Cancer and Metastasis Reviews (2025) 44:21
https://doi.org/10.1007/s10555-024-10237-z

REVIEW

Neuropeptide Y in cancer—biological functions and potential clinical implications

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Received: 31 August 2024 / Accepted: 20 December 2024 / Published online: 6 January 2025

Abstract

Neuropeptide Y (NPY) is a sympathetic neurotransmitter widely distributed in the peripheral and central nervous system, affecting many physiological functions. Consequently, dysregulation of the NPY system contributes to numerous pathological disorders, including stress, obesity, and cancer. The pleiotropic functions of NPY in humans are mediated by G protein-coupled receptors (Y1R, Y2R, Y5R), which activate several signaling pathways and thereby regulate cell growth, differentiation, apoptosis, proliferation, angiogenesis, and metabolism. These activities of NPY are highly relevant to tumor biology and known hallmarks of cancer, including sustained proliferative potential, resisting cell death, angiogenesis, invasion, and metastases. In this comprehensive review, we describe the cellular functions of NPY and discuss its role in cancer pathobiology, as well as provide the current state of knowledge pertaining to NPY and its receptors in various cancer types. Moreover, we focus on potential clinical applications targeting the NPY system, such as its role as a prognostic and predictive factor, as well as its utility in cancer diagnostics, imaging, and treatment. Altogether, growing evidence supports the significant role of the NPY system in tumor pathobiology and implicates its potential therapeutic and diagnostic value in modern oncology.

Keywords Neuropeptide Y · Cancer · Metastasis · Therapy · Biomarker

1 Introduction

Tumor growth and progression involve multiple interactions between cancer and host cells. While recent years brought significant progress in our understanding of the role the local tumor microenvironment plays in these processes, the impact of systemic factors and the overall physiological state of the patient on cancer remains understudied [1]. In this review, we will focus on one such factor, neuropeptide Y (NPY). NPY is a sympathetic neurotransmitter abundant in the central and peripheral nervous systems [2, 3]. The peptide is a crucial stress mediator, which regulates various physiological functions [4, 5]. Some of these processes, such as food intake, anxiety, and circadian cycle control, depend on NPY activity in the brain (Fig. 1A) [6]. Others, including cardiovascular and immune responses, are regulated by the peptide released from peripheral sympathetic nerves (Fig. 1B) [7, 8]. Consequently, dysregulation of the NPY system has been implicated in various disorders, with obesity and psychiatric diseases being the most extensively studied [5, 9]. However, growing evidence indicates a role for NPY in tumor biology, which is driven by its direct effects on cancer cells and their

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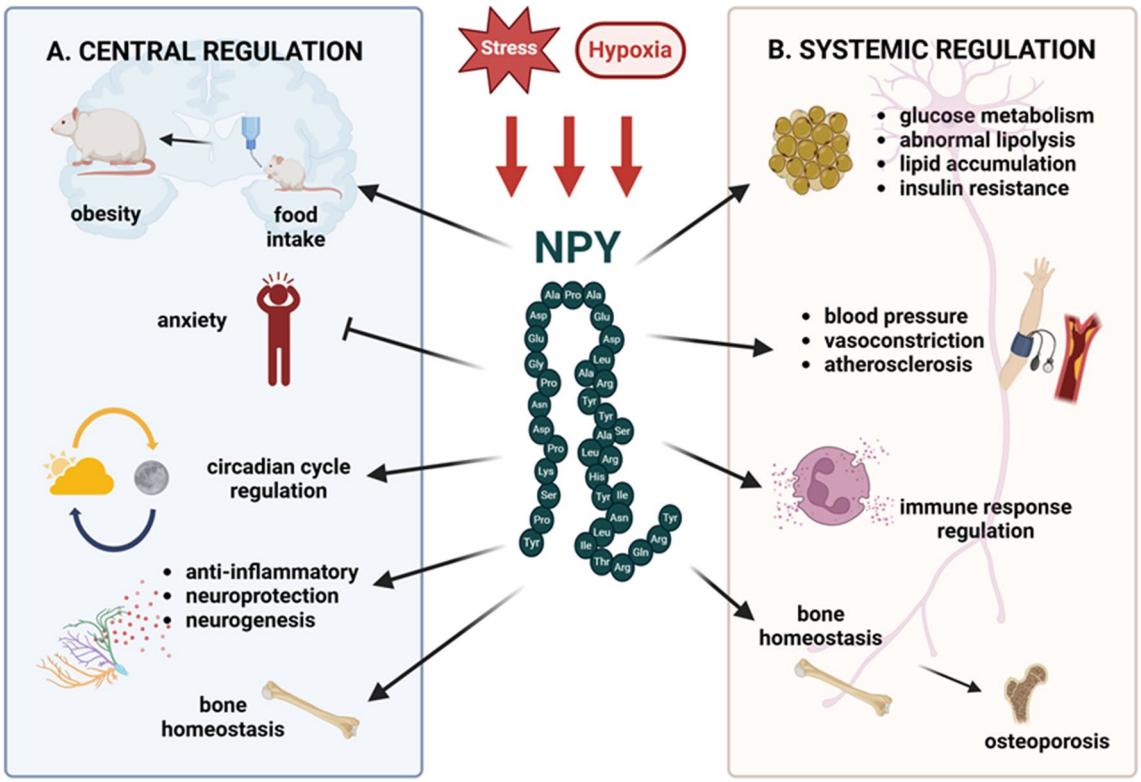

Fig. 1 Physiological functions of NPY. **A** As one of the most abundant peptides in the brain, NPY is involved in central regulation of processes involved in stress response, appetite stimulation, circadian rhythm, immunity and bone homeostasis. **B** In the periphery, NPY is co-released with norepinephrine from sympathetic neurons, affecting metabolism, cardiovascular system, bone health and immune responses (figure created using Biorender)

microenvironment, as well as indirect influence caused by altering patients' physiological responses known to affect growth and dissemination of various malignancies [10–14]. This review aims to summarize the current knowledge on the biological effects of NPY in cancer and their clinical implications, as well as delineate physiological functions of NPY that have the potential to affect cancer progression, yet have not been studied in the context of oncology.

2 Structure and components of the NPY system

NPY belongs to a family of evolutionary conserved peptides that also includes peptide YY (PYY) and pancreatic polypeptide (PP) [15, 16]. The peptides share the same receptors, although their expression patterns and affinities vary. The gene coding for NPY is located on chromosome 7p15 [17]. NPY is expressed mainly in central and peripheral nervous tissues. It is the most abundant peptide in the brain, with the highest expression in the hypothalamus, the area responsible for the control of the autonomic nervous system and its effects on metabolism, energy balance, and other crucial physiological processes [18]. In the periphery, NPY is stored in postganglionic large dense-core vesicles (LDCVs) of the sympathetic nerve endings and released with norepinephrine [19, 20]. The peptide is also present in other autonomic nerves, such as sensory or parasympathetic neurons, but at lower concentrations [21, 22]. Various factors, such as severe chronic stress, hypoxia, exercise, cold exposure, and ischemia, increase NPY release from neuronal cells and its levels in plasma and tissues [23–25]. Aside from the nervous system, NPY is also expressed in various non-neuronal cells and tissues, including endothelium, platelets, colon, kidney, testis, breast, and prostate [4]. However, it is unclear whether or not the peptide is secreted from these cells, and potential mechanisms regulating its release remain unknown. Importantly, platelets have been proposed as an NPY reservoir that could uptake and store the peptide at the time of its increased release, such as stress [26]. Consequently, under such conditions, elevated NPY levels are often detected in serum and plasma-containing platelets but not platelet-poor plasma samples [27–30].

NPY is a 36 amino acid peptide, which is derived from a 97 amino acid precursor, Pre-Pro-NPY [31–34] (Fig. 2). This large molecule is processed in the endoplasmic reticulum

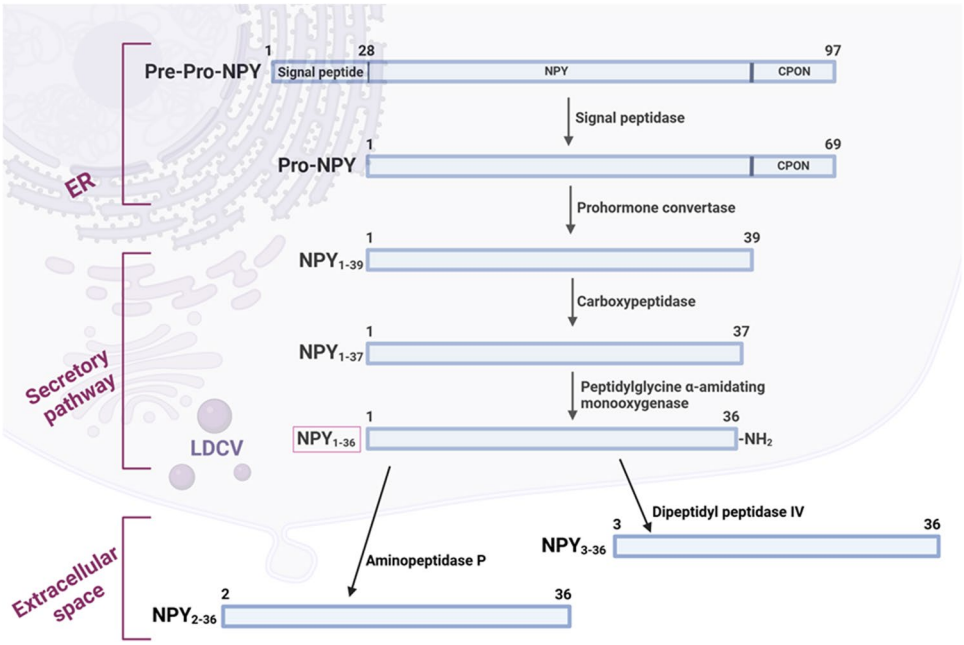

Fig. 2 The biosynthesis and processing of neuropeptide Y. NPY is synthesized as a 97-amino acid Pre-Pro-NPY, which undergoes proteolytic cleavage in endoplasmic reticulum and the secretory pathway leading to the secretion of the mature amidated 36 amino acid peptide. In the extracellular space, NPY may be further cleaved by aminopapetidase P and dipeptidyl peptidase IV to its truncated forms $NPY_{2–36}$ or $NPY_{3–36}$, respectively. ER, endoplasmic reticulum; CPON, carboxyl-terminal flanking peptide of NPY; LDCV, large dense-core vesicles (figure created using Biorender)

(ER) by removing a 28 amino acid signal sequence, so the Pro-NPY, a 69 amino acid peptide, is formed. Subsequently, Pro-NPY is transported to the Golgi apparatus, then to the trans-Golgi network, and moved towards the secretory pathway. Most of the NPY in a cell is stored in LDCVs [33, 34]. A number of processing enzymes are active during post-translational steps, including convertases, carboxypeptidases as well as the amidating enzyme—peptidylglycine α-amidating monooxygenase [33, 34]. Only the final, amidated form of NPY ($NPY_{1–36}$) is biologically active.

After its exocytosis to the extracellular space, NPY is subjected to further proteolysis (Fig. 2). Two essential enzymes which regulate the NPY system, aminopeptidase P and dipeptidyl peptidase IV (DPPIV), cleave one or two first amino acids from the N-terminus of the NPY protein, creating $NPY_{2–32}$ and $NPY_{3–36}$, respectively [35]. Both aminopeptidase P and DPPIV are membrane enzymes and their capabilities to cleave NPY are well documented. In addition, two intracellular dipeptidyl peptidases, DPP8 and DPP9 have been shown to truncate NPY to its $NPY_{3–36}$ form both as purified proteins and in cellular systems [36, 37]. However, the mechanisms by which these intracellular enzymes access NPY remain to be determined.

NPY acts through multiple membrane receptors named Y1R-y6R, which are widely distributed throughout the body and have different functions [38]. Y1R, Y2R, and Y5R are functional NPY receptors in humans, while Y4R serves mainly as the PP receptor since its affinity to NPY and PYY is low [39]. y6R is functional in mice, while in humans, it is encoded by a pseudogene, which is not transcribed [40]. The affinity of NPY to its receptors is regulated by its proteolytic cleavage. Y1R require the full-length $NPY_{1–36}$ for binding and are not activated by $NPY_{2–36}$ and $NPY_{3–36}$, while these truncated forms of the peptide preserve their ability to bind to Y2R and Y5R [35, 41, 42]. This change in the NPY receptor activation affects its functions, particularly in the cells expressing multiple types of its receptors, since $NPY_{3–36}$ will preferentially activate Y2R and Y5R, even in the presence of high levels of Y1R [36]. This shift in NPY receptor affinities modifies its activity in response to environmental stimuli, such as hypoxia [42].

NPY activity is further regulated by interactions between its heterotypic receptors. All NPY receptors have been shown to form homodimers, while Y1R can also heterodimerize with Y5R and Y4R [43–45]. However, even without direct receptor binding, heterotypic NPY receptors (e.g. Y2R and Y5R) can interact with each other, enabling cellular responses, such as cell proliferation and migration, to low peptide concentrations [44, 46, 47]. Similarly, NPY receptors have been shown to interact with other receptor types, including the β-adrenergic and TrkB receptors [46, 48]. The synergistic interactions between NPY and β-adrenergic receptors augment the mitotic effect of the peptide, while the transactivation process between TrkB and Y5R increases the pro-survival and antiapoptotic effects of NPY [46, 48]. Altogether, these interactions are crucial in regulating NPY actions, often leading to dramatic changes in its activity depending on the host's microenvironmental milieu and overall physiological state. However, the mechanisms underlying the increased NPY activity dependent on interactions of its heterotypic receptors or cross-talk with other membrane proteins are not well understood. It is not clear if such interactions increase the peptide binding or amplify receptor signalling.

All NPY receptors belong to the family of G protein-coupled receptors (GPCRs) and act mainly via the Gαi subunit [49] (Fig. 3). Consequently, all receptors inhibit adenylyl cyclase, leading to the decrease in cAMP levels and downregulation of PKA activity [44, 50]. The second mechanism of NPY signaling, which is specific for the Y1R and Y2R, involves the activation of phospholipase C (PLC), leading to Ca^{2+} mobilization and activation of calcium-dependent pathways and calcium–calmodulin (CaM)-dependent protein kinase II (CaMKII) [51–54]. Paradoxically, the NPY-induced CaMK activation may lead to an increase in the cyclic AMP response element binding protein (CREB) phosphorylation and stimulation of its transcriptional activity [55]. In concert, the above molecular events lead to the activation of the extracellular signal-regulated kinase 1/2 (ERK) belonging to the mitogen-activated protein kinase (MAPK) family, known as the main proliferative pathway [44, 54]. Moreover, NPY receptors have been shown to activate phosphoinositide 3-kinases (PI3K)/Akt axis, which is involved in regulation of cell survival [56]. The unique feature of Y5R is its ability to control cytoskeleton remodelling by activation of its key regulator, RhoA, which can promote cell migration [57, 58]. In addition to the above pathways that can directly affect cancer cell proliferation, survival, and motility, NPY modifies the activity of ion channels in receptor-specific manner, which is crucial for regulation of neuronal activity [59].

Upon their direct or indirect activation, ligand-bound NPY receptors undergo internalization. Next, they are degraded in lysosomes or returned to the cellular surface in active form. The above process depends on additional proteins, such as clathrins and arrestin [60, 61]. Such receptor internalization can facilitate drug delivery to the target cell. Consequently, several selective agonists of NPY receptors have been synthesized and proposed as vehicles for cancer imaging and nuclear medicine [62, 63].

3 Physiological functions of NPY

Due to its presence in the brain and in peripheral nerves, NPY coordinates the central and systemic regulation of various physiological processes (Fig. 1). One of the key aspects of physiology regulated by NPY is metabolism [64]. In the hypothalamus, NPY acts as an orexigenic peptide [65]. Its transcriptional gene expression is regulated by

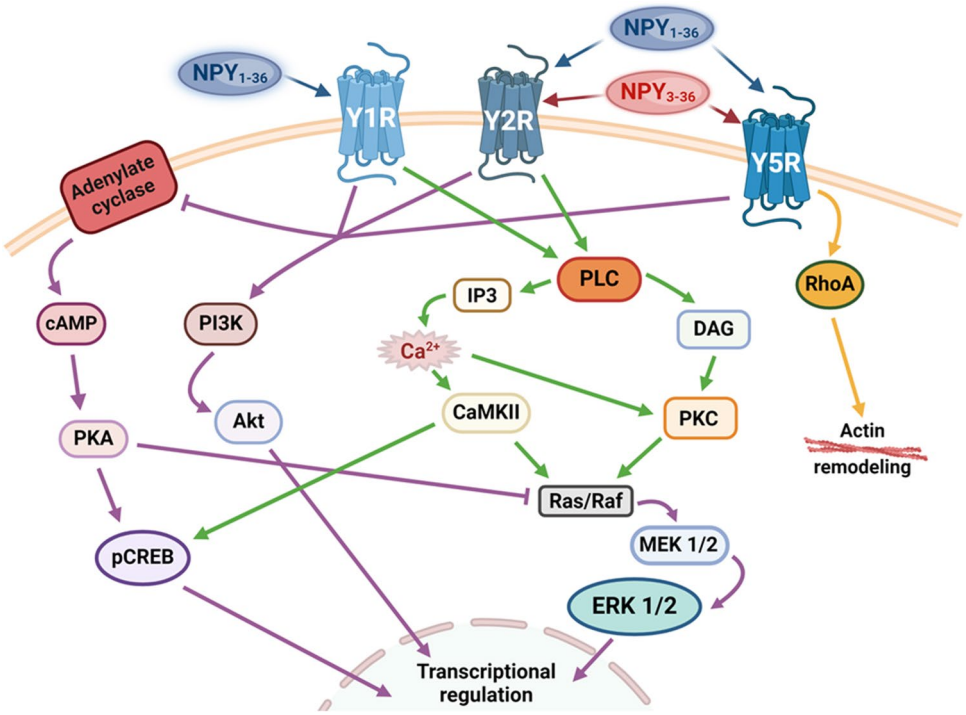

Fig. 3 The molecular mechanism of NPY actions relevant to cancer biology. In humans, NPY acts mainly via three GPCRs—Y1R, Y2R, and Y5R. Y1R requires the full length $NPY_{1–36}$ for the activation, while Y2R and Y5R can also bind to $NPY_{3–36}$. All NPY receptors signal through $G_{i/o}$ proteins and inhibit cAMP synthesis by adenylyl cyclase, which blocks the inhibitory effects of PKA on the downstream pathways. The second mechanism of NPY signaling, specific for Y1R and Y2R, involves $Ca.^{2+}$ mobilization and subsequent calcium-dependent activation of PKC and CaMKII. Concomitantly, these signaling pathways lead to the activation of ERK1/2. Moreover, all NPY receptors are capable of activating the PI3K/Akt pathway. The specific feature of Y5R is the ability to activate RhoA and thereby regulate cytoskeleton remodeling (figure created using Biorender)

ghrelin, insulin, and leptin [66]. The dysregulation in the NPY-hypothalamus-adipose tissue axis results in obesity. For example, in animal models, the overexpression of NPY in the hypothalamus led to hyperphagia and increased body weight [67]. However, the peptide can also stimulate obesity by its peripheral effects on adipocytes and vascularization of adipose tissue [23]. Here, NPY dysregulates glucose metabolism, drives abnormal lipolysis, and supports adipocyte differentiation, lipid accumulation, and insulin resistance [23, 68].

Similarly, NPY plays a dual role in the central and peripheral stress response. In the brain, NPY exerts anxiolytic effects [69]. In animals, intracerebroventricular administration of NPY had a sedative effect, which supports the anti-stress and anti-anxiety activity of the peptide [69]. Locally, the peptide plays an important role in maintaining central nervous system homeostasis by its neuroprotective and anti-inflammatory effects, inhibition of the ER stress, regulation of calcium homeostasis, stimulation of autophagy in the hypothalamus, and nutritional support [70]. Moreover, NPY promotes neurogenesis and protects neurons from injury [71].

In the periphery, NPY acts as a neuromodulator released from sympathetic nerves upon their high intensity stimulation [4, 5]. Consequently, the elevated NPY levels in the blood can serve as a marker of severe chronic stress [72]. Presynaptically, NPY inhibits catecholamine release from sympathetic nerve endings via its Y2R [73]. At the same time, the peptide takes over some functions of norepinephrine [74]. For example, in the cardiovascular system, NPY secreted from neurons innervating the vasculature, endocardium, and cardiomyocytes regulates blood pressure and vasoconstriction [8]. Moreover, prolonged exposure to high doses of NPY, for example during chronic stress, leads to an increase in smooth muscle cell proliferation and atherosclerosis [75, 76].

The NPY activity in the immune system is complex, with bimodal actions both stimulating and inhibiting inflammatory processes, depending on the receptor expression, immune cell type, and physiological context [77]. NPY regulates numerous functions of immune cells, including proliferation, migration, phagocytosis, antigen capturing, and cytokine secretion [7, 78]. This immunomodulatory effect can be exerted by neuronal NPY, acting as a critical player in the neuroimmune crosstalk, as well as endogenous peptide expressed by many immune cells, including monocytes, macrophages, lymphocytes, dendric cells, and granulocytes [7, 79–81]. Altogether, the overall role of the NPY system is to preserve immune homeostasis in response to various environmental stimuli [81].

NPY is also an important factor in the regulation of bone homeostasis. NPY receptors are expressed by osteoblasts, osteocytes, and osteoclasts. Consequently, the peptide controls both bone matrix resorption and formation via its effects on osteogenic and osteoclastic differentiation and osteoblast activity [82]. However, the reports regarding the specific role of NPY and its receptors are often contradictory, indicating both osteogenic and osteolytic effects [82]. Nevertheless, due to its impact on both bone and lipid metabolism, NPY has been implicated as a factor coordinating changes in fat tissue and bones in several disorders, including osteoporosis, cachexia, and bone metastasis [82].

Importantly, many systemic effects regulated by NPY actions in the central and peripheral nervous system are capable of affecting the course of the malignant disease (Fig. 4). For example, obesity that may result from a dysregulation of the NPY system is a well-known risk factor for development and progression of several malignancies, often associated with increased cancer-specific mortality [83]. Clinical studies confirmed that the levels of NPY are elevated in obese people, while adipose tissue itself is a rich source of growth factors and proinflammatory cytokines [84]. Similarly, NPY-induced changes in the immune system may affect anti-tumor response and inflammation, while the effect of the peptide on bone homeostasis may facilitate osseous metastasis [85].

The role of NPY as a sympathetic stress mediator may also impact cancer development and progression. Many studies suggest that chronic stress increases cancer risk and promotes its progression due to elevated levels of stress hormones and neurotransmitters [86]. While such effects are commonly attributed to the elevated cortisol levels and the resulting immunosuppression or increased catecholamine concentrations, the role of NPY in stress-induced cancer progression cannot be excluded [4]. Elevated NPY levels caused by chronic stress can exacerbate direct and indirect actions of the peptide on cancer cells and tumor microenvironment. Importantly, NPY is more stable than catecholamines and its stress-induced up-regulation lasts longer than the acute spikes in epinephrine and norepinephrine concentrations [4]. For example, psychological stress in prostate cancer patients leads to NPY-dependent immunosuppression, which in turn stimulates cancer progression [25, 87].

4 Direct effects of NPY on cancer cells

On a cellular level, NPY is involved in numerous processes crucial for cell survival and tissue repair, including growth, differentiation, apoptosis, and proliferation [88–91]. For example, the proliferative potential of NPY is observed in the central nervous system (neuronal precursors in the olfactory epithelium, hippocampus, retina, injured glial cells); muscles (smooth muscle cells, cardiomyocytes); endothelium and a variety of stem cells (mesenchymal, hematopoietic, adipose-derived and embryonic stem cells) [44, 92–95].

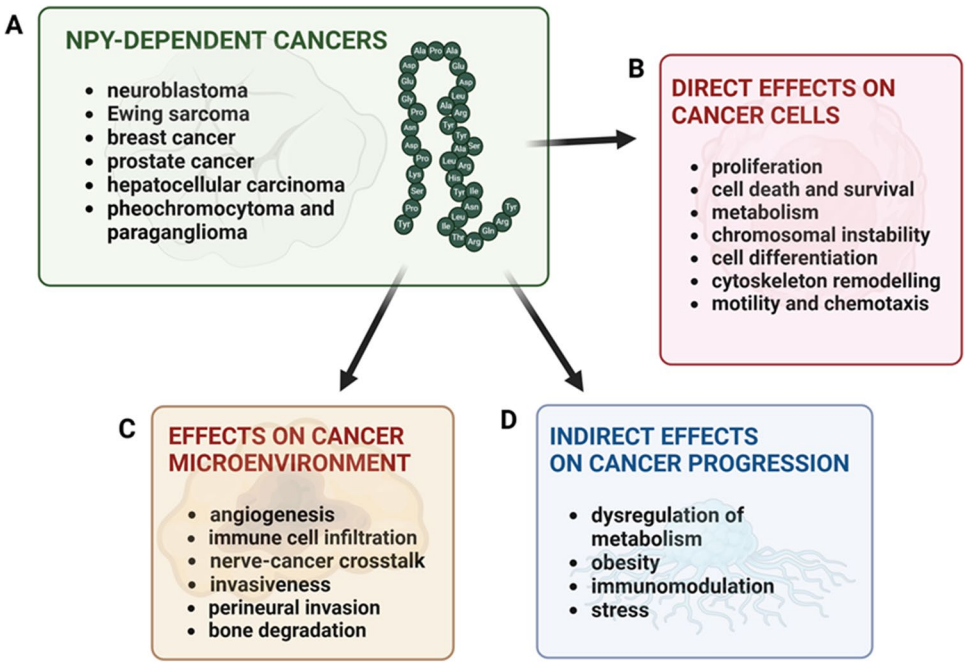

Fig. 4 The pleiotropic functions of neuropeptide Y in cancer biology. **A** Cancer types known to express NPY and/or its receptors. **B** Potential direct effects of NPY on cancer cells. **C** Processes regulated by NPY in the cancer microenvironment. **D** Systemic effects of NPY that can affect cancer development and progression (figure created using Biorender)

In multipotent embryonic cells, NPY maintains an undifferentiated state and drives their proliferation and self-renewal [95]. It also regulates the classical embryonal developmental pathways like WNT/β-catenin and Sonic Hedgehog (SHH) [96, 97]. NPY receptor expression has been detected in many cancer types, while the peptide itself can be synthesized by tumor cells or secreted from nerves and other stromal cells, e.g. immune infiltrates [87, 98–102]. Similarly, both cancer cells and host tissues, such as endothelium, express DPPIV, which can modulate NPY actions [36, 103]. Thus, the crucial role of NPY in regulation of a variety of cellular functions is highly relevant to tumor biology and can contribute to processes characterized as hallmarks of cancer (Fig. 4) [104–106].

Sustained proliferative potential NPY has been shown to stimulate proliferation of various cancer cells, including neuroblastoma, breast cancer, and some prostate cancer cell lines [91, 102, 107, 108]. These proliferative properties of NPY depend on Y1R, Y2R, and Y5R activation resulting in stimulation of the ERK1/2 MAPK pathway (Fig. 3) [44, 52, 108]. However, the receptor expression and activities vary between cancer types. Moreover, the mitogenic response to low concentrations of NPY may be enhanced by interactions between its heterotypic receptors [44]. Such proliferative properties of NPY can promote tumor growth.

Resisting cell death NPY has the ability to both stimulate and inhibit cell death. In Ewing sarcoma, NPY acting via simultaneous activation of Y1R and Y5R triggers cell death mediated by poly(ADP-ribose) polymerase (PARP-1) and apoptosis-inducing factor (AIF) [36, 91, 109]. However, this effect can be alleviated by the high activity of DPPIV in these cells, which converts the peptide to $NPY_{3–36}$ that does not bind to Y1R [35, 36]. On the other hand, in neuroblastoma, cellular stress triggered by chemotherapy or growth factor withdrawal induces expression of Y5R, which acts as a survival factor for tumor cells [48]. This effect is mediated by interactions of Y5R with brain-derived neurotrophic factor (BDNF) and its TrkB receptor [48]. However, the exact mechanisms underlying this phenomenon remain to be determined.

Deregulating cellular energetics Cancer progression is also associated with profound changes in cellular metabolism. The Warburg effect, defined as aerobic glycolysis, is a well-known phenomenon in tumor cells [110]. NPY regulates energy balance and stimulates a Warburg effect in cancer cells. In prostate cancer, NPY is necessary to maintain high metabolic activity, while in neuroblastoma it up-regulates glutaminolysis, glycolysis, and possibly tricarboxylic acid cycle activity, which confirms its function as a sensor of energy metabolism [102, 111, 112].

Genome instability Recent studies identified a new role for the NPY system in stimulation of chromosomal instability. In hypoxic Ewing sarcoma cells, over-activation of the NPY/Y5R pathway leads to abnormally high activity of the RhoA axis, which results in cytokinesis failure and leads to the formation of polyploid cells [57]. The progeny of these polyploid cells creates a unique cell population that exhibits high levels of chromosomal instability and propensity for bone

metastasis [57]. This is the first indication of the role for the NPY system in triggering genomic evolution of cancer cells [57]. Further studies are required to determine if similar processes occur in other cancer types expressing Y5R.

Unlocking phenotypic plasticity Phenotypic plasticity may result from transient transcriptional changes. One of the key aspects of such plasticity is the regulation of cell differentiation and stem cell-like properties [113]. Cancer stem cells may drive tumor initiation, metastasis, and relapse. NPY regulates the proliferation, differentiation, migration, and survival of different types of stem cells suggesting that the stimulation of tumor cell stemness may be another mechanism by which NPY can contribute to the progression of cancer [95]. Indeed, in Ewing sarcoma, NPY has been shown to selectively stimulate proliferation and migration of hypoxic cancer stem cells identified by high activity of aldehyde dehydrogenase [42]. However, understanding the contribution of the NPY signalling to cancer stemness requires further investigations.

Activating invasion and metastasis In addition to its effect on tumor growth, NPY can also contribute to cancer dissemination. The peptide stimulates the motility of various cancer cells and acts as a chemotactic factor for some of them. The pro-migratory properties of NPY were described in Ewing sarcoma, neuroblastoma, breast, prostate, and hepatic cancers [42, 58, 102, 107, 114]. However, in some colorectal cancer and cholangiocarcinoma cell lines, NPY decreased tumor invasion [115, 116]. The signaling pathways mediating these pro-migratory effects may include MAPK and PI3K [107, 117, 118]. In addition, recent studies indicated the direct effect of NPY on cytoskeleton remodelling during cell movement by Y5R-mediated RhoA activation [58]. These interactions occur in the leading and trailing edges of migrating cells, facilitating their motility [58]. The pro-migratory actions of NPY can be further enhanced by interactions between its heterotypic receptors, as previously suggested for endothelial and neuroblastoma cells [44, 47, 58].

The NPY-induced tumor cell motility can facilitate a locoregional and distant cancer spread. Clinical data strongly support the role of the NPY system in neuroblastoma metastasis [57, 99, 114]. The direct evidence for the role of the NPY/Y5R axis in local invasion were demonstrated in animal models of hepatic cancer, while the same pathway has been shown to stimulate distant bone metastasis in Ewing sarcoma orthotopic xenografts [57, 99, 114]. However, the role of the NPY system in overall cancer dissemination to other metastatic niches remains to be proven.

5 Effects of NPY on tumor microenvironment

In addition to its direct effects on cancer cells, NPY modifies the tumor microenvironment (Fig. 4C). The peptide is involved in molecular crosstalk between cancer and stroma, including neuronal, vascular, immune, and bone cells [47, 82, 119]. Some of these actions fall under the category of previously described hallmarks of cancer, while others are emerging interactions with the tumor environment that can pertain to selected cancer types [104–106].

Inducing angiogenesis Zukowska et al. discovered that NPY is an angiogenic factor that induces proliferation, migration, differentiation, and capillary tube formation by endothelial cells [24, 29, 47, 120]. The angiogenic functions of NPY are the most profound in hypoxia and ischemia when the levels of NPY increase. Y2R has been identified as the main angiogenic NPY receptor, although Y1R and Y5R also contribute to this process [24, 47, 121, 122]. The angiogenic potency and efficacy of NPY in neoangiogenesis are comparable to that of vascular endothelial growth factor (VEGF), and at least partially mediated by the VEGF system [24, 120]. The angiogenic activities of NPY are particularly important in tumors secreting endogenous NPY, such as neuroblastoma and Ewing sarcoma [42, 108, 109, 123].

Tumor-promoting inflammation and avoiding immune destruction The immune system can play a dual role in cancer development and progression. On one hand, infiltration with inflammatory cells, such as tumor-associated macrophages (TAMs), can stimulate these processes, on the other hand, the cellular immune response involving natural killer (NK) cells and cytotoxic T lymphocytes (CTL) have the capability of eliminating cancer cells [106]. As a potent immunomodulator, NPY can contribute to both of these aspects of immuno-oncology. In prostate cancer patients, stress-induced increase in NPY release has been associated with increased recruitment of myeloid-derived suppressor cells, infiltration of cancer tissues by TAMs, and secretion of immunosuppressive interleukins [25, 87]. Moreover, NPY is capable of inhibiting NK cell activity, which is a key component of the anti-tumor immune response, as well as regulating the recruitment and proliferation of lymphocytes [81]. However, the role of NPY in regulation of immune response is context-specific and the impact of NPY-dependent immune changes on cancer biology remains understudied.

Neuronal-cancer crosstalk In the last few years, the neurobiology of cancer has become an important field of

investigation into the mechanisms contributing to carcinogenesis and cancer progression [124]. Nerves constitute important components of the neoplastic microenvironment, which regulate the biology of many tumors [125]. Preclinical and clinical data indicate that NPY-positive nerves, potentially arising due to tumor-induced neurogenesis, serve as a paracrine source of NPY for tumor cells [102]. In prostate cancer, such paracrine NPY signaling exerts multifaceted effects and regulates tumor metabolism, apoptosis, motility, and therapy resistance [102]. The expression of NPY is also higher in perineural invasion areas in prostate cancer tissues, suggesting a potential role for the peptide in perineural spread [126].

Bone invasion and metastasis Among its various physiological activities, NPY is also an important regulator of bone homeostasis, suggesting its potential role in bone degradation induced by tumors expressing the endogenous peptide. Indeed, in Ewing sarcoma, tumor areas infiltrating bone had higher NPY expression [127]. In line with this observation, NPY-rich Ewing sarcoma xenografts had a high degree of bone degradation, while genetic knockdown of the peptide expression reduced tumor-induced osteolysis [127, 128]. Moreover, studies on Ewing sarcoma xenograft models indicated that cell lines, which secrete NPY to the environment have a higher capacity to metastasize to bone, as opposed to the cells that do not release the peptide [127]. The exact mechanisms by which tumor-derived NPY alters the bone environment and promotes infiltration of this tissue remains to be determined, as the peptide may affect differentiation and activity of both osteoblasts and osteoclasts. Interestingly, many NPY-rich cancer types, such as neuroblastoma, Ewing sarcoma and prostate cancer, are known to metastasize to bone [91, 98, 127].

6 Cancer type-specific effects of NPY

As described above, NPY is a pleiotropic factor with a wide range of functions that are relevant to the key hallmarks of cancer (Fig. 4). While some of these activities of NPY, such as its angiogenic potential, are universal between various malignancies, others are specific for the particular cancer types [91]. Below we outline the best-described activities of NPY in several malignancies, although its role has also been implicated in other tumor types (Table 1). This includes tumors with known endogenous NPY expression, which are capable of secreting the peptide, and those that rely on NPY present in the tumor microenvironment and the circulation.

Neuroblastoma Neuroblastic tumors constitute a heterogeneous group of pediatric malignancies arising from the precursors of sympathetic neurons [129, 130]. These tumors with wide clinical, morphological, and molecular spectrum develop in adrenal glands or autonomic ganglia [130]. Due to their sympathetic origin, neuroblastoma cells produce NPY and secrete the peptide [91, 109]. Consequently, systemic NPY levels in the serum of neuroblastoma patients are often elevated, which correlates with unfavorable prognostic factors, such as poorly differentiated phenotype and the presence of metastases [99, 131, 132]. The high NPY serum concentrations at diagnosis can also predict future relapse [99]. However, thus far, NPY is not accepted as an independent prognostic factor.

NPY secreted from neuroblastoma cells regulates their function in an autocrine manner via Y2R and Y5R. Y2R is constitutively expressed in neuroblastoma cells and its autocrine activation is crucial in maintaining tumor cell proliferation (Fig. 5A) [108]. In contrast, Y5R is an inducible NPY receptor in neuroblastoma [48]. The levels of Y5R increase under cellular stress, such as chemotherapy or serum deprivation, promoting neuroblastoma cell survival under these conditions [48]. Moreover, the NPY axis interacts with the BDNF/TrkB pathway, which induces NPY and Y5R expression and transactivates Y5R upon BDNF stimulation [48]. Altogether, these coordinated activities of the NPY and BDNF systems enhance the neuroblastoma resistance to chemotherapy [48]. Consequently, expression of NPY and Y5R is particularly high in neuroblastoma cell lines and tissues derived from patients previously treated with cytostatic therapy (Fig. 5B) [48].

In contrast, in neuroblastoma tissues not subjected to treatment, the Y5R immunoreactivity is particularly high in migratory and angioinvasive neuroblastoma cells accumulating around blood vessels (Fig. 5A) [99]. In line with this localization, the NPY/Y5R autocrine loop has been shown to stimulate neuroblastoma cell motility and invasiveness via direct effects on RhoA activity and cytoskeleton remodelling [58]. These data are in agreement with clinical correlations between high systemic NPY levels and metastatic phenotype of the disease [99]. However, the direct effect of the NPY/Y5R pathway on neuroblastoma dissemination in vivo remains to be proven.

In addition to its effect on cancer cells, NPY secreted from neuroblastoma is crucial for tumor vascularization [108, 109]. Notably, the NPY-induced angiogenesis is also dependent on the activity of Y2R and Y5R in endothelial cells, further supporting the NPY/Y2R/Y5R axis as a potential target in neuroblastoma therapy impacting multiple processes involved in the disease progression to the therapy-resistant and metastatic phenotype [47, 108, 109].

Ewing sarcoma Ewing sarcoma arises in bone and soft tissue, most often in pediatric and adolescent patients [133]. The disease develops due to a chromosomal translocation

Table 1 The NPY system in cancer

Cancer type	NPY tissue expression	Receptor expression	Preclinical data	Clinical data
Neuroblastoma [44, 48, 58, 99, 108, 109]	Yes	Y2R—constitutive Y5R—inducible	-Y2R—promotes tumor cell proliferation and angiogenesis -Y5R—promotes invasiveness, motility, cytoskeleton remodeling, and survival -NPY/Y5R axis—activated in pro-apoptotic conditions	-NPY is elevated in the serum of patients with poorly differentiated and metastatic tumors -High systemic NPY at diagnosis predicts future relapse -Y5R expression is elevated in cells with angioinvasive phenotype and in post-treatment tumors
Ewing sarcoma [42, 57, 100, 109, 127]	Yes	Y1R, Y5R—constitutive Y2R—inducible	-DPPIV-dependent receptor switch from growth-inhibitory Y1R/Y5R-induced effects to growth-promoting Y2R/Y5R-dependent actions -Y2R—promotes tumor vascularization -Y2R/Y5R—stimulate proliferation and migration of cancer stem cells -High NPY expression associates with bone metastasis -Hypoxia-inducible NPY/Y5R/RhoA axis promotes osseous dissemination	-NPY is elevated in serum of Ewing sarcoma patients with bone tumors -NPY system expression is increased in tissues from bone Ewing sarcomas, as compared to soft tissue tumors -High DPPIV activity in serum correlates with better survival in patients with localized Ewing sarcoma
Breast cancer [11, 101, 107, 145–148]	No data	Y1R, Y2R, Y5R	-Y5R promotes proliferation, migration, and angiogenesis via stimulation of VEGF release -Y1R upregulated by estrogens -Y2R promotes chemotaxis	-Y2R predominant in normal breast tissues, switch to Y1R expression in breast cancer -Luminal A breast cancer has the highest Y1R expression; high Y1R expression predicts better survival -In ER-positive breast cancer, Y1R inhibits estrogen-induced cell proliferation -The pattern of receptors is similar in primary and secondary tumors -In Egyptian breast cancer patients, high Y1R expression correlates with advanced stage, metastatic disease and perineural invasion
Pheochromocytoma paraganglioma [139, 174]	Yes	Y1R, Y2R	No data	-NPY is increased in the serum of cancer patients -NPY is higher in adrenal than in extra adrenal tumors

Table 1 (continued)

Cancer type	NPY tissue expression	Receptor expression	Preclinical data	Clinical data
Prostate cancer [12, 98, 126, 150–153, 175]	Yes	Y1R, Y2R, Y5R	-NPY acts as a chemotactic factor for LNCaP cells -Y1R inhibits proliferation of LNCaP and DU145 cells -Y1R increases proliferation of PC3 cells -Neuronal NPY regulates prostate cancer cell metabolism and treatment resistance	-Expression of NPY and all its receptors is higher in prostate cancer than in benign prostate lesions -Particularly high NPY expression is observed in ERG fusion-positive cases -Plasma NPY levels are elevated in prostate cancer patients and positively correlate with Gleason score; high NPY in platelets predicts worse survival -High percent of cancer cells positive for NPY by immunostaining correlates with increased risk of biochemical recurrence after prostatectomy -High pro-NPY protein levels are associated with increased mortality -Elevated immunostaining for Y1R, Y2R, and Y5R in areas of perineural invasion and in areas of extraprostatic extensions -Low NPY mRNA levels are associated with adverse genomic features and high-risk disease -Density of NPY-positive nerves predicts cancer-related death, biochemical recurrence, and radiation therapy resistance
Hepatocellular cancer [114, 176]	Yes	Y1R, Y2R, Y5R	-Y1R inhibits hepatic cancer cell proliferation -Y5R stimulates hepatic cancer cell proliferation, stemness, chemotaxis, and invasion -DPPIV promotes Y5R-mediated effects in liver cancer	-Y5R, but not Y1R and Y2R, are elevated in the tumor, as compared to normal liver -High Y5R expression correlates with worse overall survival -Low Y1R correlates with a shorter overall survival and more aggressive phenotype of the disease
Colorectal cancer [115, 177, 178]	Yes	Y1R, Y2R	-Y1R stimulates proliferation -Y2R stimulates angiogenesis -NPY reduces the invasiveness of colorectal cancer cells	-NPY and Y2R expression is elevated in colorectal cancer, as compared to the normal colon, and further increases in metastatic primary tumors
Cholangiocarcinoma [116]	Yes	Y1R, Y2R, Y5R	-NPY decreases proliferation and invasiveness -NPY expression is higher in the center of the tumor -NPY secretion is upregulated, as compared to cholangiocytes	-NPY expression is increased in cholangiocarcinoma, as compared to normal tissue
Pancreatic cancer [167, 179]	Yes	Y1R, Y2R	No data	-Hypermethylation of NPY gene and its low expression correlate with low patient survival -Y2R expression is increased in cancer, as compared to normal tissue, while NPY and Y1R expression does not change

Table 1 (continued)

Cancer type	NPY tissue expression	Receptor expression	Preclinical data	Clinical data
Melanoma [158, 159]	Yes	No data	No data	-NPY expression is increased in melanoma, as compared to melanocytic nevi; metastases are negative for NPY -Data on clinical correlations are conflicting and based on limited number of cases
Testicular Germ cell tumors [180]	No data	Y1R, Y2R	No data	No data
Ovarian cancer [181]	No data	Y1R, Y2R	No data	No data
Pituitary adenoma [182]	Yes	No data	No data	No data
Renal cell carcinoma [183]	No data	Y1R	No data	No data
Nephroblastoma [183]	No data	Y1R, Y2R	No data	No data

resulting in the fusion between the EWSR1 gene and gene encoding a transcription factor from the ETS family, typically FLI1 or ERG gene [133, 134]. The most common chromosomal translocation in Ewing sarcoma is t(11;22)(q24;q12), which leads to the formation of the EWS-FLI1 fusion protein [134]. These abnormal transcription factors stimulate expression of numerous genes, including NPY and its Y1R and Y5R [135, 136]. Consequently, all Ewing sarcoma tumors express high levels of these proteins (Fig. 5C) [100]. Additionally, in approximately 50% of Ewing sarcoma patients, tumors secrete NPY, resulting in elevated systemic levels of the peptide in serum [100]. Paradoxically, the initial studies indicated that the NPY/Y1R/Y5R autocrine loop, which is up-regulated by EWS-FLI1, triggers cell death mediated by the PARP-1/AIF signalling [36, 91, 109]. However, this effect can be blocked by endogenous DPPIV, which cleaves NPY to its $NPY_{3–36}$ form, inactive at Y1R [36]. This process is enhanced by hypoxia, which stimulates expression of NPY, Y5R, and DPPIV, and induces expression of Y2R, effectively shifting the activity of the peptide to the Y2R/Y5R axis [42]. Notably, this pathway increases proliferation and motility of the Ewing sarcoma cells with cancer stem cell properties [42]. In line with this, expression of the hypoxia-inducible Y2R in Ewing sarcoma tissues is associated with worse patient survival [42].

In addition to its overall effect on Ewing sarcoma cell biology, high levels of endogenous NPY have been associated with bone invasion and metastasis. In Ewing sarcoma patients, serum NPY concentrations are higher in patients with bone primary tumors, as compared to those with extraosseous lesions [100]. The tissues from tumors developing in bone had also increased expression of the NPY system [100]. In animal models, xenografts derived from Ewing sarcoma cells capable of secreting NPY metastasized to bones and had a higher degree of tumor-induced bone degradation, as compared to xenografts with low NPY expression [127]. Consequently, NPY knockdown in Ewing sarcoma cells reduced bone degradation within primary tumors, implicating NPY as an osteolytic factor [127]. However, the exact mechanisms of this effect remain to be determined.

Aside from its osteolytic activity, NPY is an important factor linking tumor hypoxia, chromosomal instability, and bone metastasis. Recent studies indicated that severe hypoxia overactivates the NPY/Y5R/RhoA pathway in Ewing sarcoma, leading to cytokinesis failure and whole genome doubling [57]. Subsequently, the progeny of these polyploid cells undergo abnormal cell divisions, creating a specific cell population, which exhibits high levels of chromosomal instability and preferentially metastasizes to bone [57]. Further studies are required to determine the mechanisms facilitating osseous dissemination of this cell

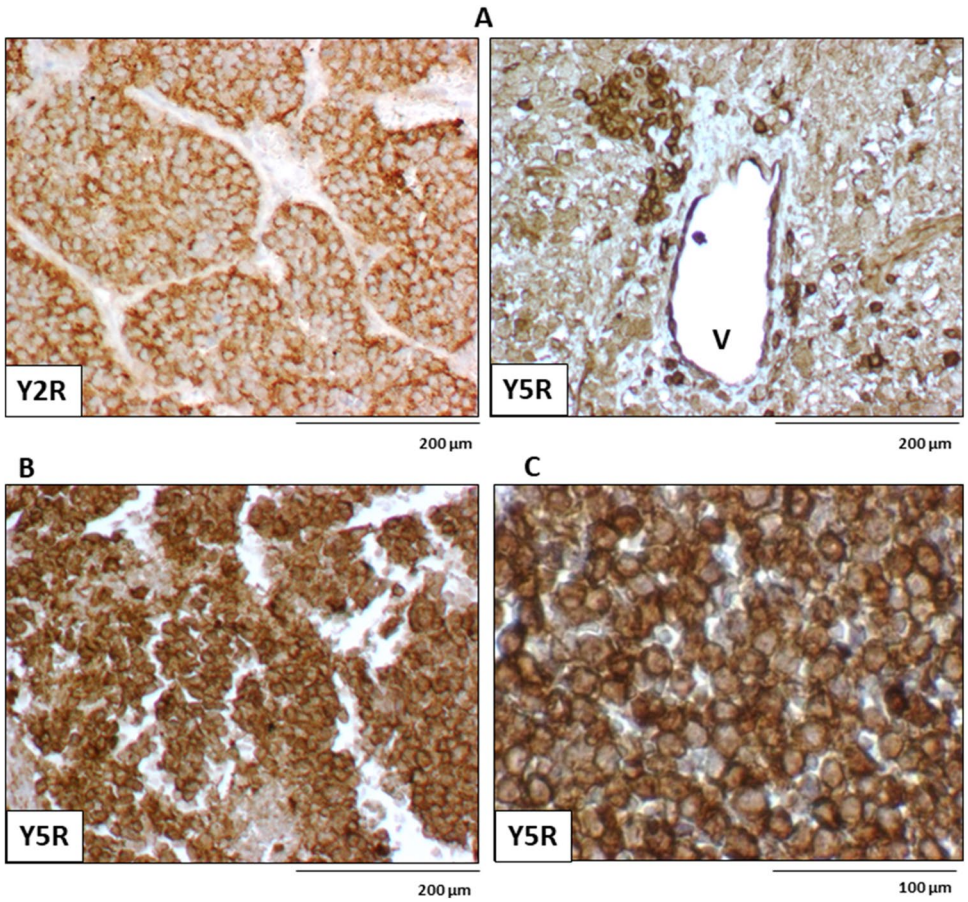

Fig. 5 NPY receptors in pediatric tumors. **A** Neuroblastoma—poorly differentiated, chemonaive tumor with strong and diffuse expression of Y2R. Y5R expression within the neoplastic cells is generally low, while high Y5R levels are seen in the endothelial cells and neuroblasts accumulated around blood vessels (200×). **B** Post-treatment chemoresistant neuroblastoma showing strong Y5R expression (200×). **C** Chemonaive Ewing sarcoma with strong Y5R expression (400×)

population, as well as the role of the NPY system in overall Ewing sarcoma metastasis to other niches.

Altogether, despite their different origins, both neuroblastoma and Ewing sarcoma tumors express high levels of endogenous NPY and are capable of secreting the peptide [99, 100, 109]. If released to the tumor environment, particularly under cellular stress, NPY exerts growth-promoting and prometastatic properties via the NPY/Y2R/Y5R axis [42, 48, 57, 58, 108]. Moreover, in both tumor types, the peptide exerts angiogenic activities via the same Y2R/Y5R pathway [42, 108, 109]. Taken together, these data warrant further investigation into the value of the Y2R and Y5R as potential therapeutic targets in these malignancies.

Sympathetic tumors of the adults Pheochromocytoma and paraganglioma are tumors of sympathetic origin developing in adults from chromaffin cells residing in the adrenal gland and their neural crest precursors in the sympathetic ganglia [137]. Under physiological conditions, NPY is secreted from these cells, where it inhibits catecholamine release [138]. Consequently, in patients with pheochromocytomas and paragangliomas, tissue expression of NPY is high and the plasma concentration of the peptide is elevated in 20 to 67% of patients, depending on the form of NPY measured [139–141]. This data suggests that NPY may be used as a catecholamine substitute in diagnostics of these tumors, especially in patients with kidney impairment, or treated with a drug that interferes with catecholamine metabolism [141, 142]. It was suggested that NPY mRNA levels might distinguish the benign from the malignant form of the tumor [143]. However, other reports indicated that the level of NPY does not correlate with the degree of malignancy [144]. Overall, despite its high expression, the biological functions of NPY in these tumors are not clear.

Breast cancer During breast carcinogenesis, NPY receptor expression switches from Y2R to Y1R, and the Y1R is present in 85% of breast cancer cases (Fig. 6) [101]. Y1R expression in breast cancer is up-regulated by estrogens [145]. The gene and protein expression of Y1R is the highest in luminal A and the lowest in the HER2-positive molecular subtype of breast cancer [146]. In a biologically unselected population of breast cancer patients, high Y1R expression in circulating tumor cells was a predictive biomarker for lymph node metastases and a prognostic factor for shorter patient survival [11]. Also, the immunohistochemical analysis of Y1R in an Egyptian population of patients with breast cancer revealed correlation of

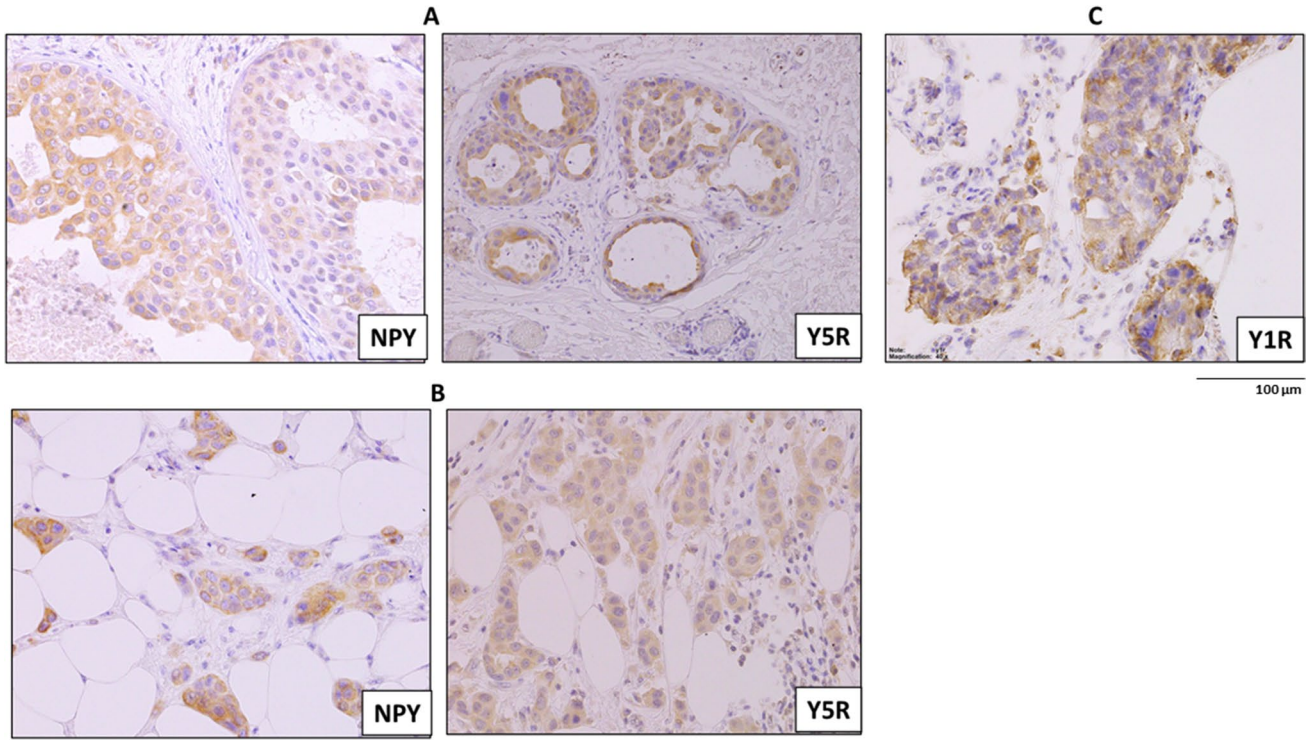

Fig. 6 NPY system in breast cancer. **A** Ductal carcinoma in situ of the breast—NPY with low and intermediate cytoplasmic expression, and intermediate Y5R expression in the preinvasive cells (400×, 200×). **B** Invasive ductal carcinoma of the breast showing intermediate NPY and Y5R immunoreactivity in the cancer cells (400×). **C** Breast cancer metastasis to the bone with intense Y1R immunostaining (400×)

its increased non-nuclear expression with the presence of metastatic disease, advanced clinical stage, perineural invasion, and luminal subtype [147]. In contrast, in luminal A breast cancer, Y1R was a positive predictive factor for longer relapse-free and overall survival [146]

Functionally, NPY has been implicated in regulation of many processes involved in breast cancer progression, including cancer cell proliferation, survival, motility, and tumor vascularization. For example, in estrogen receptor (ER)-positive breast cancer, Y1R has been shown to inhibit estradiol-induced cancer cell proliferation, while its high expression predicted endocrine sensitivity and better survival [146]. On the other hand, Y5R has been implicated as a growth- and metastasis-promoting factor, as it stimulates breast cancer cell proliferation and migration, as well as promotes the expression and release of the key angiogenic stimulator, VEGF [51, 107, 148]. Importantly, Y1R and Y5R are up-regulated by hypoxia in breast cancer cells, and both receptors have been shown to contribute to the NPY-induced breast cancer cell proliferation and migration under these conditions (Fig. 6B, C) [117, 118]. However, Y5R is also frequently lost in breast cancer tissue, while its ectopic expression in breast cancer cells led to a decrease in their proliferation and migration and an increase in chemosensitivity due to the cell cycle arrest in the G2/M phase and subsequent apoptosis [149]. This effect may reflect the supraphysiological levels of Y5R resulting from its ectopic overexpression, as overactivation of the Y5R/RhoA axis in Ewing sarcoma has been shown to result in cytokinesis failure [57]. The discrepancy in the reports pertaining to the biological activity of Y5R in breast cancer may also result from the heterogeneous nature of this disease. Hence, further studies are required to determine the role of the NPY system in specific breast cancer subtypes.

Prostate cancer According to the TCGA database, the expression of NPY in prostate cancer is the highest among all adulthood cancer types (Fig. 7A–C) [150]. However, the clinical data regarding correlations between the NPY levels and prognosis in prostate cancer patients are conflicting and the results are often dependent on the method of NPY detection. Gene expression analyses based on mRNA levels often associate lower NPY expression with a more aggressive disease course [150]. In contrast, elevated NPY protein levels in the prostate cancer tissue are typically correlated with more aggressive phenotype. Pro-NPY levels detected by proteomics analysis are increased in prostate cancer, as compared to normal prostate, and correlate with worse cancer-specific survival [151]. Likewise, a high frequency of NPY-positive

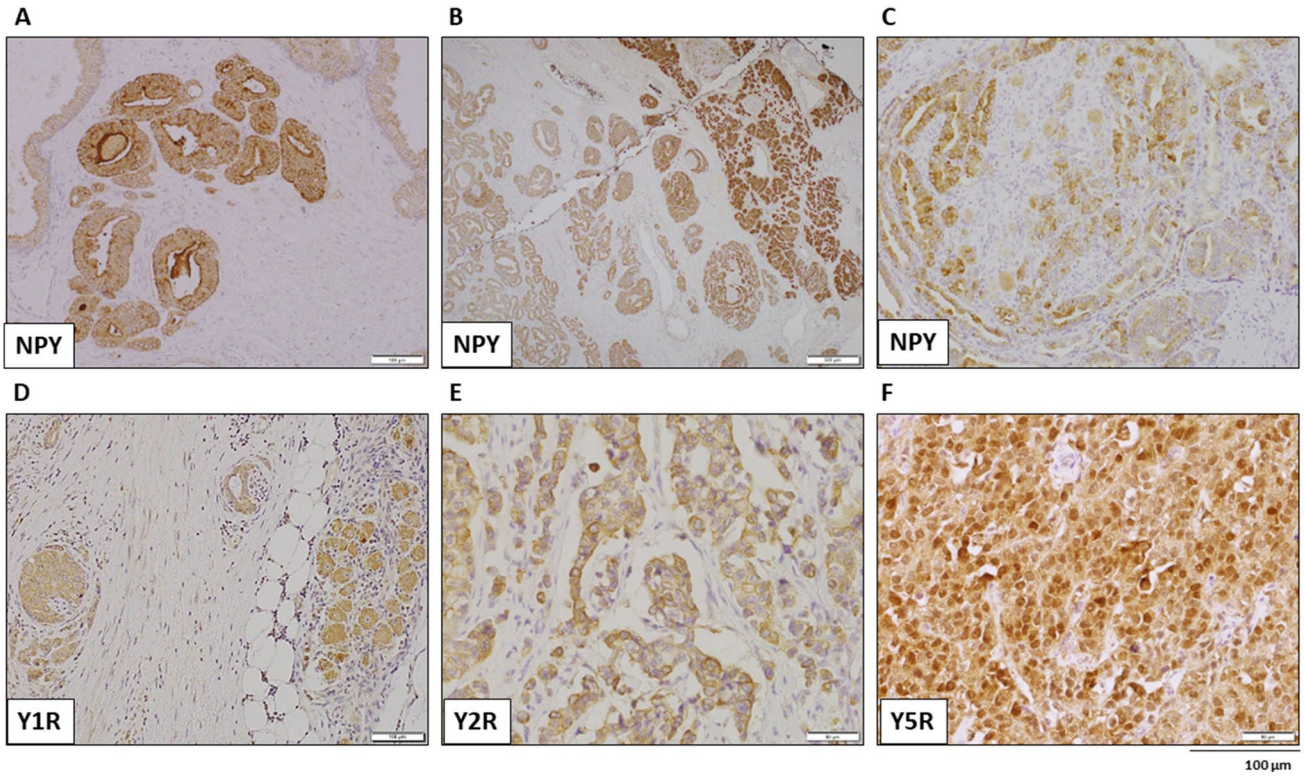

Fig. 7 NPY system in prostate cancer. **A** NPY staining is low and mostly membranous in the normal prostatic glands, while cancer cells exhibit strong and cytoplasmic immunoreactivity (200×). **B** NPY expression with gradient increasing toward the tumor invasive front and perineural invasion of the prostate cancer (100×). **C** Cancer cells infiltrating ganglion present with NPY staining comparable or higher than that observed in neuronal cells (400×). **D–F** NPY receptors within the prostate cancer in localized (Y1R, Y2R), and metastatic (Y5R) stages (100×, 400×, 400×, respectively)

prostate cancer cells identified by immunohistochemistry is associated with a high risk of relapse [152]. Particularly high expression of NPY is observed in ERG fusion-positive (ERG+) tumors, although a study by Kristensen et al. indicated that high pro-NPY and ERG levels were unrelated to unfavorable oncological outcomes [153]. Another measure of the NPY system activity in patients is the concentration of the peptide in the blood. Plasma proteome profiling identified NPY as a prostate cancer biomarker, with concentrations increasing in patients with high Gleason scores [154]. Similarly, elevated platelet NPY, which has been previously associated with the response to stress and hypoxia, correlates with worse progression-free survival in patients treated with abiraterone, the novel antiandrogen drug [23, 27, 29, 30]. Since NPY acts via membrane receptors, its secretion from neurons or cancer cells is necessary for its actions. Hence, an elevated serum NPY is the most biologically relevant biomarker of its enhanced activity in cancer patients and often correlates with metastatic disease and relapse [99, 100].

While the associations between high systemic NPY and adverse phenotype of prostate cancer are well established, it is not clear if the elevated levels of the peptide in the blood result from its release from tumors or increased neuronal activity (e.g. due to stress). For example, patients with prostate cancer and high psychological depression scores have further elevated NPY expression within tumor tissue and the peptide can be released from the prostate cancer cells upon norepinephrine stimulation, suggesting the role of the autocrine peptide [25]. This cancer cell-derived NPY acts as a chemoattractant for macrophages, increasing the infiltration by TAMs and thereby advancing the disease [25]. In contrast, studies by Ding et al. suggest the impact of the neuronal NPY on prostate cancer progression, as the high count of NPY-positive nerve fibers within cancer tissue predicted the increased risk of biochemical recurrence and cancer-specific death [102]. The mechanisms underlying these effects include changes in cancer cell metabolism, motility, proliferation, apoptosis and therapy resistance [102]

In addition to the high NPY expression, prostate cancer cells are also rich in its receptors. It has been recently shown that the entire NPY system, including Y1R, Y2R, and Y5R, is up-regulated in prostate cancer starting from the early stages of carcinogenesis (Fig. 7D-F) [98]

Current functional data implicate Y1R in prostate cancer biology [102]. However, the role of the remaining NPY

receptors in this malignancy remains to be determined. For example, our recent studies indicated accumulation of prostate cancer cells enriched in all three NPY receptors on the invasive edge of the prostate cancer and in perineural invasion areas [98]. Importantly, Y2R was the only NPY receptor with expression significantly elevated in advanced tumors, as compared to low-stage lesions (pT3 vs pT1-2), while high Y5R expression correlated with the presence of extraprostatic extensions [98]. Given the known role of the Y2R and Y5R in invasiveness and metastasis in other malignancies, these data warrant further investigations into their role in prostate cancer progression.

Hepatocellular carcinoma Liver pathogenesis is associated with changes in the local and systemic NPY. Patients with liver cirrhosis and hepatocellular carcinoma have elevated serum NPY levels as compared to the healthy control [155]. Moreover, recent studies indicated the role of NPY in cross-talk between liver cancer cells and surrounding normal tissue. Normal hepatocytes surrounding hepatocellular carcinoma release NPY, which activates Y5R present in cancer cells and stimulates their invasiveness [114].

Other cancer types The preclinical and clinical data in other cancers are usually limited to the characteristics of the expression pattern of NPY and its receptors. Among cancers of the gastrointestinal tract, the role of NPY is best characterized in colon carcinogenesis. In the animal model of inflammation-induced tumorigenesis, NPY knock-out significantly reduced the number and size of intestinal polyps, as well as decreased proliferation and increased apoptosis in colonic epithelial cells [156]. In line with these results, NPY has been identified as one of the core genes up-regulated during colon cancer progression [157]. Elevated NPY expression and secretion were also described in cholangiocarcinoma, as compared to normal cholangiocytes [116]. Moreover, NPY-positive cancer cells are detectable in stomach carcinoma (Fig. 8).

In skin cancers, the function of NPY is not well elucidated and the data is conflicting. While overall expression of NPY is elevated in melanoma, its high levels have been associated with better clinical outcomes or invasiveness and metastasis [158, 159]

7 Potential clinical applications

The pleiotropic actions of NPY relevant to tumor biology and the widespread expression of its receptors in neoplastic cells warrant further investigations into potential clinical applications based on the NPY system. Due to the growth-promoting and metastatic functions of NPY described in various malignancies, its receptors represent promising targets in cancer therapy [108, 160–162]. NPY receptors can be directly blocked by small molecule inhibitors. Thus far, a variety of selective antagonists have been developed for all NPY receptors. Some of them were effective in inhibiting tumor growth and dissemination in preclinical animal models. For example, the Y2R antagonist, BIIE0246, inhibits the growth of neuroblastoma by decreasing tumor proliferation and vascularization [108]. In Ewing sarcoma xenografts, the Y5R antagonist, CGP71683, inhibited hypoxia-induced bone metastasis [57]. Y1R and Y5R antagonists have also been shown to block proliferation, migration, and invasiveness of breast cancer cells in vitro [118]. Importantly, one of the Y5R antagonists, MK-0557, has been approved for clinical trials in obese patients and can be potentially repurposed for oncological studies [163, 164]. However, the limitation of the existing NPY receptor antagonists is their ability to cross the blood–brain barrier and thereby affect many central functions of the peptide, including food intake. Hence, future studies should focus on developing novel compounds with the activity limited to blocking the NPY actions in the periphery. Still, even in this case, potential side effects caused by interfering with broad physiological actions of NPY should be monitored, even though in animal experiments no such adverse effects were reported. Another

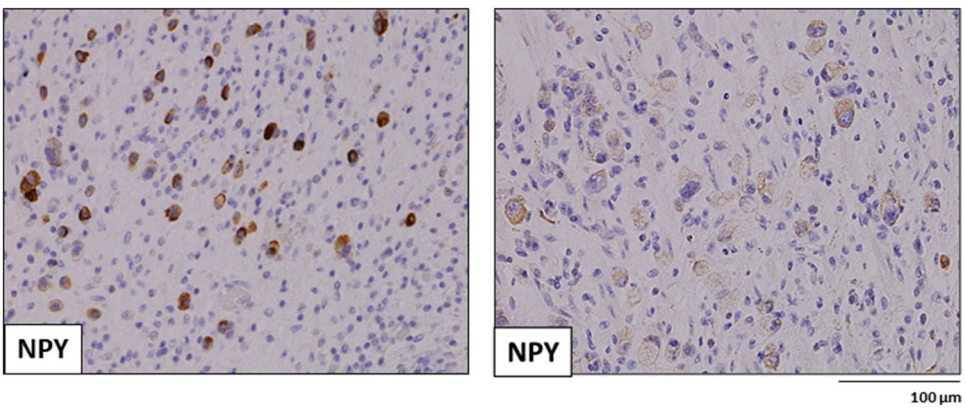

Fig. 8 NPY-positive cells in stomach cancer. Poorly-cohesive gastric cancer with heterogeneous NPY expression in the subsets of the infiltrating neoplastic cells (200×, 400×)

therapeutic option could be blocking the activity of DPPs enhancing many tumor-promoting actions of NPY. However, previous attempts were hindered by a feedback effect leading to a significant upregulation of DPPIV expression in cancer cells upon prolonged DPP inhibitor administration [36].

Carcinogenesis is often associated with changes in the NPY receptor expression. Consequently, the pattern of their distribution varies between normal and cancerous tissues, giving a scientific premise for utilizing NPY receptors in cancer diagnosis [101]. This may include standard histopathological analyses, as well as the use of labelled NPY analogs for cancer imaging [160]. Moreover, levels of NPY protein and its receptors often correlate with cancer stage, differentiation, and patient survival (Table 1), suggesting their potential value as prognostic or predictive biomarkers. For example, the status of NPY gene methylation has been proposed as a prognostic marker in some malignancies, including colorectal and pancreatic cancers [165–168].

As GPCRs, NPY receptors undergo internalization upon ligand binding [169]. This process is particularly effective in the case of Y1R [169]. This phenomenon was utilized for the delivery of therapeutics into cancer cells. The NPY receptors have been proposed as targets for drug conjugates that selectively kill cancer cells. Li and colleagues synthesized the doxorubicin encapsulating albumin nanoparticles conjugated to [Pro30, Nle31, Bpa32, Leu34]NPY(28 – 36), which allow for selective drug delivery and inhibition of cell viability by binding to Y1R on the surface of breast cancer cells [162]. The analogs of NPY were also studied in other types of cancer. Tubugi-1-SS-NPY disulfide-linked conjugates show toxic selectivity in different cell lines with Y1R expression [170]. NPY receptor ligands have also been proposed as a vehicle to deliver radionuclides and siRNA to the cells [171, 172]. Yet, while designing such therapies, consideration should be given to potential side effects that may be caused by the widespread expression of NPY receptors in normal tissues.

8 Conclusions and future directions

NPY is a pleiotropic peptide with multifaceted functions in physiology and tumor biology (9). The peptide can directly regulate cancer cell proliferation, survival, and motility, as well as impact the tumor microenvironment by stimulating angiogenesis and modifying immune infiltration [14, 77, 80, 88]. While many of these processes were initially described in tumors with high endogenous NPY expression, the peptide secreted from peripheral nerves can exert similar effects, making it an interesting mediator of cancer-nerve interactions [102, 126]. Moreover, NPY-dependent systemic effects, such as changes in energy metabolism, obesity, and the immune response can have an impact on cancer development and progression [78, 109, 173]. However, the exact role of these complex systemic activities of the peptide in the context of cancer needs to be further investigated. Another understudied aspect of NPY biology that needs to be elucidated in order to design effective therapies pertains to the interactions of its cognate receptors and cross-talk with other systems, such as adrenergic axis and BDNF; the phenomenon that can impact the efficacy of selective NPY receptor antagonists [44, 47, 48, 58]. Altogether, these findings warrant further investigations into the impact of physiological conditions resulting in elevated NPY, such as chronic stress, on cancer initiation and progression and its role in this process. Thanks to its pleiotropic functions in tumor biology, NPY and its receptors can become promising new targets in oncology, with potential applications in cancer therapy, imaging, diagnosis, and stratification. However, translation of these preclinical findings to clinical practice requires further research to fully elucidate the direct and indirect effects of NPY on tumor tissue and the impact of its complex systemic physiological functions on cancer progression.

Acknowledgements The authors would like to thank Tyler Biermann for proofreading the manuscript.

Author contribution D. S. collected and analyzed literature, wrote manuscript, prepared Figs. 5–8. A. S. collected and analyzed literature, wrote manuscript. N. A. collected and analyzed literature, wrote manuscript. E. K. wrote and edited manuscript, designed and prepared Figs. 1–4. E. I. initiated and supervised the work on the review, analyzed the literature, wrote and edited the manuscript, selected representative pictures for Figs. 6–8. J. K. together with E.I. supervised the work on the review, analyzed the literature, wrote and edited the manuscript, selected representative pictures for Fig. 5.

Funding This work was supported by the University of Warmia and Mazury in Olsztyn and the Medical University of Gdańsk, Poland, as well as grants: Walczak grant from the Polish National Agency for Academic Exchange (NAWA) to DS; the National Institutes of Health awards 1RO1CA123211, 1R03CA178809, R01CA197964 and 1R21CA198698 to JK; support from Sunbeam Foundation and Children's Cancer Foundation to JK.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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