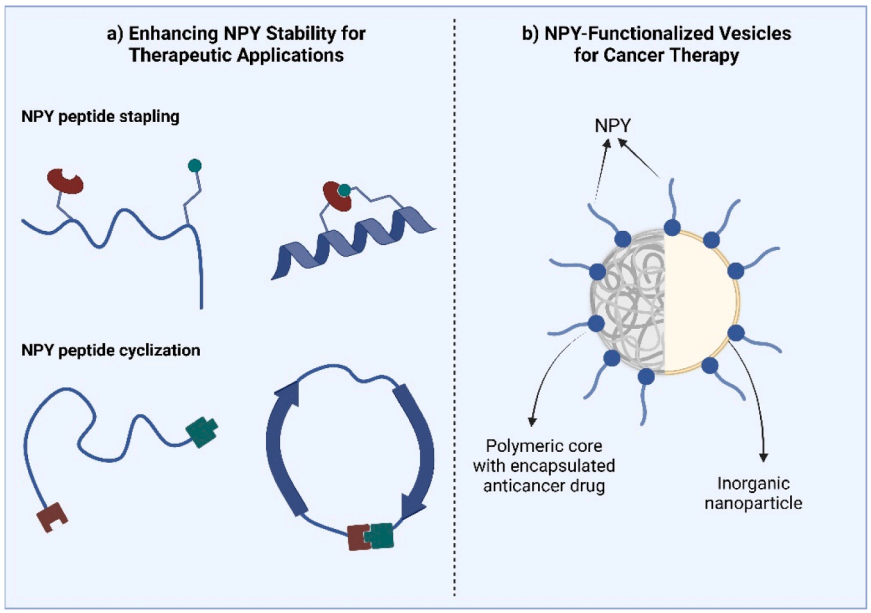

Fig. 8. Schematic representation of two effective solutions to improve peptides' stability and pharmacological profile: a) chemical peptide modification and b) conjugation of peptides to a drug delivery system. Created with BioRender.com.

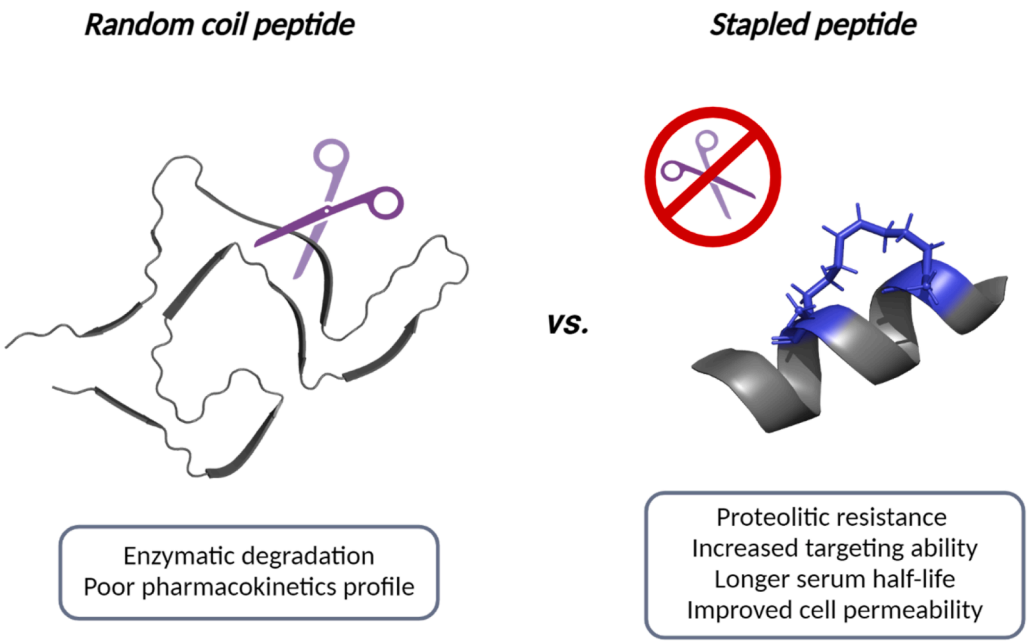

Fig. 9. Peptide stapling is an effective and practical method for designing synthetic peptides that possess improved pharmacokinetic and pharmacodynamic properties. For example, stapled peptides are more resistant to degradation by endogenous hydrolytic enzymes compared to unconstrained peptides.

uptake and stability [31,78].

Accordingly, Green et al. [87] exemplify this approach by creating stapled NPY analogs like NPY-S1 and NPY-S2, which demonstrate increased stability and improved pharmacokinetic properties compared to the unmodified NPY fragment (Fig. 10a shows the NPY-S1 structure, Fig. 10b shows the NPY-S2 structure) [87]. While peptide stapling and structural modification, in general, offer a promising avenue, the design of NPY analogs to be applied in drug discovery extends beyond these approaches. Recent research also explores the development of *minimized NPY analogs*, i.e., structures reduced to their minimal pharmacophoric groups with improved physicochemical and pharmacological profiles. One strategy involves lactam-bridge formation (Fig. 10c (i)), which has been shown to maintain receptor-binding affinities similar to unmodified peptides [87]. Additionally, hydrocarbon stapling, also known as dicarba bridge formation (Fig. 10c (ii)), has been explored to increase proteolytic resistance and enhance α-helical content for modulating protein-protein interactions within the cell. Another promising approach is click chemistry, specifically azide-alkyne cycloaddition (Fig. 10c (iii)), which allows for the formation of triazole bridges. This method facilitates selective cyclization and has the potential to improve both the stability and solubility of NPY analogs, making them more effective in drug development [87].

6.2. *NPY-based innovative nanodelivery systems*

Nanotechnology has been researched across different scientific

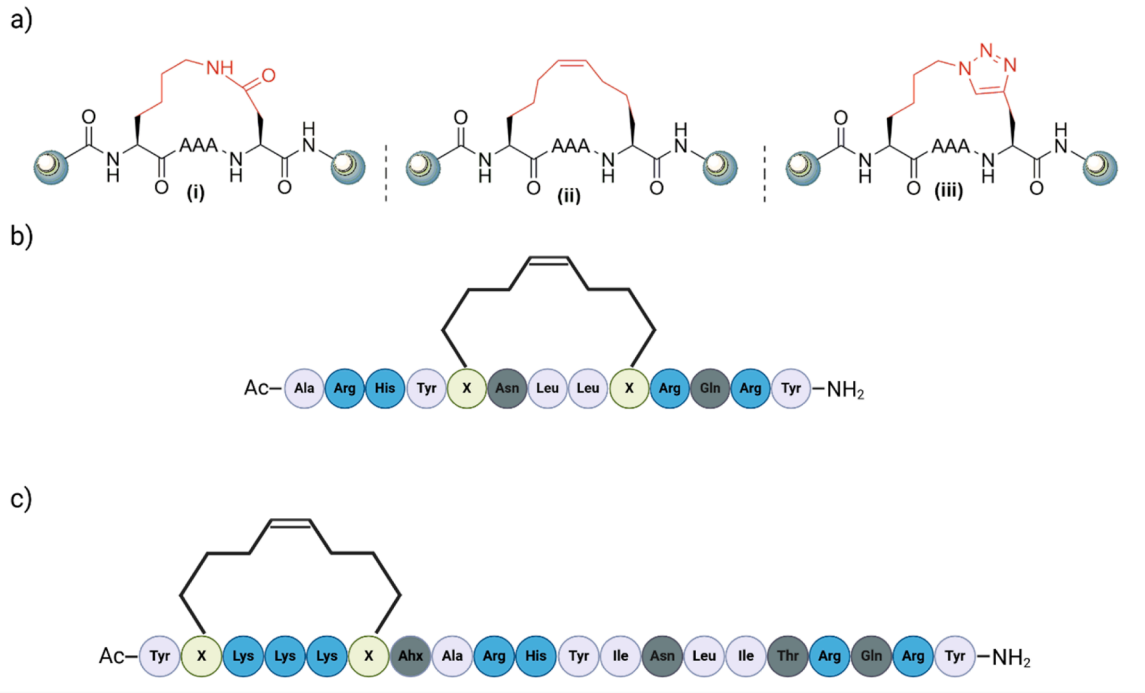

Fig. 10. Examples of peptide stapling. a) Different chemical strategies for one-component peptide stapling: (i) amide bridges, (ii) hydrocarbon, and (iii) click chemistry azide-alkyne cycloaddition. Examples of peptide stapling. b) Design of NPY analog, NPY-S2. In addition to the hydrocarbon staple, NPY-S2 includes modifications such as cationization motifs and a 6-aminohexanoic acid (Ahx) spacer to enhance its structural stability and functionality. The hydrocarbon staple helps stabilize the peptide's helical structure, with the modifications ensuring enhanced bioactivity. c) Design of another hydrocarbon-stapled neuropeptide Y analog, referred to as NPY-S1. The peptide sequence shown retains the critical C-terminal active fragment, NPY(25–36), necessary for biological activity. Created with BioRender.com, ChemDraw, and PyMOL (Schrödinger, LLC).

fields. Specifically, in drug delivery, nanoparticles (NPs) have shown potential in improving drug solubility, increasing bioavailability, facilitating the transport of substances across biological membranes, allowing controlled drug release, and improving targeting and protection against metabolism and degradation [31]. Functionalization of the surface of NPs can be a valuable technique to improve the specificity of the treatment, so-called active targeting. One way to modify NP surfaces is by attaching different active biomolecules, such as oligonucleotides, peptides, antibodies, aptamers, and more. Molecules can be conjugated to the NP surface through physical absorption with non-covalent interactions or via direct binding by covalent conjugation. Physical adsorption involves the molecule being linked to the surface by weak interactions, such as electrostatic interactions, hydrogen bonding, hydrophobic, and Van der Waals attractive forces. Therefore, advanced delivery methods could offer another avenue for overcoming the limitations of NPY. These systems not only optimize the pharmacokinetics of therapeutic agents but also offer multifunctionality for therapeutic and diagnostic applications. The development of NPY-decorated delivery systems for active cancer targeting is thus an intriguing field in which the NPYR-NPY molecular target could unveil its full potential. For instance, NPY-based drug delivery systems could leverage NPY's ability to interact with specific receptors to deliver therapeutic payloads directly to targeted areas [31,78]. Here, we will review the techniques for the conjugation of NPY on different NP systems [12]. A few inorganic nanomaterials in combination with NPY were reported in the literature.

As previously described, Avvakumova et al. [9] developed NPY-conjugated gold nanocages with three different NPY analogs tested. Conjugates have been exploited for localized photothermal therapy against in vitro prostate cancer models under NIR laser treatment at a wavelength of 800 nm. Conjugates with Au nanocages showed highly selective interaction with NPYR1 in the PC3 prostate cancer cell line and efficient uptake promoted by clathrin-mediated endocytosis.

Hild et al. devised a technique for binding neuropeptide Y (NPY) analogs to quantum dots (QDs) by introducing cysteine residues via lysine side-chain modification. This modification established specific binding sites for connecting the analogs to the QDs. Subsequently, thiol-reactive amino-PEG-coated QDs were prepared using sulfosuccinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (sulfo-SMCC), enabling the conjugation with peptides and subsequent purification via gel filtration chromatography. This method facilitated the precise attachment of ligands to the QDs, resulting in ligand-modified QDs capable of selectively interacting with the NPYR1 on cell surfaces. Various peptidic agonists and a nonpeptidic antagonist were covalently linked to QDs, allowing specific targeting of the NPYR1. Experimental findings revealed that both agonist-modified and antagonist-modified QDs exhibited highly selective binding to the NPYR1 receptor on MCF-7 breast cancer cells. Importantly, only the agonist-modified QDs were capable of being internalized by the cells. Furthermore, both agonist- and antagonist-modified QDs demonstrated significantly enhanced receptor affinity compared to free ligands, indicating potential applications in targeted drug delivery for cancer treatment. Cell culture experiments confirmed the specific binding of ligand-modified QDs to the NPYR1 on MCF-7 cells, with fluorescence imaging demonstrating localized QD binding primarily on the cell surface [77].

Also, graphene-based materials (GBMs) have gained increasing attention in the field of biomedical applications because the nanoscale dimension of GBMs is similar to that of subcellular elements in the nervous tissue [4]. This characteristic makes them suitable as selective nanocarriers targeting essential brain functional units. Graphene oxide (GO), in particular, has shown good dispersibility in water solutions and, because of a large number of functionalizable groups, is capable of carrying biologically active materials [88]. Cellot et al. [88] used NPY peptide terminated at the N-terminus with 5-hexynoic acid. Graphene oxide was functionalized with 11-Azido-3,6,9-trioxaundecan-1-amine (TEG-N3) and then coupled with NPY peptide with copper-catalyzed azide-alkyne click cycloaddition. In this case, NPY was selected as a

model because of its modulatory action on synaptic transmission. Still, Cellot et al. wanted to verify if NPY would retain its pharmacological activity after being conjugated to GO [88].

Also, Li et al. [89] have conjugated NPY analog [Pro30, Nle31, Bpa32, Leu34]NPY(28–36) (with Bpa = p-benzoyl-L-phenylalanine) with a potent anticancer drug doxorubicine, which was loaded into albumin nanoparticles. To avoid affecting the biological activity of NPY analogue, the carboxylic groups of albumin nanoparticles were activated by using 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDAC) and N-hydroxysuccinimide (NHS), and only then reacted with amine groups of NPY. This was necessary because the biologically active site of NPY to NPYR1 is located on its C-terminus. This drug delivery system showed the ability to selectively recognize and bind to NPYR1 that are present in high amounts on the surface of the breast cancer cells MCF-7. They have shown that 97.2 % of cancerous MCF-7 cell lines were able to internalize nanoparticles, while 79.2 % of cells were able to internalize nanoparticles without NPY attached to them. In addition, normal cells (MCF-10) have 70 % lower internalization than the MCF-7 cell line.

In summary, these examples demonstrate that conjugating peptides on the surface of nanoparticles offers a powerful strategy to enhance the specificity and efficacy of drug delivery systems in cancer treatment. This innovative approach holds significant potential to improve therapeutic outcomes by precisely targeting cancer cells while minimizing off-target effects.

7. Conclusions and future perspectives

Since GPCRs are abundant in the human body, ensuring the specificity of GPCR-targeting therapeutics is essential to minimize off-target effects. Selecting appropriate receptors that are predominantly expressed in cancer cells remains a critical but challenging step, often limiting the available options for targeted approaches. Notably, neuropeptide Y (NPY) and its receptors (NPYRs) have emerged as promising targets in cancer therapy. However, the literature reveals that the effects of NPY and its synthetic agonists or antagonists are highly tumor-dependent, with both growth-promoting [10,11] and growth-inhibitory [10] effects reported across various cancer cell lines. In addition, more signaling pathways may coexist and cross-talk with other ligand-receptor-based pathways, which leads to adaptive effects in cellular mechanisms [26]. Furthermore, NPY-dependent systemic effects, including alterations in energy metabolism, obesity, and immune responses, can influence cancer development and progression [90]. These findings underscore the urgent need for systematic studies that link receptor subtype selectivity with functional outcomes in phenotypic assays involving both tumor and normal cells. In other words, the precise role of these complex systemic activities of the peptide in the context of cancer remains to be explored in greater depth.

Off-target effects could represent a significant concern in the development and clinical application of NPY analogs as cancer-targeting agents. NPY analogs are designed to exploit the overexpression of specific NPY receptors on tumor cells; however, many of these receptors (e.g., Y1, Y2, Y5) are also expressed in several normal tissues, leading to possible unintended pharmacologic actions. For instance, physiological roles in the regulation of food intake, cardiovascular function, and stress response may be inadvertently affected when these analogs bind to receptors outside the tumor microenvironment [30]. Studies have highlighted that even though molecular modifications can increase selectivity towards tumor-associated receptor subtypes, such as those targeting NPYR1 [89], the distribution of these receptors in non-malignant tissues raises potential issues regarding therapeutic windows and safety profiles. In the context of cancer targeting, several strategies have been investigated to mitigate off-target interactions. One promising approach involves the use of nanocarrier systems that can encapsulate or conjugate NPY analogs, as demonstrated by the utilization of gold nanocages functionalized with NPY analogs for the imaging and photothermal therapy of prostate cancer cells [9]. This strategy aims to enhance accumulation in tumor tissue while minimizing exposure to healthy tissues, thereby reducing potential off-target effects. Chemical modifications on the peptide backbone, which refine the binding affinity and pharmacokinetics, have similarly been advanced to favor the desired receptor subtype engagement [85]. Such modifications are critical because subtle changes in the peptide sequence can significantly alter receptor selectivity, potentially reducing interactions with receptor subtypes not predominantly expressed in cancerous tissues. Another significant consideration is the biodistribution and metabolism of these analogs. While truncated and modified NPY peptides have been developed to target specific receptor profiles, many still experience rapid metabolism, which not only diminishes tumor uptake but may also lead to metabolites that interact with off-target receptors [91]. Consequently, optimizing in vivo stability is essential to ensure that the pharmacological activity remains confined to the tumor region, thereby enhancing efficacy and reducing off-target adverse effects.

To outline the future roadmap, we have identified the current challenges and limitations in developing NPY-NPYR-based therapeutics, along with strategies to address these obstacles. The challenges and solutions are categorized into three pillars, encompassing the full spectrum of drug discovery and development, as illustrated in Fig. 11 and discussed here.

To advance the clinical translation of NPYR-based therapeutics, it is vital to address several key challenges. First, a comprehensive understanding of the interactions between neuropeptides (NPY, PYY, PP) and their off-target effects is needed, along with detailed investigations into factors regulating NPYR subtype expression in cancer cells and their surrounding normal tissues. Moreover, most of the studies show in vitro cell line data, which are unable to fully mimic tumor heterogeneity and microenvironmental influences. Therefore, studying tumor-specific microenvironments in vivo, beyond traditional in vitro models, will provide critical insights into receptor activity and therapeutic potential. For example, NPYR1 has shown promise in breast [27,28] and prostate [44,45] cancer due to its selective overexpression in these tumor types compared to normal tissues, particularly in triple-negative breast cancer, which lacks effective therapeutic options.

Regardless of these concerns, the NPYR-NPY complex still represents a promising molecular target, especially in cancer imaging and the development of sophisticated delivery systems [7], [8]. These applications, in fact, do not require that the NPY (or its ad hoc-designed synthetic analogs) would be antiproliferative agents in themselves, but they "just" need to act as potent and selective molecular hooks for a specific NPYR subtype, selected based on the profiling of specific cancer lines. An example of this strategy has been presented by Avvakumova et al. [9], who developed theranostic systems for prostate cancer based on the decoration of gold nanocages with synthetic (shortened) NPY analogues. Their system was able to selectively target the NPYR1-expressing PC3 cell line and was efficiently internalized by clathrin-mediated endocytosis. This allowed, *in vitro*, the localized and selective photothermal therapy of prostate cancer models, besides properly visualizing them for diagnostic purposes.

To enhance the translational potential of these strategies, the rational design of NPY analogs using structure-based approaches is crucial. Techniques such as in silico docking, conformational analysis, and molecular dynamics simulations could help develop analogs with high receptor selectivity, minimal off-target effects, and improved pharmacokinetic profiles. However, predicting peptide structures in silico from their sequence remains a complex task that is far from being trivial [92–94]. Despite the challenges of predicting peptide structures and interactions, advancements in computational modeling and peptide-based biotechnologies are paving the way for innovative solutions.

Finally, considering the complexity of cancer biology, NPY-based therapies are unlikely to succeed as standalone treatments. Instead, they hold significant promise in combination with existing

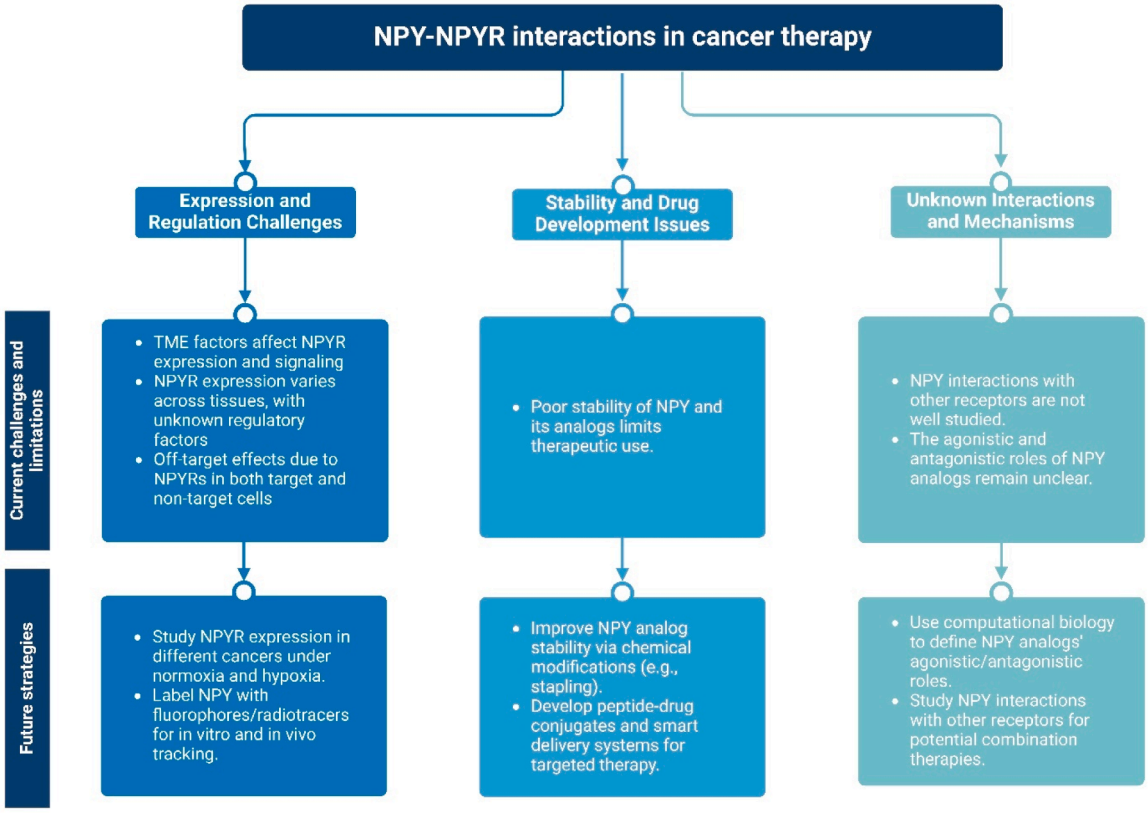

Fig. 11. Overview of NPY-NPYR interactions in cancer therapy, highlighting current challenges and future strategies. The challenges are categorized into three pillars: (1) Expression and Regulation Challenges, including variability in NPYR expression and signaling across tissues; (2) Stability and Drug Development Issues, focusing on the poor stability of NPY and its analogs; and (3) Unknown Interactions and Mechanisms, addressing the lack of understanding regarding NPY interactions with other receptors and the roles of NPY analogs. Proposed strategies to address these challenges are outlined below each category.

chemotherapeutics or other targeted agents, where their high-affinity binding to cancer cells could enhance drug delivery and sensitize tumors to treatment. For example, NPY analogs, in combination with the potent chemotherapeutic drug doxorubicin, proved to be highly effective on breast cancer cell lines [40,89]. As a strong immunomodulator, NPY can influence the immune response [89,90] and, thus, the cancer development, progression, and treatment outcome. In prostate cancer, inhibition of NPY suppressed macrophage migration and IL6 secretion and showed to be a good complementary therapy in prostate cancer patients with high psychological depression [95]. Therefore, the combination of NPYR-targeted therapy with chemotherapy and/or immunotherapy could be exploited as a new cancer therapeutic strategy. Overcoming the current limitations in receptor specificity, delivery system design, and preclinical validation will be critical for realizing the full clinical potential of NPYR-targeted therapies (Fig. 11).

CRediT authorship contribution statement

kostevšek nina: Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization. **Tomić Katarina:** Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation. **Bassanini Ivan:** Writing – review & editing, Conceptualization. **Romeo Sergio:** Writing – review & editing.

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Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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