Review

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Review

Peptidergic GPCRs Signaling Systems and Cancer: Into New Pharmacological Approaches

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Abstract: Peptidergic GPCR systems are broadly distributed in the human body and regulate numerous physiological processes by activating complex networks of intracellular biochemical events responsible for cell regulation and survival. Their failure due to overaction, ill-function, or blockade gives rise to cell disturbances, eventually causing disease if compensatory mechanisms do not suffice. Revision of updated experimental research provided an evident relationship associating peptidergic GPCR malfunction with tumor formation and maintenance resulting from uncontrolled cell proliferation and migration, colonization, inhibition of apoptosis or altered metabolism, and increased angiogenesis in tumoral tissues. Determination of the implication of GPCR peptide signaling in specific neoplasia is crucial to designing tailored pharmacological treatments to counteract or dismantle the origin of the signaling circuitry causing cellular disruption. In some cases, particular ligands for these receptors may serve as concomitant treatments to aid other pharmacological or physical approaches to eradicate neoplasias.

Keywords: G-protein-coupled receptors (GPCR); peptide; cell growth; apoptosis; peptide antagonists; peptide signaling pathways; cancer; cancer therapy; drug design

1. Introduction

The word "cancer" refers to several complex diseases with various etiological factors, acting alone or combined, including genetic background/predisposition, environmental influences, aging, hormonal factors, and lifestyle choices. These factors can trigger abnormal biochemical processes and disrupt the cell's available compensatory mechanisms against damage. If the cells cannot recover from these disruptions, tumors can be generated and progress. Each neoplasia has distinct characteristics based on the type of cell and tissue involved and the specific molecular events and transcriptional programs affected. By studying the biochemical processes for every kind of tumor, we may uncover new biochemical incidences and new opportunities for developing targeted therapies to combat these diseases [1–6].

G-protein-coupled receptors (GPCRs) form an extensive family of proteins (more than 800) that convey extracellular chemical or sensory information to trigger intracellular changes affecting short-term intermediary metabolic events and long-term gene expression programs through signaling cascades. GPCRs participate in numerous biochemical events associated with physiopathological processes, including cell transformation, apoptosis, growth, and migration [7].

Different classification criteria categorize GPCRs into several families or groups. Approximately 350 GPCRs are non-sensory receptors (processing amines, peptides, proteins, lipids, ions, nucleotides, or hydroxycarboxylic acids), whereas the rest (approximately 450) respond to sensory (odors, tastes, photons, or pheromones) stimuli. Based on sequence homology and functional resemblance, GPCRs are included in eight classes, six of which are present in humans. These include class A (rhodopsin), class B (secretin and adhesion), class C (glutamate), class T (taste), class O

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(orphans), class E (frizzled/smoothened), and classes D (fungal mating pheromone receptors), and E (cAMP receptors) absent in vertebrates [8,9].

Several GPCRs are presently druggable targets for numerous pathological conditions, including cardiovascular, chronic inflammation, pain, or cancer pathologies [10,11]. The weaponry of therapeutic compounds includes synthetic ligands acting on the receptor as molecules displaying full agonism (triggering maximal signal), partial agonism (eliciting activity below the maximum response), inverse antagonism (inhibition of constitutive receptor activity), and neutral antagonism (the constitutive receptor activity is not changed). Other drugs not binding at the orthosteric but at an allosteric site (outside or within the transmembrane helices) are PAMS (positive allosteric modulators) and NAMS (negative allosteric modulators), which activate or inhibit, respectively, the responses raised by orthosteric ligands. Furthermore, the list grows with the so-called bitopic ligands (binding both the orthosteric and the allosteric sites within the GPCR) [12,13].

Approximately 90 class A and 20 class B GPCRs bind and process around 180 peptide signals. The majority of GPCRs binding peptides belong to class A (for example, receptors for opioids, tachykinins, galanin, neuropeptide Y, orexin, cholecystokinin, bradykinin, somatostatin, endothelin, neurotensin, bombesin, vasopressin, kisspeptin, thyrotropin-releasing hormone, melanocortin, or apelin). A few GPCR class B (secretin) include receptors for glucagon, secretin, corticotropin-releasing factor, parathyroid, vasoactive intestinal peptide, calcitonin or pituitary adenylate cyclase-activating peptide [9].

The interaction between peptides and GPCRs is intricate. It encompasses various possibilities, such as one peptide binding to one GPCR, one peptide binding to multiple GPCRs, or multiple peptide ligands binding to multiple GPCRs [14,15]. The complexity is compounded by many GPCRs being labeled orphans, and their peptide activation awaits identification and corroboration [11,13].

This report reviews recent evidence and findings concerning the fine structure, dynamic conformations, and plausible mechanistic involvement of eight peptidergic GPCRs in tumorigenesis. It also discusses the examination of these systems as pharmacological objects.

2. GPCR Peptides and Their Receptors

Several natural peptides bind with different affinities and activate specific GPCRs with varied localization, triggering a complex network of intracellular signals that control essential cell activity in short, mid, and long-term periods. Peptidergic GPCR systems share common properties concerning their broad distribution and shared signaling pathways regulating an array of pleiotropic cell responses affecting vital physiology [14,16,17]. We focus on advances concerning eight GPCR peptidergic systems and their relationship with tumorigenesis and expansion. Additionally, we describe some synthetic and specific activating or silencing compounds modulating peptidergic GPCR signaling that may be valuable tools for treating some types of cancer alone or as concomitant measures [18]. The systems comprising the main endogenous ligands and their respective GPCRs are schematically shown in Figure 1.

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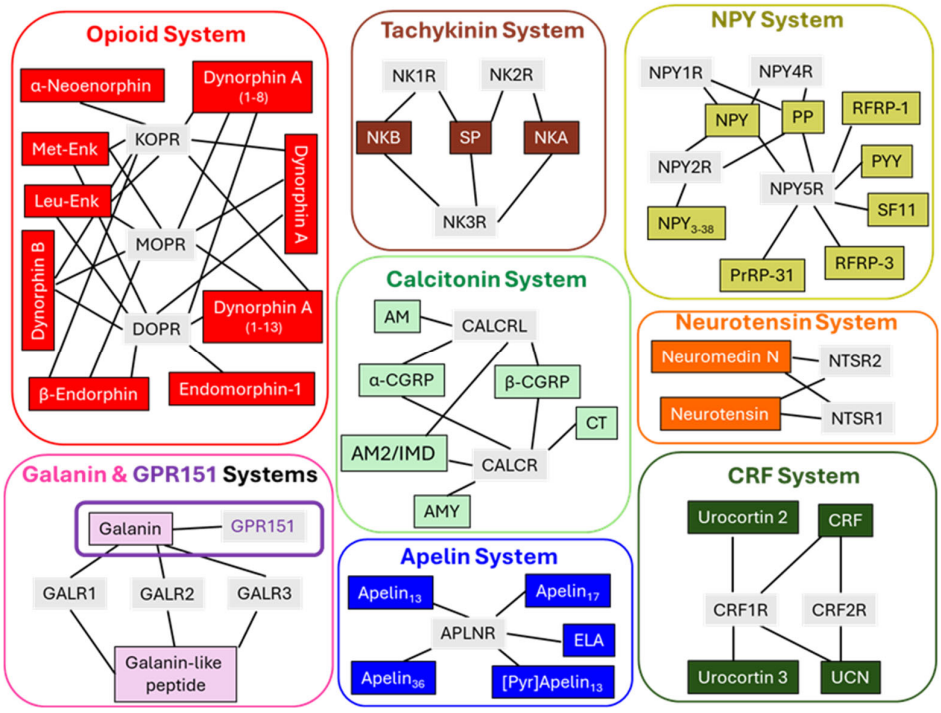

Figure 1. Natural peptide ligands for eight representative GPCRs. The lines indicate the binding affinity (in different degrees) of peptides for the receptor type. The figure is based on data on pairing peptides and GPCRs reported by Foster et al. [14]. Abbreviations: AM2/IMD, Adrenomedullin/Intermedin; AMY, amylin; APLNR, apelin receptor; CALCR, calcitonin receptor; CALCRL, Calcitonin receptor-like; α-CGRP, Alpha-calcitonin gene-related peptide; CRHR, Corticotrophin releasing hormone receptor; CT, Calcitonin; DOPR, delta-opioid receptor; ELA, Elabela; GALR, Galanin receptor; GPR151, G protein-coupled receptor 151 KOPR, kappa opioid receptor; Leu-Enk, Leucine-enkephalin; Met-Enk, Methionine-enkephalin; MOPR, Mu opioid receptor; NKA, Neurokinin A; NKB, Neurokinin B; NKR, Neurokinin receptor; NPYR, neuropeptide Y receptor; NTSR, Neurotensin receptor; PP, pancreatic peptide; PYY, Peptide YY; PrRP, Prolactin-releasing peptide; RFRP, R(Arg) F(Phe)-amide-related peptide SF11, [N-(4-ethoxyphenyl)-4-(hydroxydiphenylmethyl)-1-piperidinecarbothioamide]; SP, substance P; UCN, Urocortin.

Several opioid peptides (for instance, enkephalins, endorphins, or dynorphins) and three main types of opioid receptors, mu (MOPR), delta (DOPR), and kappa (KOPR) [19,20] form the endogenous opioid system, a primary regulator of intrinsic analgesia, cardiovascular responses, gastrointestinal and hepatic function, respiration, thermoregulation, or immunological reactions (see the recent review series by [21]).

The tachykinin system comprises 3 receptors, namely neurokinin 1, 2, and 3 (NK-1R, NK-2R, and NK-3R), and three main natural ligands, substance P (SP), neurokinin A and neurokinin B, showing different affinities for the receptors. Also, hemokinin-1 is a natural ligand for NK-1R in peripheral tissues [22]. The tachykinin system is widely distributed in the nervous system and other non-neural cells and tissues and influences numerous physiological functions, such as smooth muscle contraction, cell proliferation, pain, inflammation, or tissue regeneration [23,24].

Four neuropeptide Y (NPY) receptors, NPYR 1, 2, 4, and 5, and a handful of peptide ligands with different affinities for the receptors coordinate multiple activities (analgesia, angiogenesis, allergy responses, inflammation mechanisms, or energy homeostasis) from their ubiquitous presence within the human body [25–27].

The galanin and GPR151 systems include two ligands, galanin and galanin-like peptide activating three galanin receptor types: GALR1, GALR2, and GALR3 [28]. The galanin system is distributed widely in neural and non-neural tissues and impacts many physiological processes, from pain to metabolism, innate immunity, and inflammation coordination, involved in brain and

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gastrointestinal function [29,30]. A galanin-binding receptor, GPR151 [14,31], previously considered an orphan GPCR, exhibits high structural similarity with galanin receptors and influences metabolic routes controlling glucose homeostasis [32] and trigeminal neuropathic pain [33]. However, it is still unclear and requires further elucidation whether galanin behaves as a proper functional endogenous ligand for GPR151 in CNS structures [34] and the precise role of the GPR151 receptor system.

The calcitonin system is made up of two secretin GPCRs, CACRL (calcitonin receptor-like) and CALCR (calcitonin receptor), regulated by receptor-activity modulating proteins (RAMPs) [34–36], and several endogenous ligands comprising calcitonin (CT) adrenomedullin/intermedin (AM2/IMD), adrenomedullin (AM), alpha- and beta-calcitonin gene-related peptides (α-CGRP and a (β-CGRP) and amylin (AMY) [37,38]. The calcitonergic system is vastly distributed and assists many functions like, for example, hormone secretion, pain control, blood calcium regulation, food intake modulation, maintenance of vascular tone, angiogenesis, or immunoregulation [39–41].

Two neurotensin receptors, NTSR1 and NTSR2, bind two principal endogenous peptides, neurotensin (NT) and neuromedin N. The neurotensin system [42] influences energy balance, pain control, stress, gastrointestinal motility and secretion, and brain reward mechanisms [43–46].

Two families of peptides, apelin [47] and elabela (ELA, an acronym meaning "epibole late because endoderm late") [48,49], activate the apelin receptor (APLNR). This system distributes in several tissues (placenta, heart, lungs, brain) and influences basic physiological mechanisms affecting cardiovascular function, inflammation events, insulin secretion, or fluid homeostasis [47,50,51].

A family of endogenous ligands, including corticotropin-releasing hormone (CRH), urocortins, and two receptor types, CRH1 and CRH2, build up the corticotropin-releasing hormone system. A broad and scarce overlapping distribution of these peptides and their distinct receptor affinities determine a complex collection of actions modulating stress and neuroendocrine responses [52–55].

3. Peptidergic GPCR Structural Features

Recent advances in determining GPCR three-dimensional structures and alternative conformations revealed orthosteric binding sites' geometry and the functional significance of insurmountable antagonism, activation/inactivation states, or allosteric regulation [20,56–58]. The detailed understanding of the setting up of signaling complexes at the plasma membrane and in intracellular endosomes provides new insights for understanding the role of peptidergic GPCR function and cell dysfunction. Still, challenging questions defy basic research to understand how and to what extent peptidergic GPCR function influences biochemical pathways responsible for cancer development, cell survival, or cell resistance to different therapy interventions. Some peptidergic GPCR systems have been the focus of research focused on mechanisms responsible for the appearance of various pathologies, including cancer, and the design of selective pharmacological therapies [14,18,59–61].

Where available, some essential features about structure-activity and delineation of orthosteric and allosteric sites in some peptidergic GPCRs are described next. Figure 2 shows representative structures of peptidergic GPCR objectives of this report taken from the Protein Data Bank repository [62].

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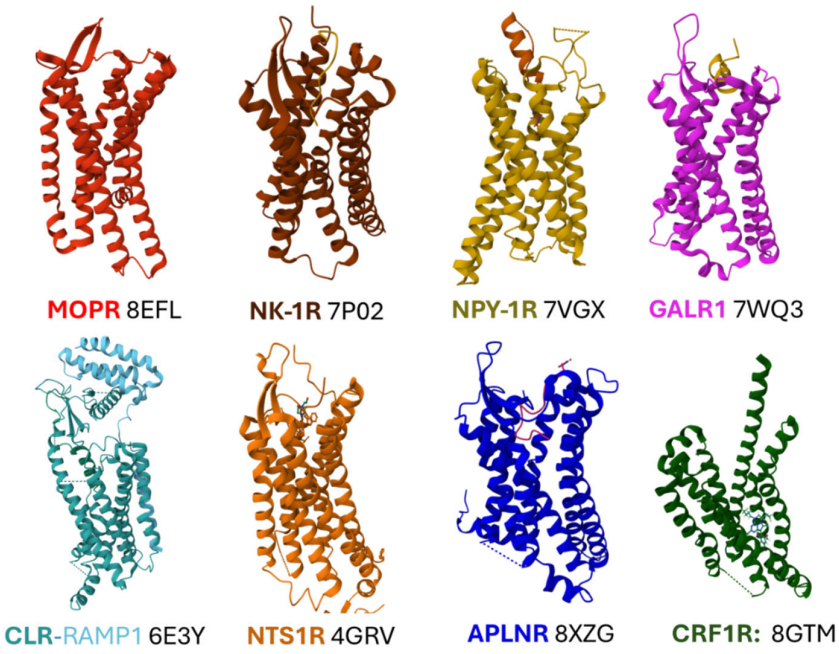

Figure 2. Three-dimensional structures representing peptidergic GPCRs determined by X-ray crystallography or cryo-electron microscopy. All structures shown were obtained from the protein data bank [62] and drawn with the Molstar online free software [63]. Each figure depicts the receptor abbreviated name and the PDB identity code (four characters). Abbreviations: APLNR, apelin receptor; CLR-RAMP1, calcitonin-like receptor—receptor activity modifying protein 1; CRF1R, corticotropin releasing factor receptor 1; GALR1, galanin receptor 1; MOP, mu-opioid receptor; NK-1R, neurokinin 1 receptor; NPY-1R, neuropeptide receptor 1; NTS1R, neurotensin receptor 1.

Structural studies of three opioid receptors, MOPR, DOPR, and KOPR [20,64,65], have revealed details connected with their relationship with effector proteins, such as protein effectors, agonists, and antagonists. This work will not detail the ligand binding interactions of all peptidergic GPCR mentioned. However, a few structural examples intend to illustrate the importance of defining orthosteric and allosteric grooves in designing and developing drugs to abrogate or modulate their activities. For example, the cryo-electron microscopy (EM) structure of MOPR bound to agonist fentanyl reveals its position within the orthosteric site (PDB ID 8EFL) [64], between helices 2, 3, 5, 6, and 7 of the transmembrane regions. The phenylethyl structure of fentanyl directs towards a "minor pocket" defined by residues of transmembrane 2 (TM2) and 3 (TM3), whereas the propionyl moiety establishes hydrophobic contacts with residues of TM5 and TM6. Fentanyl, like many other MOPR agonists, interacts with Asp^{149} in TM3 (Figure 3, A).

Interestingly, when comparing the interaction of morphine, an agonist, and naloxone, an antagonist of the MOPR agonist, it revealed that these drugs with similar chemical structure (but different bond torsion profiles) interact with MOPR in a similar groove where they accommodate distinctly [66] explaining their different outcomes when binding to the receptor. One of the main features of their diverse coupling lies in the differential dynamic recognition of residues Ile^{322} and Y^{326} (mouse MOPR) by the drugs. The mutation I322A (shortening of the aliphatic chain of Ile) attenuated morphine activity and converted naloxone into a full agonist [66].

The analysis of the structure of the NK-1R bound to the selective antagonist aprepitant unmasked the characteristics of its binding pocket compared with the binding site of the natural ligand substance P. The Met^{11} residue in the N-terminal region of the undecapeptide poses deep into the structure of the receptor, and the carboxy-terminal region interacts with the extracellular domains defined by extracellular domain 2 (ECL2) and the outer part of transmembrane helices I, VI and VII [58]. Aprepitant occupies a site defined mainly by the lateral chain of some amino acids. Figure 3 (B)

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compares the binding grooves of SP and aprepitant. The morpholine central ring of aprepitant lies between residues F^{268} in helix VI and Q^{165} in helix IV, and residues N^{109}, $P^{112,}$ and I^{113} provide hydrophobic contacts in the surface of helix III. The positioning of F^{264}, $F^{268,}$ and W^{261} in helix VI towards the trifluoro methyl groups of the aromatic ring fixes the positioning of W^{261} and determines the inactivation state of NK-1R induced by the antagonist (Figure 3, inset in B) [58,67].

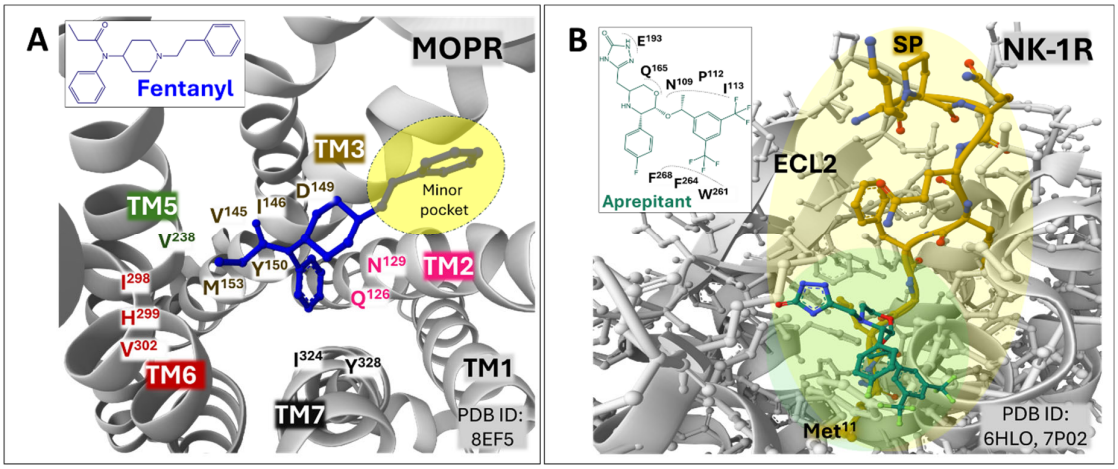

Figure 3. A depicts the binding site of the opioid agonist fentanyl to MOPR. Transmembrane domains (TM) are indicated, as well as the amino acid residues with a one-letter code and the number in superscript indicating their position in the primary sequence of the receptor protein. The color code of the residues denotes the TM where the residues belong. A so-called minor pocket lodging the aromatic ring, as defined by TM2 and TM3, appears to be highlighted in yellow. The inset in A presents the two-dimensional structure of fentanyl. B shows the binding pockets of substance P (SP) and aprepitant within the NK-1R. ECL2 denotes the extracellular domain 2. The inset in B contains the two-dimensional structure of aprepitant with the positioning of amino acids that delimit the binding cavity. The three-dimensional representations were from the protein data bank [62] sketched with the Molstar online free software [63]. The two-dimensional chemical structures were drawn using KingDraw, a free chemical structure editor (version 3.0 for Windows).

The binding site of neuropeptide Y (NPY_{1-36}) to NPY-1R is tightened mainly through weak interactions of residues of the C-terminal region with amino acids situated deeply within the core of the protein [68]. The A panel of Figure 4 represents the position of amino acid residues in the agonist and the receptor and indicates their interactions with dashed-colored lines. Briefly, NPY Tyr36 interacts through a hydrogen bond with Q^{219} in transmembrane domain V (TM V) and through hydrophobic engagement with I^{124} in TM III. Arg33 connects with N^{283} (TM VI) through a hydrogen bond and with F^{286} (TM VI) and F^{302} (TM VII) through π-cation interactions. Arginine35 sets electrostatic bonding with D^{287} (TM VI) and van der Waals forces with F^{173} (TM IV). Residues Thr32 and Gln34 establish non-polar interactions with Y^{100} (TM II).

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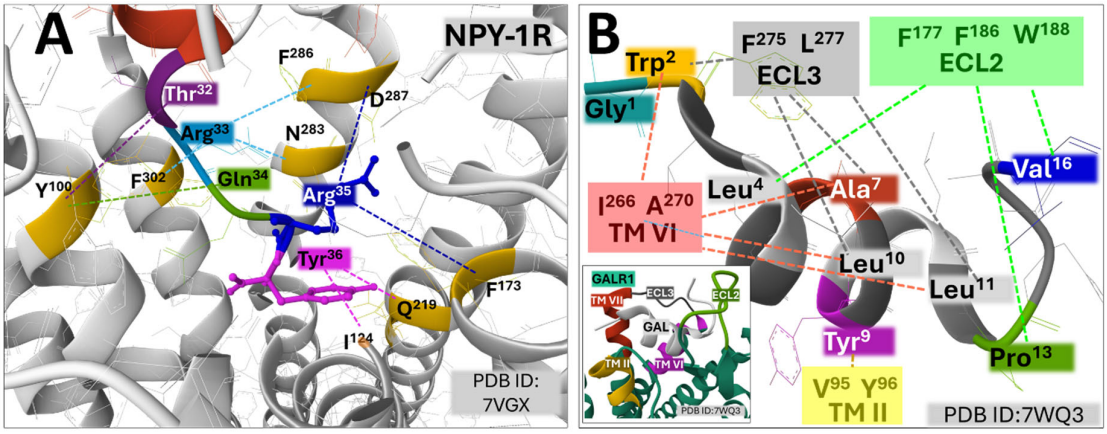

Figure 4. Panel A depicts the accommodation of the C-terminal region of NPY to the NPY-1R (see texts for details). Panel B represents the N-terminal end (residues 1 to 16) of galanin (GAL), with the network of weak interactions defining the binding site in the GALR1. The B inset represents the galanin receptor site delimited by extracellular loops 2 and 3 (ECL2, ECL3) and transmembrane domains (TM) II, VI, and VII. Dashed colored lines in both panels represent weak interactions between the ligands and their respective receptors. The amino acid residues were drawn with a one-letter code for the receptor proteins and a three-letter code for the peptide ligands. The superscript number denotes their position in the primary sequence of the receptor or the peptide ligand. The three-dimensional representations were acquired from the protein data bank [62] sketched with the Molstar online free software [63].

Cryo-EM structure analysis of galanin receptor 1 (GALR1) bound to N-terminal truncated galanin (GAL_{1-16}) showed that the peptide interacts with the receptor uniquely, with the N-terminus adopting an alpha helix conformation. The C-terminus would adopt a more disorganized loop random structure. Galanin sits on the extracellular moiety of the transmembrane helices of the receptor with an almost horizontal positioning [69]. The receptor hosts the ligand peptide in a cavity delimited by TM VI and VII and extracellular loops ECL2 and ECL3 (the main weak bonding is illustrated in Figure 4 (B).

Cryo-EM maps of the complex formed by Gs, the human calcitonin (CAL) receptor (CLR), the receptor-activity modifying protein (RAMP1), and calcitonin gene-related peptide (CGRP) have unmasked relevant features that explain the function of this peptidergic system [70]. RAMP1 extensively contacts CLR both in the extracellular domain and the transmembrane region. The carboxy-terminal region of CGRP (residues Phe^{37}, Ala^{36}, Lys^{35}, and Ser^{34}) contacts with residues F^{83} and P^{85} in the extracellular part of RAMP1 (Figure 5, A), whereas the N-terminal region of the peptide buries deeply into the transmembrane region of CLR arranging contacts with CLR residues (Figure 5, B). Residues I^{298} and L^{302} (in TM V) and M^{223} and Y^{227} (in TM III) delimit a hydrophobic bottom of the binding cavity. Positions in ECL1 and ECL2 are essential for arranging the protein receptor as an active conformer [70,71].

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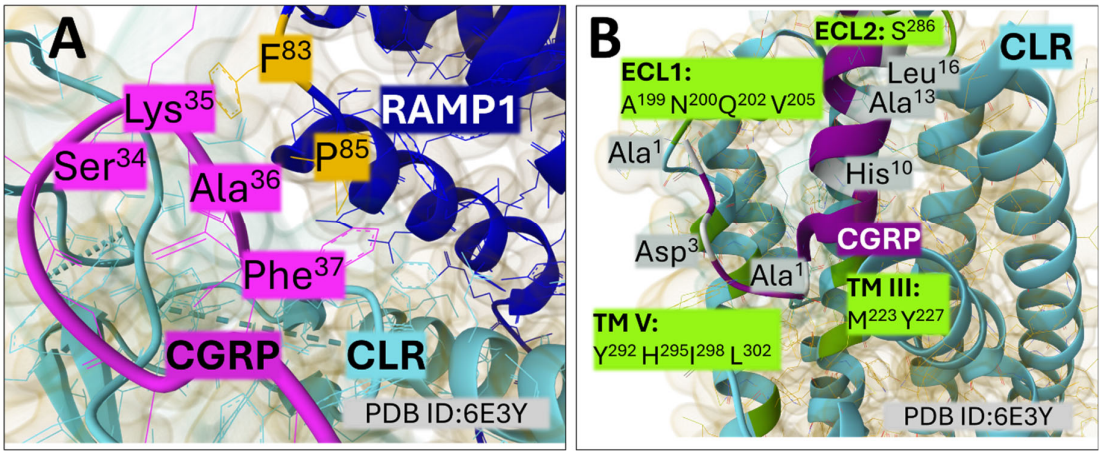

Figure 5. Panel A represents the contacts between the C-terminal region of CGRP and RAMP1 (see texts for details). Panel B depicts the N-terminal end of CGRP with the circuitry of weak interactions defining the binding site in CLR. Some residues in extracellular loops 2 and 3 (ECL2, ECL3) and transmembrane domains (TM.) V marking out the peptide binding site are highlighted. The amino acid residues were drawn with a one-letter code for the receptor proteins and a three-letter code for the peptide ligand. The superscript number denotes their position in the primary sequence of the receptor or the peptide ligand. The three-dimensional representations were acquired from the protein data bank [62] drawn with the Molstar online free software [63].

Crystallographic and X-ray diffraction analysis of rat neurotensin (NTS) 1 receptor (NTS1R) bound to the C-terminal sequence of NTS (amino acids 8 to 13, RRPYIL), capable of activating the receptor offers structural details worth considering. The peptide places itself into the core of the receptor protein almost perpendicularly to the plasma membrane [72]. The ligand binding hole is delimited by amino acid residues in ECL2, TM III, TM VI, and TM VII (Figure 6, A). Inverse agonist SR48692 (2-[[1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)pyrazole-3 carbonyl]amino]adamantane-2-carboxylic acid) shares the same binding site as NTS_{8-13} but establish some distinct interactions. For example, in contrast with the NTS positioning, the inverse agonists hydrogen bond with Y^{351} (in TM VII) instead of Y^{146} (in ECL2). On the other hand, the antagonists, through their adamantyl group, share the same hydrophobic surface contacted by the carboxy-terminal end of NTS_{8-13} in the receptor core, although this surface appears more extended [73]. Recent studies emphasize the importance of recognizing the different conformers adopted by the receptor and its molecular dynamics when hosting different ligands to attain efficacious drug design [74].

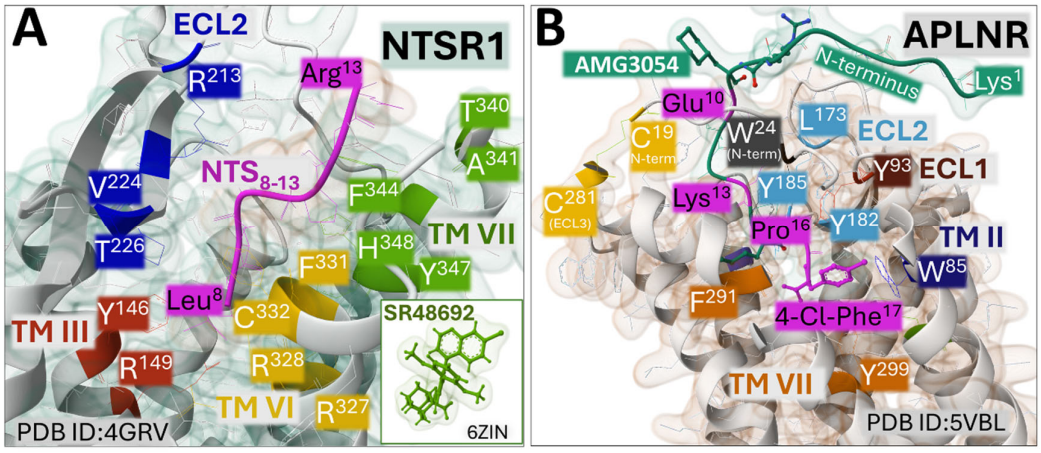

Figure 6. Panel A highlights the primary contacts between the C-terminal region of NTS_{8-13} and NTS1R (PDB ID: 4GRV). The inset in A illustrates the structure of inverse agonist SR48692 (PDB ID: 6ZIN)

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(refer to text for details). Panel B depicts the binding site of the apelin17 mimetic peptide, AMG3054 (PDB ID: 5VBL), in the apelin receptor (APLNR). The amino acid residues were drawn with a one-letter code for the receptor proteins and a three-letter code for the peptide ligands. The superscript number denotes their position in the primary sequence of the receptor or the peptide ligand. Transmembrane domains (TM) and extracellular loops (ECL) are indicated. The three-dimensional representations were acquired from the protein data bank [62] drawn with the Molstar online free software [63].

The structure of AMG3054 (the apelin17 mimetic peptide), bound to the apelin receptor (APLNR), was resolved with X-ray diffraction analysis, giving details concerning the coupling coordinates of the peptide within the receptor [75]. The C-terminal end of the peptide deepens into the transmembrane structure of APLNR, whereas the N-terminus extends to the outer surface. A cavity delimited by hydrophobic amino acids provides hydrophobic interactions with the peptide: L^{173}, Y^{185}, and Y^{182} in ECL2, W^{85} in TM II, and F^{291} and Y^{299} in TM VII (Figure 6, B). Recently, cryo-EM analysis of APLNR bound to different ligands revealed fundamental structural features explaining biased signaling (Gs protein versus β-arrestin activity) with relevant implications in drug design [56].

Early studies confirmed that the peptide ligand CRF, adopting an alpha-helix conformation, binds primarily to the extracellular part of the CRF1R, a class B secretin GPCR, and also provided relevant details concerning the interaction of the C-terminal amidated residue of the peptide into the structure of the receptor [76]. Recent structural analysis employing X-ray free-electron laser methodology applied to a monoclinic crystal of CRF1R, bound to antagonist BMK-I-152 ([8-(4-bromo-2,6-dimethoxyphenyl)-2,7-dimethylpyrazolo[1,5-α][1,3,5]triazin-4-yl]-*N*, *N*-bis-(2-methoxyethyl) amine and docking simulations solved the structure of the complex [77]. The antagonist is accommodated in a cavity delimited by weak interactions with CRF1R residues in TM II, III, V, VI, and VII (Figure 7). The inset depicts the Gaussian volume representation of BMK-I-152.

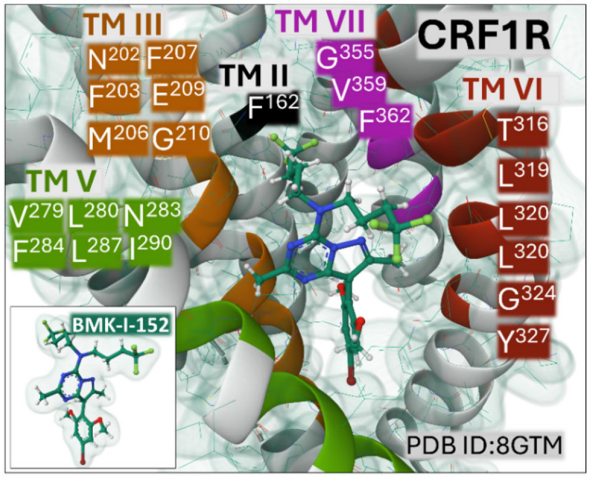

Figure 7. The allosteric antagonist of BMK-I-152 binds to the core of CRF1R (PDB ID: 8GTM), depicting the surface contacts of the receptor with the antagonist. The site has three regions: the upper, defined by residues in TM II, III, VI, and VII; the middle, by residues in TM III, V, and VI; and the bottom, by residues in TM III, V, and VII. The inset illustrates the Gaussian volume representation of BMK-I-152 (PDB ID: 8GTM). The amino acid residues were drawn with a one-letter code. The superscript number denotes their position in the primary sequence of the receptor and the background color of the TM to which they belong. The structures were acquired from the protein data bank [62] and drawn with the Molstar online free software [63].

4. Peptidergic GPCR Signaling Circuitries and Cancer

Once bound to their respective GPCRs, agonist peptides induce versatile and adaptable intracellular signaling cascades, leading to cell metabolism control and switching on or off

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transcriptional programs. Peptidergic GPCRs may signal through the alpha or beta-gamma subunits of different heterotrimeric G proteins and β-arrestins, as well as other ancillary proteins, for example, regulators of G-protein signaling (GRS) or receptor kinases [78], generating adaptable signaling pathways from the plasma membrane or from intracellular compartments that share intracellular effectors and mutually regulate with other signals, peptidergic or not, processed by the cell. Intracellular effectors regulate intracellular messengers (cAMP, DAG, Ca^{2+}), modulating biochemical events encharged of short-term metabolic effects or long-term events related to gene expression (Figure 8, A and B) [16,33,79–82].

Peptidergic GPCR systems may fail in several ways, affecting harmonic cell functioning should compensatory mechanisms override. Among the causes of malfunction, we could consider abnormal overexpression, overstimulation by ligand overproduction, protein mutations, aberrant dimerization or oligomerization, truncation, and altered internalization. When the receptor does not adequately govern cellular responses associated with cell growth, migration, inflammatory outcomes, oxidative stress, increased angiogenesis, apoptosis inhibition, or mitochondrial dysfunction, it may provoke the appearance of cancer (Figure 8, right panel) [61,83–85].

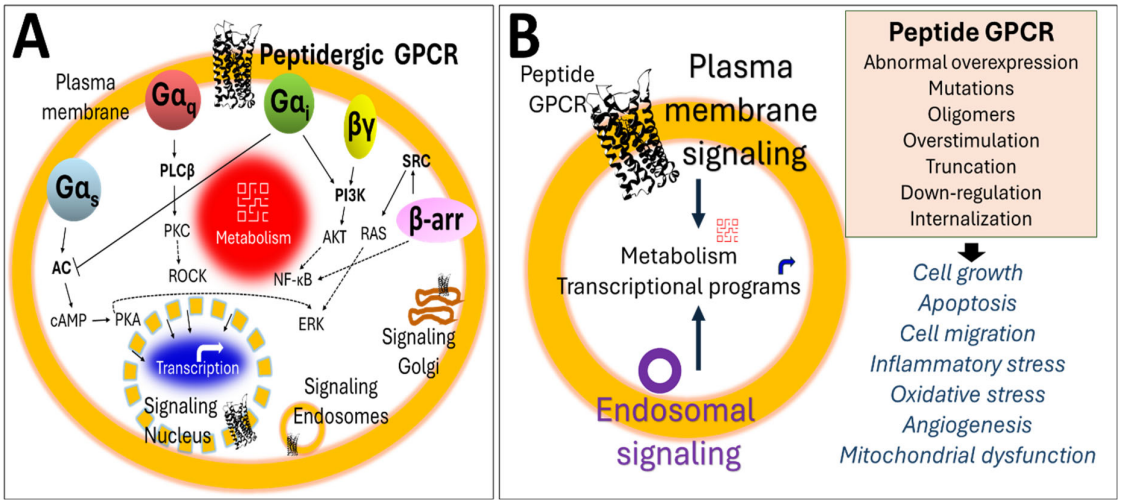

Figure 8. Schematic representation of signaling pathways triggered by peptides binding to their respective receptors (A). B indicates how GPCRs activated by peptides may signal from the plasma membrane or, once internalized in endosomes, from signalosomes, determining complex and modulated intracellular signals that affect cell metabolic processes and transcriptional programs. Alteration of GPCR homeostasis through uncontrolled overexpression, down-regulation, truncation, dimerization, or overstimulation leads to changes inducing transitory or sustained cellular events, leading to aberrant cell growth, cell migration, inhibition of apoptosis, or mitochondrial dysfunction compromising energy metabolism and adaptative cell responses, ending eventually in abnormal cell division and growth, among other disturbances. The three-dimensional representation of GPCR was drawn from the protein data bank (PDB ID: 8XZG) [62] with the Molstar online free software [63]. Abbreviations: AC, adenylyl cyclase; AKT, Ak strain transforming, a protein kinase B; β-arr, beta-arrestin; cAMP, cyclic 3′-5′ adenosine monophosphate; ERK, extracellular receptor kinase; NF-kB, nuclear factor kappa B; PI3K, phosphatidyl inositol 3-kinase; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; RAS, rat sarcoma, ; ROCK, Rho-associated coiled-coil kinase.

It's important to note that not all peptidergic GPCR systems function alike or regulate cell activity in similar ways. Therefore, it is crucial to understand the specific mechanisms governed by these systems and the implications of their altered function concerning the onset and perpetuation of cancer [7,86].

Recent experimental evidence associating peptidergic GPCR systems with cancer is summarized below to underlie the importance of these receptors as possible targets in cancer therapy through strategic drug design.

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4.1. The Opioid System

Opioid receptors are broadly distributed in the nervous system and peripheral tissues. Their involvement in cancer has been reported for different types of neoplasia [87]. In colorectal cancer, for example, the epithelium is struck by opioid-induced changes in the microbiota populations and altered inflammation, angiogenesis, and apoptosis mechanisms [88]. Delta opioid receptors (DOPR) appeared highly expressed in human and murine breast cancer tissues, and their stimulation-induced metastasis via the JAK1/2 signaling pathway was abolished by the administration of a DOPR antagonist [89]. The opioid drug agonist tramadol (2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol) activated mu-opioid receptors (MOPR) and exerted an antitumor effect in pancreatic ductal carcinoma cell lines by reducing their invasive and proliferative profile through decreasing proteins controlling the cell cycle [90].

Notwithstanding some controversy concerning whether opioids stimulate or suppress cancer development and durability, further studies should clarify their precise role in cell proliferation and migration, angiogenesis, and immunity responses in several tumors to adequately apply agonists and antagonists in potential specific treatments [91]. Also, deciphering the participation of opioids in cancer is very important since they may sustain cancer when frequently used as analgesics, for example, in neoplasia-associated pain [92,93].

4.2. The Neurokinin System

Extensive basic research performed in different experimental models, from cell cultures to in vivo procedures, has reported the involvement of neurokinin receptors, especially the NK-1R type, in various cancers [67,94–99].

In vitro and in vivo studies reported the antiproliferative activity of an NK-1R antagonist, aprepitant (a morpholine derivative), combined with 5-fluorouracil in colorectal cancer cells. The drugs displayed a synergistic effect, which upregulated genes implicated in apoptosis (Bax, p53) [100]. The undecapeptide substance P, through NK-1R, induced oxidative stress and promoted cancer progression in prostate cancer lines PC3 and LNCaP by increasing reactive oxygen species (ROS) and decreasing oxidoreductase activities. The antagonist aprepitant reversed the observed effects [101]. The NK-1R system appeared overstimulated in intrahepatic cholangiocarcinoma cells, and aprepitant showed an antiproliferative profile by activating apoptosis and autophagia via ROS increase and JNK signaling activation [102]. L760,033 (a phenyl piperidine derivative), an NK-1R antagonist, increased apoptosis in SiHa cells (a human cervical cancer line) through silencing ERK signaling and the controlling of anti-apoptotic Bcl-2 and pro-apoptotic BAX proteins [103]. The synergistic action of aprepitant and 5-aminolevulinic acid abolished cell viability and migration of glioblastoma multiforme [104].

4.3. The NPY System

The malfunction of the NPY GPCR system has been associated with more than 20 types of cancer [105].

An immunohistochemistry study showed high expression of NPY peptide and the NPY receptors 1, 2, and 5 in cells from primary prostate cancer and bone metastasis, compared with healthy tissue, suggesting the implication of this peptidergic system from the first phases of the tumor progress to advanced metastatic stages of the disease [106]. Hypoxia-induced stimulation of the NPY5R system in Erwin sarcoma cells caused mitotic alterations, leading to metastasis and colonization in other tissues [107]. NPY5R has also been implicated in the capacity of neuroblastoma cells to migrate and invade other tissues through cytoskeleton modifications mediated by RhoA [108]. In a study centered on the analysis of genes altered in colon cancer, an ensemble of 10 genes, including the one encoding NPY, acted as an altered network associated with the early and advanced stages of the disease [109]. Antagonizing NPY1R and NPY5R in breast cancer cell lines MDA-MB.231 and MCF7 in low oxygen partial pressure blocked NPY-dependent cell proliferation and migration [110]. In hypoxia conditions, the sensitivity of breast cancer cells to NPY stimulation may be due to the

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modulation of 5' promoter regions of the genes *NPY1R* and *NPY5R* by the hypoxia-inducible factor, HIF [111]. Applying NPY2R antagonists in human colorectal adenocarcinoma cells (HT29) reduced angiogenesis and tumor growth. ERK/MAPK was the main signaling pathway involved in the process [112].

4.4. The Galanin System

The galanin peptide is highly expressed in neuroendocrine and non-neuroendocrine tumors [86,113,114].

Galanin immunohistochemical analysis of human colorectal adenocarcinoma samples revealed a higher expression of the peptide in lower stages of the disease (I-III, TMN tumor grading) than in advanced stage IV. On the other hand, a transcriptome study in the same samples showed an upregulation of specific genes concerned with the modulation of cell cycle and cell division, transcriptional control, and immunity regulation in the late stages of the disease compared with early stages[115]. This report, thus, correlates galanin downregulation with advanced colorectal adenocarcinoma stages. Immunohistochemistry studies of GAL3R in colorectal adenocarcinoma samples indicated that the high immunoreactivity signal for GAL3R correlated with a favorable clinical prognosis and extended patient survival [116]. In human glioma cell lines U251 and T986, galanin activating the GAL1R exhibited an antiproliferative profile [117].

On the other hand, secreted galanin by head and neck squamous carcinoma cells favored tumor progression [118]. A specific agonist (M89b, a truncated galanin analog) of GAL2R showed an antitumoral effect of patient xenografts of pancreatic ductal adenocarcinoma in mice [119]. Additionally, to the presence and role of the galanergic systems in different tumors, their occurrence in macrophages and other immune cells infiltrating tumors represents an essential feature of galanin as a participant in the neoplasia microenvironment regulation [86,114,120].

The effects of galanin on GAL receptors appear complex and inconspicuous and may result in proliferative or antiproliferative outcomes depending on the tumor type and the receptor ligand-triggered. Therefore, further exploration of their participation in neoplasias is needed [113].

4.5. The Calcitonin System

Several receptors and peptides build up the calcitonin signaling crossroads. The implication of this peptidergic complex in cancer is supported by in vitro and in vivo studies in different tumors [71,121–123].

Determination of high calcitonin levels is an excellent marker of medullary thyroid cancer; however, it is not a pathognomonic mark [124–126]. CGRP associated with altered dendritic development in a single cell model of medullary thyroid cancer. This association exhibited a close relationship with activated adenylyl cyclase activity, leading to augmented Kruppel-like factor 2 and consequent impairment of T-cell tumor infiltration [127]. Calcitonin receptor was found augmented in glioblastoma cells in stage T0; however, its participation in carcinogenesis mechanisms in these tumors remains undefined [128]. The upregulated presence of CGRP and its receptor CALCRL in tumor samples from patients with colorectal cancer provided novel diagnosis and prognosis tools concerning tumor stage definition. The overactivation of this system and mechanisms triggered to impulse tumor appearance require better clarification [123]. CALCR appeared as a critical regulator of cell proliferation, stemness, apoptosis, and expression of SOX2 and Oct4 in acute myeloid leukemia cells. It also has a prominent involvement in resistance mechanisms to chemotherapy [129]. Secretion of CGRP from afferent neurons added a microenvironmental tumor element influencing oral cancer growth, pain, and inflammation, making the receptor a putative target for therapy intervention [130]. A mechanistic relationship between KRAS mutation and adrenomedullin in prostatic ductal carcinoma has resulted in a disarray of the alternative complement pathway by altering the secretion of complement factor B [131]. The different types of receptors (CLR associated with varying proteins of RAMP 1, 2, and 3) impact the development of several tumors [132].

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4.6. The Neurotensin System

Sound experimental evidence in patient samples and cell cultures sustains the connection of neurotensin with different neoplasias, from breast cancer to prostate, lung, brain, colon, or pancreas tumors [133–138].

By activating NTS1R and NTS2R, neurotensin augmented the appearance of neuroendocrine differentiation in animal models and cell lines related to castration-resistant prostate cancer. The NTS1R antagonist SR48692 also delayed the transdifferentiating process associated with cancer progression [139]. Some neurotensin analogs tested in a colorectal cancer cell line HCT116 diminished cell survival and reduced the percentage of cancer stem cells (CD133 positive) [140]. By triggering the NTS1R/Akt signaling route, neuron-released neurotensin promoted cell invasion and propagation in pancreatic cancer (ductal carcinoma) cell lines. NTS1R knockdown and applying GDC0941, a PI3K inhibitor, counteracted the observed effect [141]. Functional interaction between NTS2R and TRKB favored chronic lymphocytic leukemia B cell viability. The use of a peptide able to disrupt the interaction disrupted Src signaling and apoptosis mechanisms, making the protein-protein contact a sound target for therapy [142].

4.7. The Apelin System

Non-comparable methodological approaches and procedures have made it challenging to use bloodstream apelin as a marker and predictor of different neoplasias (lung, breast, prostate, ovary, brain, and more) [143–145]. Hence, there is an urge to standardize methods and apply them to specific tumors to ascertain the actual value of the circulating peptide in diagnosis and prognosis procedures.

The apelin/APLNR system has been shown to exhibit an antineoplastic effect in colon26 (a murine colon adenocarcinoma cell line) by increasing immunological mechanisms against the tumor cells. The agonist ligand [Pyr1]Apelin-13 administration induced $CD4^+$ and $CD8^+$ T cell accumulation [146]. On the other hand, the effect of apelin in immune macrophage responses to tumoral progression was analyzed in a co-culture of mouse macrophage cell line RAW264.7 with neck carcinoma cell line SCCLMT1 with suppressed (RNA interference) apelin expression. Apelin was found in this experimental model to promote the accumulation of M2 macrophages, thus facilitating tumor expansion by interfering with the microenvironment controlling tumor behavior [147]. By activating the APLNR, apelin augmented cell proliferation, migration, invasiveness, and increased glycolysis rate in cervical cancer cells (human samples and cell lines) via the PI3K/AKT signaling pathway [148]. Apelin has also been associated with liver fibrosis and hepatocellular carcinoma; moreover, the impact of this peptide on the gastrointestinal microbiota and its influence on cancer pathologies await deeper analysis and clarification [149].

One may suppose that the proliferative or antiproliferative effects observed for the apelin system may be related to experimental procedures (methodological bias) or specific characteristics of tumor types and tumor microenvironmental milieu (physio-pathological importance). In consequence, further research from this perspective should be encouraged.

4.8. The CRF System

CRF may directly influence colon cancer, as is the case for CRF-induced cell proliferation through Janus kinase activation and activation, via NF-κB, of VEGF (vascular endothelial factor) with subsequent augmented angiogenesis [150]. Indirectly, CRF and urocortins intervene in cancer progression by participating in stress and inflammation mechanisms [151]. In a murine breast cancer model, tumor-associated anxiety was partially responsible for tumor advancement. Interestingly, CRF neurons forming a circuit between the central amygdala and the lateral paragigantocellular nucleus were relevant in modulating anxiety and related tumor progression [152]. Also, CRF neurons of the paraventricular nucleus of the hypothalamus seem to contribute to tumor progression by altering the equilibrium of immune control of tumors [153]. These findings suggest an interesting salient central brain control over tumors developed in other tissues.

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In human bladder cancer cell lines, CRF augmented cell migration capacity and favored metastasis in a murine-implanted xenografted tissue (originated from cancer cells). The effect was mechanistically associated with the activation, through ERK, of supervillin, an actin-binding protein involved in cell proliferation processes [154]. The participation of CRF and related peptides in cancer has also been indirectly associated with their increased expression in malignant tissues. For example, immunohistochemical analysis of CRF urocortin and CRFRs in human vulvar samples (from control to premalignant and malignant specimens) established a clear correlation between the expression patterns and malignity [155]. Also, immunolocalization analysis of CRF and its receptors, CRF1R, and CRF2R in human pancreatic cancer samples unveiled a positive correlation between augmented CRF1R signal and poor prognosis [156].

This report concerns a few peptidergic GPCR systems and their relationship with molecular mechanisms sustaining cancer. However, the list is far from complete. Other systems are also implicated. For example, glucagon-like peptide-1 (GLP-1) receptor agonists, besides their application in diabetes and obesity therapies, behave as antitumoral agents against hepatocellular carcinoma[157]. Analogs of cyclic peptide somatostatin were reported to be effective in treating neuroendocrine tumors [158]. In human-derived colorectal cancer cell lines (T84, Caco-2, HCT116), the nonapeptide bradykinin increased interleukin 6, leading to tumor cell invasion and migration [159]. Gastrin-releasing peptide, cholecystokinin, endothelins, and Tat-NTS/Tat-Cx43266-283 are associated with glioma tumors through different mechanisms [160].

5. Peptidergic GPCRs as Targets for Cancer Treatment

It is relevant to place peptidergic GPCR systems in a position of consideration and inclusion in basic and clinical studies that aim to treat different cancers by advancing structure-activity analysis, exploring molecular mechanisms, reviewing possible drug candidates, and underlying the relevance of setting appropriate drug design strategies. Since agonist and antagonist activity may regulate receptor activation and inactivation, a few representative structures that bind and impact structure conformation to the receptors under study in this review are presented.

Noteworthy, the obtention of ligands with the capacity of abrogating or stimulating peptide GPCR activity, directly related to the mechanism of cancer development and progression, would be beneficial since complex downstream signaling networks may be disconnected and complex biochemical cascades may be silenced from their initial stages. On the other hand, careful and reliable with comparable methodological procedures studies should previously establish if silencing, aborting the signaling, or promoting the signal does not interfere with physiological mechanisms in healthy cells. Hence, there is an insistence on properly unraveling the signaling circuitries for every system and in specific types of tumors.

Therapeutic strategies not only pertain to specific receptor ligands modulating orthosteric and allosteric sites and influencing dynamic receptor conformations but also include strategies aiming at obtaining safe, stable, durable, and specific drugs and compound envoys targeting the altered peptidergic system in tumor cells without affecting healthy cells. It may also include a bivalent drug designed to aim for two or more molecular objectives of the same signaling pathway or a different one. Additionally, these drugs may complement other treatments (radiotherapy, surgery, classical chemotherapy) tailored for specific cancers, tumor stages, and individuals. In practice, a canopy of pharmaceutical approaches may serve the purposes mentioned above, for example, peptides with anticancer cargo, antibodies directed against specific receptors, antibodies conjugated to drugs, or specific signaling proteins, or silencing of receptors and other proteinaceous targets with siRNA technology [161,162].

Table 1 summarizes peptidergic GPCR antagonists and agonists that, based on current research, offer promising opportunities for treating tumor development, extension, metastasis, or resistance to tumor therapy. The purpose is to underlie the importance of continuing to test and deepen knowledge in preclinical, experimental models, and clinical trials.

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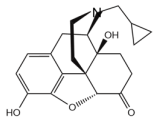
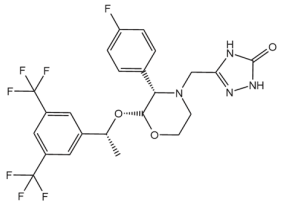
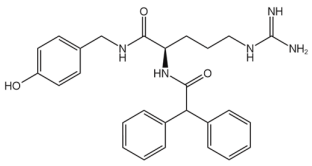
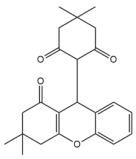
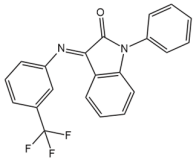
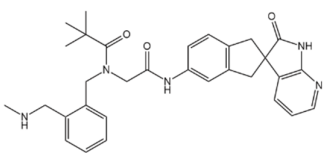
Table 1. Representative compounds with antineoplastic properties when tested in different types of tumors in experimental preclinical models and clinical trials. Chemical structures were drawn with KingDraw free chemical structure editor software (version 3.0 for Windows).

Antitumoral compounds	Receptor	Tumor	References
Naltrexone (antagonist) 	OPR	Metastatic breast cancer (phase II study) Gastrointestinal cancer Cervical cancer	[163–167]
Aprepitant (antagonist) 	NK-1R	Gall bladder cancer Colorectal cancer Gynecologic cancer Cholangiocarcinoma Neuroblastoma Prostate cancer Thyroid cancer Pancreatic cancer	[96,102,168–172]
BIP3226 (antagonist)  L152,804 (antagonist) 	NPY1R (BIBP 3226), NPY5R (L152,804)-	Breast cancer Colon cancer Prostate cancer	[105,110,112,173]
M89b, a galanin analogue (agonist) Amino acid sequence: pEWNLNAAGYLLATHACG (pE stands for pyro-Glu)	GAL2R	Pancreatic ductal adenocarcinoma Colorectal cancer	[114,119,174,175]
SNAP 37889 (antagonist) 	GAL3R	Promyelocytic leukemia cells	[176]
Indolinone Compound 25 (antagonist) 	CLR-RAMP3 (AM2R)	Human pancreatic cancer cell lines In vivo models of breast cancer	[132,177,178]

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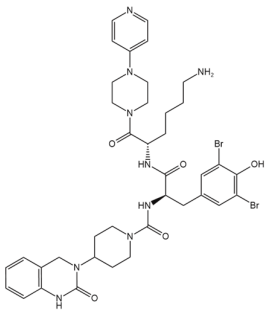
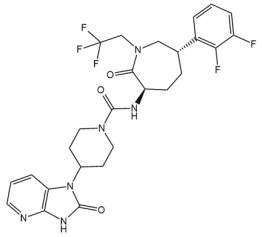
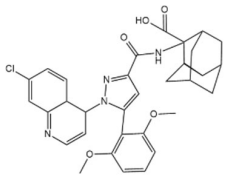
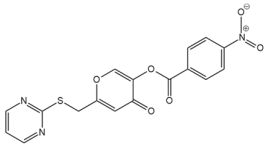
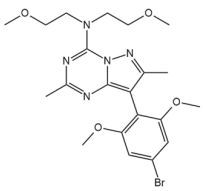
Olcegepant (antagonist)  Telcagepant or MK0974) (antagonist) 	CLR-RAMP1	Prostate cancer Acute myeloid leukemia	[179–181]
SR 48692 (antagonist) 	NTS1R	Ovarian cancer	[182]
ML 221 (antagonist) 	APLNR	Cholangiocarcinoma Breast cancer Infantile hemangioma	[143,183,184]
BMK-I-152 (antagonist) 	CRFR1	Not tested in cancer therapy* Tested for major depression	[77]

6. Conclusion and Future Directions

Peptidergic GPCRs have gained positions for their consideration as druggable targets for cancer management and treatment. Also, peptides, their GPCR, transducer, and effector intracellular proteins associated may serve as reliable biomarkers for diagnosing and prognosis of specific types of tumors and their stages [185].

Procedures of organic synthesis by using reference structures (for example, pyrazole rings) [186] and other synthetic strategies aimed at exploring the orthosteric and allosteric GPCR sites better may generate new compounds with possible applications in adapting, modulating, and mending GPCR

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dysfunction. The routes for the obtention of receptor cavity-flexible and safe, though specific, non-toxic, and potent compounds targeting and modulating conformation receptor states with functional outcomes and biased signaling routes may result in rewarding the efforts [187]. Therefore, a three-dimensional analysis that unravels receptor-drug interfaces' structural and docking characteristics is paramount.

Also of consideration is that some drugs are already marketed for other purposes, and therefore, setting clinical trials to define their therapeutical potential in cancer may result in a lower burden [188,189]. The case applies, for example, to aprepitant, an NK-1R antagonist used in clinical practice to annihilate vomit and nausea induced by chemotherapy or surgery [169]. Also, CGRP antagonists, generically called “gepants” (rimegepant or atogepant), are currently used to treat migraine [190]. Another example is a CRF1R antagonist, MK-I-152, tested for treating major depression [77].

The task is challenging, time-consuming, resource-consuming, and effort-consuming. Still, it may yield encouraging results in the context of new approaches in cancer therapy, including the repurposing of drugs already in use in different nosological entities.

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