**Review**

The Galaninergic System: A Target for Cancer Treatment

Manuel Lisardo Sánchez 1,* and Rafael Coveñas 1,2

1 Laboratorio de Neuroanatomía de los Sistema Peptidérgicos (Lab. 14), Instituto de Neurociencias de Castilla y León (INCYL), Universidad de Salamanca, c/Pintor Fernando Gallego 1, 37007 Salamanca, Spain; covenas@usal.es
2 Grupo GIR USAL: BMD (Bases Moleculares del Desarrollo), University of Salamanca, 37007 Salamanca, Spain
* Correspondence: lisardosanchez8@gmail.com; Tel.: +34-923294400 (ext. 1856); Fax: +34-923294549

**Simple Summary:** Peptidergic systems play an important role in cancer progression. The galaninergic system (the peptide galanin and its receptors: galanin 1, 2 and 3) is involved in tumorigenesis, the invasion and migration of tumor cells and angiogenesis and it has been correlated with tumor stage/subtypes, metastasis and recurrence rate in many types of cancer. Galanin exerts a dual action in tumor cells: a proliferative or an antiproliferative effect depending on the galanin receptor involved in these mechanisms. Galanin receptors could be used in certain tumors as therapeutic targets and diagnostic markers for treatment, prognosis and surgical outcome. This review shows the importance of the galaninergic system in the development of tumors and suggests future promising clinical antitumor applications using galanin agonists or antagonists.

**Abstract:** The aim of this review is to show the involvement of the galaninergic system in neuroendocrine (phaeochromocytomas, insulinomas, neuroblastic tumors, pituitary tumors, small-cell lung cancer) and non-neuroendocrine (gastric cancer, colorectal cancer, head and neck squamous cell carcinoma, glioma) tumors. The galaninergic system is involved in tumorigenesis, invasion/migration of tumor cells and angiogenesis, and this system has been correlated with tumor size/stage/subtypes, metastasis and recurrence rate. In the galaninergic system, epigenetic mechanisms have been related with carcinogenesis and recurrence rate. Galanin (GAL) exerts both proliferative and antiproliferative actions in tumor cells. GAL receptors (GALRs) mediate different signal transduction pathways and actions, depending on the particular G protein involved and the tumor cell type. In general, the activation of GALR promoted an antiproliferative effect, whereas the activation of GALR induced antiproliferative or proliferative actions. GALRs could be used in certain tumors as therapeutic targets and diagnostic markers for treatment, prognosis and surgical outcome. The current data show the importance of the galaninergic system in the development of certain tumors and suggest future potential clinical antitumor applications using GAL agonists or antagonists.

**Keywords:** galanin; galanin receptor; galanin receptor antagonist; galanin receptor agonist; neuroendocrine tumors; signaling pathways

1. Introduction

The GLOBOCAN 2020 database (World Health Organization (WHO)) states that of the 7,794,798,844 inhabitants of our planet, 19,292,789 of them were diagnosed with some type of cancer and 9,958,133 died, with prevalence cases at 5 years of 50,550,287. Female breast cancer is the most diagnosed cancer and the leading cause of cancer death is lung cancer (1.8 million deaths) [1]. In 2040, 28.4 million patients suffering from cancer are expected in the world [1]. These data are sufficiently representative of the health problem that cancer represents today. Cells, escaping from normal behavior, acquire distinctive characters (evading growth suppressors, maintaining proliferative signaling, allowing replicative immortality, resisting cell death, activating invasion/metastasis, inducing
angiogenesis) that make them cancerous [2] (Figure 1). Moreover, the reprogramming of energy metabolism and evasion of immune destruction have also been added to the previous hallmarks of cancer [2]. These behaviors arise from the instability of the genome that produces genetic diversity, and inflammatory mechanisms that promote the multiple actions described above (Figure 1). Tumors are not currently considered as simple masses of cancer cells; they are more complex in that they contain a repertoire of apparently normal recruited cells that contribute to the acquisition of distinctive features by regulating the tumor microenvironment [2]. The full knowledge of the previously mentioned hallmarks will help to develop new therapeutic strategies against cancer.

![Image](image_url)

**Figure 1.** Ten keys of cellular/tissue behavior that make a cell a cancer cell, contrary to its normal biological destiny, leading to the formation of a primary tumor and later a secondary one. Red arrows show the involvement of the galaninergic system in these mechanisms: note that GAL is involved in six of them.

Neuropeptides such as galanin (GAL), angiotensin II, apelin, adrenomedullin, endothelin-1, bombesin, orexin, substance P, neuropeptide Y, calcitonin gene-related peptide, vasoactive intestinal peptide and neurotensin are involved in cancer [3–9]. The overexpression of the peptidergic systems has been involved in the progression of some types of cancer [3,5,9]. In general, the mentioned peptides promote the proliferation, invasion and migration of tumor cells, angiogenesis and lymphangiogenesis and exert an antiapoptotic effect in these cells. However, other peptides exert an anticancer action; this is the case of the heptapeptide angiotensin (1–7) which blocks cell proliferation and angiogenesis. For
these reasons, it is necessary to investigate the roles played by the peptidergic systems in cancer in more depth. This line of research has been developed over the last several years and the knowledge of the roles played by peptides in tumor progression has notably increased [9]. It is important to note that the galaninergic system has been involved in six (e.g., proliferative action, invasion, metastasis, angiogenesis) of the ten cancer hallmarks previously mentioned (Figure 1). Unlike what happens with other peptides (e.g., substance P, neurotensin), which exclusively exert a proliferative action on tumor cells [9,10], GAL exerts this proliferative action, but also suppresses the development of certain types of cancer (e.g., neuroblastoma, head and neck squamous cell carcinoma, gastric cancer) [11–13]. Thus, due to the crucial role that GAL plays in cancer, the aim of this review is to show the involvement of the galaninergic system in this disease and to suggest potential therapeutic strategies to block the development of tumors using GAL receptor antagonists or agonists. The latter is an important point that must be developed in the future to identify potential antitumor targets and to better evaluate the involvement of GAL in cancer.

2. The Galaninergic System: Galanin and Its Receptors

GAL was discovered in porcine intestinal extracts and contains 29 amino acids [14]; however, in humans, the peptide contains 30 amino acid residues (Figure 2) and, unlike porcine GAL, the carboxy-terminus is not amidated [15–17]. The amino acid sequence of GAL is highly conserved among species (almost 90%) [18]. The C-terminus of GAL is involved in its receptor-binding affinity and the N-terminus is crucial for its biological activity [19]; the fifteen N-terminal residues of GAL are highly conserved throughout evolution [20]. GAL and other peptides (GAL message-associated peptide (GMAP), GAL-like peptide (GALP), alarin) belong to the GAL family of peptides. In addition, the peptide spexin (neuropeptide Q, 14 amino acids) is the most recently discovered member of this family; spexin has been shown to be involved in reproduction, nociception, renal function and energy homeostasis [21]. GALP, an endogenous ligand that activates the three known types of GALRs, was isolated from the porcine hypothalamus, contains 60 amino acids and is involved in reproduction and energy homeostasis [22,23]. Alarin (25 amino acids) is a splice variant of GALP mRNA [24]. The human chromosome 11q13.3-q13.5 contains the pre-pro-GAL gene encoding GAL, which shows five introns and six exons, which in turn are translated into a pre-pro-hormone (123 amino precursor) containing the signal peptide, GAMP and GAL [17,25] (Figure 2). Some oncogenes have been located in the abovementioned region, which is also the breakpoint for the translocation t (11; 14) (q13; q32) in diffuse B-cell lymphoma and chronic lymphocytic leukemia [26]. The gene spans 6.5 kb and its first exon only encodes the 5′ untranslated sequence. In the pre-pro-GAL gene, its 5-prime flanking sequence shows a TATA box preceded by binding sites for transcription factors (e.g., NF-κB) and contains a CT-rich region that is flanked by two Alu repeats, 2.3 kb upstream of the transcriptional start site; the region (500 bp) preceding this site contains 79% CG [27]. GALP and alarin are encoded by the pre-pro-GALP gene, which is located on the human chromosome 19q13.43 and comprises six exons [28]. The region encoding GALP is contained in exons 2–5 and alarin is formed when post-transcriptional splicing leads to the exclusion of exon 3, resulting in a frame shift and a novel precursor peptide [24].
Figure 2. Transcription–maturation–translation processing of GAL, from human chromosome 11. Human GAL contains 30 amino acids residues. 1–6: exons; aa: amino acids.

The galaninergic system (GAL and GAL receptors (GALRs)) is widely distributed by the mammalian gastrointestinal tract, testis, ovary, uterus, kidney and heart, and by the immune, endocrine, peripheral and central nervous systems (e.g., endocrine pancreas, pituitary gland, paravertebral sympathetic ganglia, myenteric plexus, glial cells, dorsal root ganglion, spinal cord, brainstem, thalamus, hypothalamus, hippocampus, amygdala) [25,29–36]. The half-life of GAL in plasma is about five minutes and GAL coexists with many other neuroactive substances (e.g., enkephalin, vasopressin, calcitonin gene-related peptide, substance P, neuropeptide Y, cholecystokinin, growth hormone, luteinizing hormone-releasing hormone, dopamine, glutamate, noradrenalin, serotonin, acetylcholine) [29,37–44]. In general, GMAP in the rat central nervous system showed a similar profile of expression to GAL; however, GALP and alarin showed a more restricted expression than GAL [45]. Due to the widespread distribution of the galaninergic system by the whole body, GAL has been involved in many physiological actions after binding to
specific G protein-coupled receptors: smooth muscle contraction, acetylcholine release inhibition, energy metabolism, food and water intake, hyperglycemia, osmotic and metabolic homeostasis, spinal reflexes, injury response, nociception, reproduction, memory, cognition, learning, arousal, sleep, neural growth, glucose-induced insulin release inhibition and respiratory, cardiovascular, neuroendocrine and gastrointestinal mechanisms [8,14,18,20,25,29,33,38,46–50]. Moreover, GAL regulates the level of growth hormone, prolactin, dopamine, pancreatic peptide, luteinizing hormone, luteinizing hormone-releasing hormone, somatostatin and insulin [18,42,51–53]. GAL acts as a neurotransmitter and neuromodulator in the central nervous system and the peptide has been involved in several diseases (e.g., anxiety, depression, stroke, alcoholism, Alzheimer’s disease, Parkinson’s disease, epilepsy); the galaninergic system also plays an important role in inflammatory bowel diseases and diabetes [18,20,25,54–58]. In addition to the nervous system actions mediated by the galaninergic system (e.g., GAL exerts a neuroprotective action in the hippocampus and favors neurite outgrowth) [49,50], GAL also mediates non-neural functions including the emerging roles played by the peptide in tumorigenesis [20] and in tumor-infiltrating immune cells (e.g., glioma-associated macrophages, microglia, neutrophils) [30]. GAL regulates the expression of chemokines (CCL2, CCL3, CCL5, CXCL8) and anti-inflammatory cytokines (tumor growth factor-β, interleukin-10, interleukin-1Ra) in macrophages [59]. The expression of GALRs in tumor-related immune cells suggests that GAL regulates the homeostasis of the tumor microenvironment. In humans, the expression of GAL is regulated in a cell type-specific manner by the brain-derived nerve growth factor, dexamethasone, progesterone, thyroid hormone, nerve growth factor, activity-dependent neuroprotective protein, leukemia inhibitory factor, vasoactive intestinal peptide and gonadotropin-releasing factor [20]. Protein kinase A (PKA) and protein kinase C (PKC) are inducers of the expression of the GAL gene, and the expression and release of GAL is promoted by axotomy, chronic stress, ischemic brain damage, orofacial pain, virus infection and chronic constriction nerve injury [20] (Figure 3).
Figure 3. Pathological situations and bioactive molecules promoting and regulating, respectively, the expression of GAL.

GALRs (GAL 1 receptor (GAL1R), GAL 2 receptor (GAL2R), GAL 3 receptor (GAL3R)) belong to the rhodopsin-like (class A) G protein-couple receptor family (seven transmembrane receptors or 7TM) [60]. They contain three extracellular loops, three intracellular loops, an extracellular N-terminus and three intercellular loops [60,61]. The helix 8 acts as a conformational switch at the C-terminus [62]. GALRs have sequence homologies in the transmembrane region: GAL1R-GAL1R (33%) and GAL2R-GAL3R (54%) [20], whereas human GAL1R and GAL3R respectively show 89% and 92% sequence homology with their receptor homologs present in the rat [63]. Human GAL has tens of nanomolar affinity at GAL1R, subnanomolar to nanomolar affinity at GAL2R and subnanomolar affinity at GAL3R [64]. Although the structure of GALRs is quite similar, different binding characteristics and intracellular signaling pathways have been reported after the activation of these receptors by ligands [60,61]. Thus, the lengths of the N-terminus (which plays an important role in the binding of ligands) and C-terminus are different in GALRs (C-terminus: GAL1R, 37 residues; GAL2R, 30; GAL3R, 13; N-terminus: GAL1R, 47 residues; GAL2R, 80; GAL3R, 62) [60]. The physiological actions of GAL are mediated by GAL1R, GAL2R and GAL3R; several signaling pathways are activated after the binding of GAL to these receptors: the stimulation of phospholipase C (PLC, mediated by GAL1R) or the inhibition of cyclic adenosine monophosphate (cAMP)/PKA (mediated by GAL1R/GAL3R) [26]. Moreover, GAL1R mediates the inhibition of adenylate cyclase (AC) via coupling to Gi type G protein [65,66]. GALR type is determined by the region between the transmembrane helix 7 and the extracellular loop 2 (a variable region affecting the binding of ligands) and by the cavity size (e.g., the GAL1R binding cavity is narrower than that observed
in GAL1R or GAL3R) [60]. Human GAL1R and GAL3R genes have respectively been localized in chromosomes 17q25 and 22q12.2-13.1 [66].

GAL1R was isolated from a human melanoma cell line [67]. It is coupled to Gβγ/Gai signaling pathways and promotes, via a PKC-independent mechanism, the activation of mitogen-activated protein kinases (MAPKs) [17,68]. Moreover, the activation of GAL1R inhibited AC activity via an interaction with G-proteins (Gai/o), leading to G protein-coupled inwardly-rectifying potassium (GIRK) channels opening [32,67,69]. GAL1R activation can also inhibit the transcription factor cAMP regulatory element binding protein (CREB)-dependent signaling pathway [70], and the expression of GAL1R (but not that of GAL3R or GAL1R) was controlled by cAMP via CREB [71,72]. The GAL1R gene (located in chromosome 18q23) in humans shows three exons that are translated into a long protein containing 349 amino acids; GAL1R homology is high between species (e.g., in mouse, 93% of the residues are identical to those observed in humans) [73]. GAL1R has been located in the central (e.g., cortex, amygdala, hippocampus, thalamus, hypothalamus, locus coeruleus, medulla oblongata, spinal cord) and peripheral (e.g., dorsal root ganglion) nervous systems [33,34] and in the gastrointestinal tract [67,74].

GAL1R was first identified in the rat central nervous system [35,75,76] and was cloned in rat hypothalamic cells for the first time [35]. GAL1R contains His252/His253 (transmembrane domain 6) and Phe264/Tyr271 (extracellular loop 3) residues, which play a crucial role in the binding of ligands and in the activation of the receptor [77]. The sequence of human GAL1R shows a high homology with that observed in the rat (85–92%) and it was 39% identical to human GAL3R [33,63,78]. In the rat, GAL1R shows 38% amino acid identity with GAL1R [35]. In comparison with GAL1R, the distribution of GAL3R is more widespread since it has been observed in the nervous system (piriform cortex, dentate gyrus, amygdala, hypothalamus, mammillary nuclei, spinal cord), skeletal muscle, liver, testis, ovary, uterus, spleen, heart, kidney, lung, gastrointestinal tract and pituitary gland [33,35,63,79,80]. GAL1R mRNA expression has been reported in the neocortex, dentate gyrus, hypothalamus, cerebellar cortex, substantia nigra, vestibular complex and dorsal root ganglion [7,80,81]. GAL1R expression was modified in the thalamus and cerebral cortex during brain development; this suggests that the receptor is involved in important mechanisms during the establishment/maturation of synaptic circuits and during neural damage/repair in the mature nervous system [82]. GAL1R activates the G protein (Gαq/11) pathway by triggering the intracellular phosphoinositide turnover, the activity of PLC and the release of Ca2+ into the cytoplasm [35,63,68]. GAL1R, via PKC and G protein (Gαq), activated MAPKs, favoring the downstream phosphatidylinositol 3-kinase (PI3K)-dependent phosphorylation of PKB and blocked the activity of caspases 3 and 9 [68,83]. GAL1R, via GAL1R, induced the nuclear factor of activated T-cells and the cytoplasmic 2 (NFATC2)-mediated transcription of cyclooxygenase 2 and GAL1R, leading to the secretion of prostaglandin E2 and GAL1R, which favored cell invasion and neuritogenesis, respectively [84]. GAL1R can block forskolin-stimulated cAMP production; this suggests the activation of Gαi/o [66,85], and CREB [70]. GAL1R, via GAL1R, activated extracellular-regulated protein kinase (ERK) and the phosphorylation of the serine/threonine kinase Akt signaling pathway [86]. The activation of GAL1R promoted, via the Akt (PKB) pathway, cell survival and proliferation; both processes were MAPK1/MAPK3-dependent [87]. GAL1R mediated the neuroprotective effect promoted by GAL after injury and also activated PKC, PLC and ERK via Gq/11 [17,68,88]; this means that after binding to GAL1R, GAL agonists could be used to treat neurodegenerative diseases (e.g., multiple sclerosis) [49]. This is an important line of research that must be developed in the future; in particular, research must be focused on the search of GAL1R-specific agonists.

GAL1R was first isolated from rat hypothalamic cDNA libraries [89]. Human GAL1R (368 amino acids long) shows 36% amino acids identity with human GAL1R, 58% with human GAL3R and 90% with rat GAL1R [63]. The distribution of GAL1R (olfactory cortex, hippocampus, hypothalamus, medulla oblongata) is more restricted than that reported in the brain for GAL1R or GAL3R [33,63,77,89-91]. GAL1R mRNA has been located in the
amygdala, periaqueductal gray, locus coeruleus, brainstem reticular formation, spinal cord, pancreas, adrenal gland and testis [63,91]. GAL R promotes the activation of Goi/o, blocking AC activity and opening GIRK channels [63,90]. Spexin binds to human GAL2 Rs (not to GAL R), exerting a higher potency toward GAL R than GAL [21,92].

GAL agonists or antagonists (e.g., galantide, M35, M40, C7) have been used for the treatment of several disorders: GAL antagonists have been administered for the treatment of food intake disorders and Alzheimer’s disease, whereas GAL agonists have been used for the treatment of chronic pain [18,93]. Some fragments of GAL (GAL1-15; GAL1-16, GAL1-29), exerting physiological actions through GALRs (e.g., mood or cardiovascular regulation, alcohol intake), have been reported [94–97]. The conformational changes observed in GAL R lead to a higher affinity of this receptor for GAL1-15 than for GAL, increasing the signaling (mediated by Gi/o) and decreasing AC activity and CREB level [98]. GALRs may form heteromers with each other and with other types of G protein-coupled receptors in the central nervous system [99]. Thus, the GAL R/GAL R heteroreceptor complex [98] and heteromers of GALRs with alpha2-adrenoceptors and 5-hydroxytryptamine (HT), dopamine 1, neuropeptide Y1 or Y2 receptors have been reported [20]. The formation of the heterotrimer GAL R-GAL R-5-HT1A receptor complex could explain why GAL1-15, but not GAL1-29, antagonistically moderated the serotonin receptor [99]. In addition, this heterotrimer has been suggested as a potential target to reverse the actions mediated by fluoxetine on memory mechanisms [94,100]. Thus, heteromers can alter the recognition of GAL ligands, and they are promising new targets for therapeutic interventions.

3. The Galaninergic System and Cancer

Peptides and their receptors are one of the molecular bases for the therapeutic targeting of tumors [101]. The galaninergic system is expressed in normal tissues and, in cancer cells, is involved in tumorogenesis, invasion and migration (metastasis) [30,36,39,101–112], although in some tumors, GAL and GALRs are silenced [113]. This system has been observed in neuroendocrine (e.g., pheochromocytoma, pituitary adenoma, gangliocytoma, paraganglioma, neuroblastoma) and non-neuroendocrine (e.g., glioblastoma and other brain tumors, melanoma, basal cell carcinoma, head and neck squamous cell carcinoma, embryonic carcinoma, colon cancer, breast cancer, gastrointestinal cancer, prostate cancer) tumors [30,36,39,75,101–112,114–121]. For example, in squamous cell carcinoma, GAL R was involved in tumor suppression and GAL R favored tumor development and decreased survival [122,123]. GAL exerted a tumor-reducing effect in experimental murine models (gastrointestinal cancer), but in other models (adenoma formation), GAL promoted cell proliferation and tumor formation [101]. Thus, GAL can promote or inhibit the development of tumors; this is an important characteristic of the galaninergic system: to exert both proliferative and antiproliferative actions on tumor cells. Importantly, GAL/GALR expression has been correlated with tumor subtypes (colon carcinoma, squamous cell carcinoma, neuroblastic tumors, pituitary adenoma) or with tumor stage [101] and the activation of GAL R was generally antiproliferative, whereas the activation of GAL R showed antiproliferative or proliferative effects [101]. The stage and tumor size in colon cancer have been related to the GAL mRNA level: the higher the GAL expression, the shorter the disease-free survival [30,106]. In general, the data reported above suggest that the galaninergic system is a promising target for the diagnosis, prognosis and treatment of tumors expressing GAL and GALRs. In this section, the involvement of this system in neuroendocrine tumors (pheochromocytomas, insulinomas, neuroblastic tumors, pituitary tumors, small-cell lung cancer), gastric cancer, colorectal cancer, head and neck squamous cell carcinoma and glioma will be reviewed as well as other cancer types in which the galaninergic system has been less studied.
3.1. Galanin and Neuroendocrine Tumors

Neuroendocrine tumors (NETs) are a very heterogeneous tumor group including: (1) carcinoid gastroenteropancreatic tumors; (2) non-carcinoid gastroenteropancreatic tumors (vasoactive intestinal peptide (VIP)oma, gastrinoma, insulinoma); (3) catecholamine-secreting tumors (neuroblastoma, sympathoblastoma, ganglioneuroblastoma, ganglioneuroma, paraganglioma, pheochromocytoma); (4) chromophobe pituitary tumors; (5) medullary carcinoma of the thyroid; (6) Merkel cell tumors; and (7) small-cell lung cancer. NETs originate from neuroendocrine cells, which release peptides (e.g., GAL, somatostatin, pancreatic polypeptide, chromogranins) and express their corresponding receptors [124–126]. Thus, a high expression of peptidergic receptors has been reported in NETs for neutotensin, gastrin-releasing peptide, cholecystokinin, somatostatin and vasoactive intestinal peptide [125]. Importantly, the expression of the peptidergic systems in NETs has been correlated with prognosis and tumor stage [127].

Regarding the galaninergic system, many data demonstrated its involvement in NETs pathophysiology and carcinogenesis; for example, high doses of estrogens or dopamine agonists reversed rat pituitary hyperplasia and decreased the expression of GAL, suggesting that the peptide acted as a proliferative agent [128–132]. GAL expression is restricted to some NETs [107]: the peptide was observed in adrenal pheochromocytoma (62%), jugulo tympanic paraganglioma (40%) and carotid body paraganglioma (18%), but it was not found in metastatic or recurrent paraganglioma, extra-adrenal pheochromocytoma and carcinoid tumor [107,108]. Moreover, endocrine tumors from gastrointestinal tract, pancreas and lung did not show GAL [107]. This means that the utility of GAL as a diagnostic marker is limited to certain NETs. In this section, the involvement of the galaninergic system in those NETs (pheochromocytoma, insulinoma, neuroblast tumor, pituitary tumor, small-cell lung cancer) expressing this system will be reviewed (Table 1). The methodology (e.g., immunohistochemistry, in situ hybridization, Western blot) applied in the studies appearing along the text in different tables is reported. However, it is important to note that antisera directed against G protein-coupled receptors (including GALRs) are frequently unspecific [133,134]; accordingly, the findings found regarding GALRs should be taken with caution and only accepted when using valid controls, with the specificity of these antisera fully confirmed.

Table 1. Involvement of the galaninergic system in neuroendocrine tumors.

| Cancer                          | Actions/Presence                                                                 | References |
|---------------------------------|----------------------------------------------------------------------------------|------------|
| Corticotroph adenoma Human      | - High GAL expression (RIA)                                                      | [102]      |
|                                 | - GAL in 84% of tumors (IH)                                                      | [103]      |
|                                 | - GAL expression: smaller adenomas and better prognosis (IH)                    | [105]      |
|                                 | - GAL release and responded to corticotropin-releasing factor                   | [135]      |
| Ganglioneuroma Human            | - No correlation between prognosis/tumor markers and GAL level (RIA)            | [136]      |
|                                 | - GAL:R/GAL:R immunoreactivity decrease (IH)                                    | [137]      |
| Insulinoma Rat Rin14B cell line | - GAL:R expression (Northern blot, in situ hybridization)                       | [32]       |
| Insulinoma Rat RINm5F cell line | - GAL moderately suppressed insulin accumulation, but did not affect cell proliferation | [138]      |
|                                 | - Pancreatic beta-cells: GAL inhibited adenylate cyclase activity and insulin secretion | [53]       |
| Insulinoma                      | - Beta TC-1 cells: GAL, released from                                            | [139]      |
| Cancer                  | Actions/Presence                                                                                                                                                                                                 | References |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Mouse                  | sympathetic nerve terminals, inhibited pro-insulin gene expression stimulated by glucagon-like peptide-I (Northern blot)                                                                                           | [137]      |
| Neuroblastic tumors    | - GAL mRNA, GAL immunoreactivity and GAL binding sites expression (IH, in situ hybridization)                                                                                                                                 | [137]      |
| Human                  | - Low level of GAL binding sites correlated with survival; GAL/GALR expression related to tumor differentiation stage (RIA, IH, in situ hybridization)                                                           | [136,137] |
| Neuroblastoma Human    | - No correlation between prognosis/tumor markers and GAL concentration                                                                                                                                            | [136]      |
|                        | - GAL expression; GAL:R mRNA was less common than GAL:R mRNA (IH, in situ hybridization)                                                                                                                                 | [104]      |
|                        | - GAL:R/GAL:R highly expressed; GAL promoted tumor growth (IH, in situ hybridization)                                                                                                                             | [137]      |
| Neuroblastoma Human IMR32 cell line | - Dense core secretory vesicles: coexistence of GAL and beta-amyloid (IH)                                                                                                                                 | [140]      |
| Neuroblastoma Human SH-SY5Y cell line | - GAL:R mediated apoptosis, GAL antiproliferative potency: 100-fold higher in SY5Y/GAL:R cells than in SY5Y/GAL:R cells                                                                                                                                 | [12]       |
|                        | - GAL:R transfection: cell proliferation was blocked and caspase-dependent apoptotic mechanisms induced                                                                                                                                                                  | [12]       |
| Neuroblastoma Human    | - GAL, GAL:R and GAL:R mRNAs were detected, but not GAL:R mRNA (reverse transcription-PCR)                                                                                                                                 | [141]      |
| Rat B104 cell line     | - GAL expression (IH)                                                                                                                                                                                                 | [108,112,142] |
| Paraganglioma Human    | - GAL was detected in 18% of tumors (IH)                                                                                                                                                                           | [108]      |
| Paraganglioma Human carotid body | - GAL was detected in 40% of tumors (IH)                                                                                                                                                                          | [108]      |
| Paraganglioma Human jugulo tympanic | - High GAL:R mRNA expression (Western blot)                                                                                                                                                                        | [143]      |
|                        | - Higher GAL concentration than in normal adrenal glands (RIA)                                                                                                                                                     | [144]      |
| Phaeochromocytoma      | - GAL inhibited cell proliferation and GAL:R, GAL:R and GAL:R mRNA expression, but not GAL mRNA (reverse transcription-PCR)                                                                                      | [141]      |
| Human                  | - GAL/GALR expression correlated with tumor stage (IH)                                                                                                                                                               | [101]      |
| Phaeochromocytoma      | - High GAL:R levels found in some patients who relapsed shortly after                                                                                                                                              | [145]      |
| Rat PC12 cell line     |                                                                                                                                                                                                                   |            |
| Pituitary adenoma      | - GAL/GALR expression correlated with tumor stage (IH)                                                                                                                                                               |            |
| Human                  |                                                                                                                                                                                                                   |            |
| Pituitary adenoma      | - High GAL:R levels found in some patients who relapsed shortly after                                                                                                                                              |            |
Cancers 2022, 14, 3755

| Cancer                        | Actions/Presence                                                                 | References |
|-------------------------------|---------------------------------------------------------------------------------|------------|
| Pituitary adenoma Rat         | surgical intervention (q-PCR)                                                   | [38]       |
| Pituitary adenoma Rat MfTW-10 cell line | - GAL promoted pituitary cell proliferation and tumor development | [146]     |
| Prolactinoma Rat              | - Estradiol increased GAL mRNA level                                             | [147,148] |
| Prolactinoma Rat              | - GAL concentration increased and GAL promoted tumor development               |           |
|                               | - Levonorgestrel decreased GAL mRNA expression and GAL-expressing cells (IH, in situ hybridization) | [149] |
| Small-cell lung cancer Human H345, H510 cell lines | - GAL, via GAL:R, mediated cell proliferation                                    | [88,150] |
| Small-cell lung cancer Human H69, H510 cell lines | - GAL, via GAL:R, activated G proteins and promoted cell proliferation            | [88]       |
| Small-cell lung cancer Human H345, H510 cell lines | - GAL, via GAL:R, activated G proteins and promoted cell proliferation            | [151]      |
| Small-cell lung cancer Human H345, H510 cell lines | - Ca²⁺-mobilizing peptides (e.g., GAL) promoted cell growth. Broad spectrum antagonists directed against multiple Ca²⁺-mobilizing receptors inhibited cell growth | [150,152] |
| Small-cell lung cancer Human H69, H345, H510 cell lines | - GAL, via the p42MAPK pathway, promoted cell growth. Protein kinase C inhibitors blocked cell growth induced by GAL | [153,154] |
| Small-cell lung cancer Human SBC-3A cell line, mouse SBC-3A tumor | - SBC-3A cells secreted the pre-pro-GAL precursor which was extracellular processed to GAL1-20 by plasmin | [155,156] |
| Somatotroph adenoma Human     | - Low GAL level (RIA)                                                             | [102]      |
| Somatotroph adenoma Rat GH1 cell line | - GAL increased circulating growth hormone level and growth hormone-producing tumors expressed GAL (IH) | [157] |
| Somatotroph adenoma Mouse     | - GAL blocked growth hormone release                                             | [158]      |
| Somatotroph adenoma Rat GH1 cell line | - GAL inhibited growth hormone release                                           | [159]      |
| Somatotroph adenoma Mouse     | - GAL mRNA level and peptide concentration increased                             | [147]      |
| Thyrotrhop adenoma Rat        | - GAL gene expression blocked                                                    | [147]      |
| Thyrotrhop adenoma Mouse      | - GAL synthesis inhibited                                                        | [160]      |

IH: immunohistochemistry; q-PCR: quantitative real time PCR; RIA: radioimmunoassay.

3.1.1. Phaeochromocytoma

GAL and GALRs have been observed in human phaeochromocytomas (Table 1). Compared with normal adrenal glands, the concentration of GAL was much higher in
phaeochromocytomas; however, the authors of the study reported that in both phaeochromocytoma patients and normal individuals, the concentration of GAL in plasma was below the detection limit of the assay (less than 10 pmol/liter) [144]. The last observation is surprising, since GAL plasma levels are usually not below the detection limits of the assays. In the latter study, GAL was localized in 5 of 11 of the phaeochromocytomas studied, and in normal adrenal glands, the peptide was only observed in a few cortical nerve fibers/chromaffin cells. A high GALR mRNA expression was observed in human phaeochromocytomas [143] and GAL inhibited the proliferation of phaeochromocytoma tumor cells [141]. GAL blocked the proliferation of rat PC 12 cells in which the expression of mRNAs encoding the three GALRs, but not GAL mRNA, was observed [141].

3.1.2. Insulinoma

Insulinomas appear sporadically or can be related with multiple endocrine neoplasia type 1 (MEN1 syndrome: an autosomal dominant condition due to MEN1 gene inactivating mutations) [161]. This syndrome is characterized by the presence of tumors in duodenum/endocrine pancreas, anterior pituitary adenomas and primary hyperparathyroidism, with gastrinomas and insulinomas being the most common functioning islet cell tumors [161]. The expressions of GALRs and GALR have respectively been reported in RINm5F [162] and Rin14B [32] insulinoma cells. GAL (released from sympathetic nerve terminals located in the endocrine pancreas) in insulinoma beta TC-1 cells (mouse) blocked the expression of the pro-insulin gene promoted by glucagon-like peptide-1 (7-37) [139] (Table 1). It has been reported that GAL did not block the secretion of insulin by simply decreasing the Ca$^{2+}$ level [163]. In the RINm5F insulinoma cell line, GAL inhibited the activity of AC and moderately suppressed the accumulation of insulin, but did not affect cell proliferation [138]; Gi3, a G protein coupled to GALRs, was involved in this inhibition [53]. In pancreatic beta-cells, GAL blocked the secretion of insulin and the activity of AC via pertussis-toxin-sensitive G proteins [53]. Finally, the chimeric peptide M35 (galanin (1-13)-bradykinin (2-9) amide) showed a dual effect depending on the concentration administered: acting as a GALR antagonist (at low concentrations) or as a GALR agonist (at high concentrations) [93].

3.1.3. Neuroblastic Tumor

The expression of GAL mRNA, GAL immunoreactivity and GAL binding sites has been reported in neuroblastoma tumors [136,137] (Table 1). Neuroblastoma and ganglioneuroma are neuroblastic tumors, and in both, no correlation between prognosis or tumor markers and the concentration of GAL has been reported [136]. However, a low level of GAL binding sites has been correlated with survival [136] and GAL/GALR expression has been related to the tumor differentiation stage [137].

Neuroblastoma is the result of an aberrant sympathetic nervous system development, usually arising from the paraspinal ganglia or adrenal medulla [116,164]. Thus, neuroblastoma appears in very young children (median age: 17 months; 10.2 cases/million children under 15 years) [165,166]; GAL and GAL mRNA have been detected in this disease [12,104]. The coexistence of GAL and beta-amyloid peptide in dense core secretory vesicles has been reported in the human neuroblastoma IMR32 cell line; this finding suggests that both substances are involved in the regulation of brain functions [140]. Moreover, GALR and GALR are highly expressed (immunoreactivity) in neuroblastoma, whereas the presence of GALR mRNA is less common than that of GALR mRNA [104,137]. By contrast, the immunoreactivity for both GALR and GALR decreased in ganglioneuromas [137]. GAL, GALR and GALR mRNAs were detected in the rat neuroblastoma B104 cell line, but not GALR mRNA [141]. It is important to note that the galaninergic system (by autocrine/paracrine mechanisms) exerts an anticancer action or a proliferative effect on neuroblastoma tumor cells; these effects are mediated by different GALRs, which induce different signaling pathways after the binding of GAL [141]. Thus, GAL promoted the growth and development of human neuroblastoma in an
autocrine/paracrine manner [137], and in the rat B104 neuroblastoma cell line, the peptide also increased the proliferation of tumor cells [141]. By contrast, GAL exerted an antiproliferative effect via GALr in the human neuroblastoma SH-SY5Y cell line [12]. GALr mediated apoptosis in the latter cell line; however, the GAL antiproliferative potency was 100-fold higher in SH-SY5Y neuroblastoma cells overexpressing GALr than in SH-SY5Y neuroblastoma cells overexpressing GALr, suggesting that a high level of GALr is able to block tumor cell proliferation [12]. In this sense, GALr transfection into neuroblastoma SH-SY5Y cells inhibited cell proliferation and promoted a caspase-dependent apoptotic mechanism [12]. Finally, the expression of GAL has been reported in human parangliomas [108,112,142] and the peptide was respectively found in 18% and 40% of carotid body or jugulo tympanic parangliomas [108].

3.1.4. Pituitary Tumor

GAL and the three GALrs have been observed in normal pituitary glands [30,36,145]. GAL was located in cells also containing growth hormone, prolactin, thyroid-stimulating hormone or adrenocorticotropic hormone (ACTH) [38,167], GALr was the most abundant receptor observed in normal anterior pituitaries, followed by GALr, whereas GALr was not found [30]. In another study, GAL, GALr and GALr mRNAs were found in human pituitaries, but not GALr mRNA [145]. Estrogens increased GAL mRNA and peptide levels in the rat anterior pituitary [167].

GAL was detected in some, but not all, pituitary tumors [36] (Table 1). Importantly, GAL/GALr expression is related to the pituitary tumor stage. Human pituitary adenomas display an increased expression of GALr [145], while high levels of GALr have been reported in some patients who relapsed shortly after surgical intervention [145]. This suggests that GALr could be a marker for relapsing pituitary tumors and that GALr antagonists could be a therapeutic approach for the treatment of pituitary tumors [145]. GAL may promote pituitary cell proliferation and tumor development in an estrogen-dependent or independent manner. Thus, in the rat MtTW-10 pituitary tumor cell line, GAL mRNA levels highly increased after the administration of estradiol. These cells secreted GAL, a process that was blocked by somatostatin. In rats, a sexual dimorphism was observed in estrogen-induced anterior pituitary tumorigenesis (female tumors averaging twice the size of male tumors); this could be due to a differential expression of GAL [168].

GAL and prolactin coexist in lactotrophs [38,167]. In transgenic mice, the overexpression of GAL in these cells promoted the synthesis and release of prolactin favoring hyperprolactinemia; moreover, this study showed that pituitary GAL favored pituitary hyperplasia (especially lactotrophs) in an estrogen-dependent manner [169]. In fact, high estrogen levels promoted prolactin-secreting pituitary tumors, which in turn released GAL [148] and, in estrogen-induced prolactinomas, the expression of the GAL gene and the level and secretion of GAL increased in the rat anterior pituitary [147,160]. Thus, GAL acts as an autocrine/paracrine hormone, regulating the secretion of prolactin [160]. It has been reported that the synthetic progestin levonorgestrel reduced the pituitary growth by decreasing the expression of GAL [149].

The coexistence of growth hormone (GH) and GAL has been reported in somatotrophs [38,167]. GAL promoted the release of GH from normal rat pituitary cells, but the peptide blocked this release from rat somatotroph adenoma cells [158,159]. The GAL level was low in GH secreting adenomas, but the level of the peptide was high in corticotroph adenomas [102]. In humans, GAL increased the circulating level of GH and GH-producing tumors expressed GAL [157]. A high increase in both GAL mRNA and GAL expression/secretion was observed in GH-releasing hormone transgenic mouse (somatotroph hyperplasia) [147,160]. The data show that GAL plays an important role in pituitary hyperplasia mechanisms by promoting cell proliferation [38].

Most of the corticotroph adenomas express GAL [102]. In normal pituitaries, the coexistence of GAL and ACTH has been reported in corticotrophs and, in the same cells, GAL and ACTH were also co-expressed in nonfunctioning and functioning pituitary
tumors [102,105]. GAL expression is related to smaller adenomas and better prognosis [102,105]. GAL has been observed in 84% of the corticotroph cell tumors associated with Cushing’s disease [103], although another study has reported that GAL did not play an important pathophysiological role in this disease because corticotroph adenomas can function irrespective of the presence of GAL [105]. GAL is secreted by human tumoral corticotrophs and responds to the corticotropin-releasing factor [135].

The expression of the GAL gene was blocked in thyrotroph adenomas; this means that GAL did not exert a stimulatory proliferative action on thyrotrophs [147]. Another study has shown that the synthesis of GAL was inhibited in thyrotroph adenomas [160].

3.1.5. Small-Cell Lung Cancer

Small-cell lung cancer (SCLC) is a poorly differentiated neuroendocrine carcinoma [170]. Approximately, it accounts for 15% of all lung cancers, is very aggressive, and is the leading cause of cancer death worldwide in men [170–172].

GAL mediated, via GAL-R, the proliferation of SCLC cells [113,150,151,173] (Table 1). Ca2+-mobilizing peptides (e.g., GAL, neurotensin, cholecystokinin) promoted the growth of SCLC cells through autocrine and paracrine mechanisms [150]. This finding suggests that broad spectrum antagonists directed against multiple Ca2+-mobilizing receptors could exert a therapeutic antitumor action and, in fact, these antagonists inhibited SCLC cell growth [152]. In H69 and H510 SCLC cell lines, GAL increased the formation of inositol phosphate and the intracellular level of Ca2+, and the peptide also promoted the growth of both cell lines, which was dependent on the concentration of GAL [151]. GAL, mediated by the p42MAPK pathway dependent on the activity of PKC, promoted the growth of SCLC cells, which was blocked with PKC inhibitors [153,154]. SBC-3A SCLC cells release pre-pro-GAL precursors, but not active peptides; however, extracts from mouse SBC-3A tumors contained pre-pro-GAL precursors and GAL1-20 (a cleaved lower-molecular mass of GAL) [156]. This means that pre-pro-GAL precursors were extracellularly processed to GAL1-20 and, in fact, it was demonstrated that the protease plasmin (present in SBC-3A tumors) was responsible for the processing of the pre-pro-GAL precursors to GAL1-20 [155,156]. GAL promoted the release of the promatrix metalloproteinase-2/9 from SBC-3A SCLC cells [156], and SCLC cells produced and released GAL, which exerted, via GAL-R, a mitogenic action on these cells by activating Gq, Gi and G12 G proteins [88]. Thus, GAL activates multiple signals through the G12/Rho pathway and the Gq phospholipase C/calcium sequence and also promotes Ca2+ mobilization [88].

3.2. Galanin and Gastric Cancer

In nerve cells, the galaninergic system plays an important role in tumor development. In human stomach samples, obtained from the vicinity of invasive cancer cells, neurons located in the myenteric plexus showed a high expression of both caspases 3 and 8, but a low expression of GAL [45,174] (Table 2). In carcinoma-affected regions of the human stomach, an increase of the GAL-immunoreactive fibers in the longitudinal muscle layer, lamina muscularis mucosae and in the vicinity of the neoplastic proliferation was observed; thus, carcinoma invasion affected GAL stomach wall innervation [175]. In patients suffering from gastric cancer, lower levels of GAL were observed in pre-operative samples (and in plasma) when compared with those found in post-operative samples obtained from the same patients or from samples of healthy donors [176]. Moreover, the levels of GAL/GAL-R were lower in gastric cancer tissues compared with those found in adjacent regions; however, the GAL-R/GAL-R levels did not change [176]. The low level of GAL could be used as a biomarker in gastric cancer and, importantly, in these patients (pre-operative samples), the GAL protein/mRNA levels have been related to tumor size, tumor node metastasis stage and lymph node metastasis [176].
### Table 2. Involvement of the galaninergic system in gastric and colorectal cancer.

| Actions/Presence                                                                 | References |
|----------------------------------------------------------------------------------|------------|
| **Gastric Cancer**                                                               |            |
| - Fibers containing GAL: increased in longitudinal muscle layer, lamina muscularis mucosae and neoplastic proliferation vicinity (IH) | [175]      |
| - Myenteric plexus: neurons showed a high expression of caspases 3/8 and low GAL expression (IH) | [175]      |
| - GAL/GAL1R level reduced                                                       | [176]      |
| - GAL1R/GAL1R level unchanged (RT-PCR)                                            | [176]      |
| - Lower level of GAL in pre-operative samples (and plasma) when compared with that found in post-operative samples or in healthy donors. Gastric cancer tissues: GAL/GAL1R level was lower compared with that found in adjacent regions GAL1R/GAL1R: no change (Western blot; RT-PCR; ELISA) | [176]      |
| Human                                                                            |            |
| Human                                                                            | [176]      |
| Human                                                                            | [176]      |
| Human                                                                            | [176]      |
| Human                                                                            |            |
| Human                                                                            | [177]      |
| Human                                                                            |            |
| Human                                                                            | [178]      |
| Human                                                                            | [13]       |
| **Colorectal Cancer (CRC)**                                                      |            |
| - GAL/GAL1R silencing: apoptosis in drug-sensitive/resistant cell lines and enhanced the effects mediated by chemotherapy. GAL mRNA: overexpressed. High GAL level: related to poor disease-free survival of early-stage CRC patients (IH, ELISA, RT-PCR, Western blot) | [7,106,117,121] |
| Human                                                                            |            |
| Human                                                                            | [8]        |

**Human**

**Gastric cancer cell lines**

- GAL blocked gastric carcinogenesis by inhibiting antral epithelial cell proliferation

**Rats**

- GAL expression decreased: restored with a demethylating agent. GAL hypermethylation: impaired GAL tumor suppressor action. GAL downregulation: due to epigenetic inactivation (Q-MSP, Western blot)

- GAL: decreased cell proliferation
| Actions/Presence | References |
|------------------|------------|
| - CRC patients: more GAL-immunoreactive neurons in comparison to healthy samples (IH, ELISA) | [121] |
| - GAL in the vicinity of cancer cell invasion (IH, ELISA) | [121] |
| - Blood samples: increased GAL concentration. High GAL level: cancer cells. Lowest GAL level: muscular layer placed distant from tumors. GAL: CRC tumor biomarker (ELISA, IH) | [179] |
| - GAL mRNA level: related to adenocarcinoma size/stage. Correlation between higher GAL expression and shorter disease-free survival (RT-PCR) | [106,117] |
| - CRC cells showed a high GAL expression: more malignant and involved in tumor recurrence. High GAL expression: spread of cancer stem cells (metastasis) (RT-PCR) | [180] |
| - High GAL expression: associated with poor prognosis (stage II) and tumor recurrence. GAL expression: related to CRC aggressive behavior (RT-PCR) | [180] |
| Human (tissue and cell lines) | |
| - CRC cells/tissues: higher GAL levels than non-tumor cells/tissues | [106,117,179,180] |
| - CRC tissue: increased GAL gene/protein expression. CRC cell lines: GAL/GAL1R silencing promoted apoptosis. GAL1R silencing promoted FLIPL down-regulation (IH, ELISA, RT-PCR) | [106,117,121] |
| Human HCT116 cell line | |
| - Cells overexpressing GAL1R were more chemosensitive to bevacizumab than control cells | [181] |
| Rat | |
| - GAL decreased the incidence of colon tumors | [182] |

IH: immunohistochemistry; Q-MSP: quantitative methylation-specific PCR; RT-PCR: real time-PCR.

A prolonged administration of GAL (4 μg/kg) blocked gastric carcinogenesis by inhibiting the proliferation of antral epithelial cells [13]. Human gastric cancer cells (AGS, KATOIII, SNU-638, SNU-601, SNU-1) showed a low endogenous GAL expression, which was restored with a demethylating agent (5-aza-2′-deoxycytidine) [177]. In addition, the hypermethylation of GAL impaired its tumor suppressor action in gastric cancer, and the exogenous GAL expression in silenced cells promoted a decrease in phosphorylated Akt expression and apoptosis [177]. This means that the downregulation of GAL in gastric tumor cells was due to an epigenetic inactivation. Finally, GAL decreased the proliferation of human gastric cancer cells in vitro [178].
3.3. Galanin and Colorectal Cancer

Colorectal cancer (CRC), the third most prevalent cancer worldwide, is an invasive tumor process due to the proliferation of epithelial cells that acquire a neoplastic phenotype [8]. This process is known as epithelial-to-mesenchymal transition, in which epithelial cells lose many morphological and functional characteristics (e.g., shape, cell polarity, intercellular junctions) [8]. Tumor cells digest the extracellular matrix of the intestine wall, activating growth factors that promote cell proliferation, the blockade of apoptotic mechanisms and also favor the spreading of cancer cells [8]. Then, the invasion of cancer cells destroys the enteric nervous system, leading to the atrophy of the submucosal/myenteric plexuses. The galaninergic system is involved in colon cancer [106,117,121] (Table 2); thus, for example, the siRNA-mediated silencing of the GAL gene reduced both invasive and proliferative potential in CRC cells [117].

CRC tissues showed higher GAL levels than the corresponding non-tumor tissues [106,117,179,180], and human colon cancer cell lines (LOVO, HCT15, SW480, SW620) showed higher levels of GAL than those found in non-colon cancer cell lines [106]. In blood samples of CRC patients, an increased concentration of GAL (2.4 times higher) has been reported [179]. GAL mRNA is overexpressed in CRC and its level has been related to adenocarcinoma size/stage and a correlation between shorter disease-free survival of early-stage CRC patients and high expression of GAL has been reported [7,106,121]. In CRC patients, a high GAL expression was related to tumor recurrence, and CRC patients (stage II) who showed a high GAL expression had a poorer prognosis than those showing a low expression of the peptide [180]. In addition, a relationship between a high GAL expression and the spread of cancer stem cells (metastasis) has also been reported in CRC (stage II) [180]. However, an association between survival and GAL expression was not observed in CRC patients (stage III) [180]. The data show that the expression of GAL is related to CRC aggressive behavior and it seems that CRC cells showing a high GAL expression are more malignant and are also involved in the recurrence of the tumor [180]. However, a recent study has shown that GAL downregulation is correlated with advanced CRC stages in northern African individuals and it is linked to autophagy, cell cycle and division, immune system response and the transcriptional regulation of TP53 [183]. Compared to epithelial cells of the large intestine, a stronger immunoreactivity for GAL-R/GAL-R was observed in CRC cells and it has been reported that the high expression of GAL-R in CRC tissue was associated with a better prognosis and longer survival of CRC patients; this means that GAL-R is a prognostic factor for these patients [184].

The number of neurons containing GAL was higher in CRC patients than in those showing a healthy intestine, and an increased GAL gene/protein expression was observed in CRC tissues [106,179]. Compared to control individuals, a higher percentage of neurons containing GAL was reported in the myenteric plexus of CRC patients; however, no change was observed regarding the density of the immunoreactive fibers containing GAL located in the myenteric and submucosal plexuses [121]. The number of neurons containing GAL also increased in the tissue regions located close to CRC; thus, the release of GAL from these neurons could block apoptotic mechanisms favoring tumor cell survival and proliferation [8,185]. In fact, GAL promoted CRC cell proliferation and improved cell survival [8], and then cancer cell invasiveness increased and tumor development was accelerated. In another study, CRC tumor samples were collected as well as colon wall tissues located close to and distant from the neoplastic tissue: a high GAL immunoreactivity was observed in myenteric/submucosal plexuses, intestinal epithelium and cancer cells, whereas the lowest GAL level was found in the muscular layer located distant from the tumor [179]. The author concluded that GAL could be a potential biomarker for CRC tumors.

GAL-R is mainly expressed in the human colon. The silencing of this receptor or GAL promoted apoptosis in drug-sensitive/resistant cell lines and enhanced the effects mediated by chemotherapy; thus, GAL-R regulates drug resistance [117]. The GAL-R gene has been suggested in CRC as a chemosensitive methylation candidate to bevacizumab, since
HCT116 CRC cells overexpressing GAL·R were more chemosensitive to the monoclonal antibody than control cells [181]. GAL·R silencing promoted a downregulation of the FLIPL-like inhibitory protein long form (FLIPL, a caspase 8 inhibitor), meaning this inhibitor is a key downstream effector of the anti-apoptotic signaling mediated by GAL/GAL·R [117]. Thus, the downregulation of the inhibitor favors the induction of caspase 8-dependent apoptotic mechanisms. Finally, GAL decreased the incidence of colon tumors in rats and it seems that this effect was due to the inhibitory action exerted by GAL on cancer cell proliferative mechanisms [182].

3.4. Galanin and Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinoma arises from mucosal surfaces of the head and neck [186] (Table 3). Perineural invasion (PNI), a mechanism of tumor dissemination via nerves, predicts poor survival in some cancers including head and neck squamous cell carcinoma (HNSCC), pancreatic cancer, stomach cancer and colon cancer, and is a sign of cancer cell invasion and metastasis [187]. An interaction between nerves and tumor cells occurs in PNI. PNI, mediated by molecular signals, promoted neuritogenesis and the survival, proliferation and invasion of tumor cells [84,188–190]. These cells are attracted to nerves and communicate with them. GAL (released from nerves) exerted a nerve–tumor crosstalk by activating GAL·R expressed in tumor cells and by inducing NFATC2-mediated transcription of cyclooxygenase-2 and GAL; then, GAL released from tumor cells promoted neuritogenesis, favoring PNI [84].

| Table 3. Involvement of the galaninergic system in head and neck squamous cell carcinoma. |
|-----------------------------------------------|------------------|
| Actions/Presence                           | References       |
| Human                                       |                  |
| - High GAL level (RT-PCR)                   | [120]            |
| - GAL·R gene promoter: frequently methylated (Q-MSP) | [191] |
| - Methylation status of some peptide-encoding genes, including GAL, is related with survival and recurrence. Methylation changes: possible molecular marker for HNSCC risk/prognosis (Q-MSP) | [192] |
| - GAL/GALR epigenetic variants: markers for prognosis prediction (Q-MSP) | [193,194] |
| - Poor survival: associated with methylation of GAL/GAL·R genes. Hypermethylation: inactivation of GAL/GAL·R/GAL·R genes (Q-MSP) | [195] |
| Human Cell lines                            |                  |
| - Apoptosis: mediated by GAL·R but not by GAL·R. GAL·R/GAL·R: tumor suppressors in a p53-independent manner | [11] |
| - GAL·R transfection into HNSCC cells: cell proliferation inhibited. GAL·R re-expression: blocked cell proliferation (showing | [113,196,197] |
mutant p53)
- GAL/R/GAL:R negative HNSCC cells: GAL:R re-expression suppressed tumor cell proliferation via ERK1/2-mediated actions on cyclin-dependent kinase inhibitors and cyclin D1
- GAL/GAL:R blocked HNSCC and oral tumor cell proliferation by cell-cycle arrest (RT-PCR, ELISA, Q-MSP)
- GAL:R blocked tumor cell proliferation through the activation of ERK1/2
- GAL:R promoted an antitumor effect by inducing cell cycle arrest and apoptotic mechanisms (caspase 3-dependent)
- GAL:R suppressed HNSCC cell viability. HEp-2 cells: GAL:R mediated apoptotic mechanisms (caspase-independent) by downregulating ERK1/2 and inducing Bim

- GAL:R overexpression: favored survival/proliferation by activating PI3K/Akt and MAPK/ERK-dependent pathways. Ras-related protein 1 (Rap1): involved in HNSCC progression.
- GAL/GAL:R: tumor suppressor. GAL:R absent in some cell lines (Q-MSP, RT-PCR)
- GAL:R promoter: widely hypermethylated and related to reduced GAL:R expression. GAL:R/GAL:R hypermethylation: associated with higher recurrence rate and reduced disease-free survival (RT-PCR, Q-MSP)
- GAL:R methylation status: potential biomarker for predicting clinical outcomes. Methylation: related to carcinogenesis and decreased GAL:R expression (RT-PCR, Q-MSP)

References

[113,197]
[123,177,196,198]
[196]
[197]
[199]
[122]
[177,178,198]
[191,194,198,200]
[193,194,198]
The promoter methylation status of the peptide-encoding gene GAL was studied in HNSCC samples; methylation was observed in 20% of them [192]. The authors showed that the methylation status of some peptide-encoding genes, including GAL, was related to survival and recurrence in HNSCC, and they also suggested that methylation changes could be a possible molecular marker for HNSCC risk/prognosis. In fact, poor survival has been associated with the methylation of GAL/GAL:R genes, and a hypermethylation promoted the inactivation of GAL/GAL:R/GAL:Rs genes [195]. The GAL:R gene promoter is widely hypermethylated in HNSCC (cell lines, primary tumor); this is related to reduced GAL:R expression, which can be restored by treating with a histone deacetylase inhibitor (trichostatin A) or with a methyltransferase inhibitor (5-azacytidine) [191,198]. This is important, since the methylation of the GAL:R gene promoter has been related to HNSCC carcinogenesis [193]. GAL:R/GAL:R hypermethylation has been associated with a higher recurrence rate and reduced disease-free survival [194,200]. GAL/GALR epigenetic variants are excellent markers for the prognosis prediction of patients suffering from HNSCC [193,194]; thus, the GAL:R methylation status could be a biomarker for predicting HNSCC clinical outcomes. Importantly, because methylation suppresses GAL/GALRs expression in some tumors and because GAL/GAL:R act as tumor suppressors (see below) [177], these findings suggest that the methylation-based suppression of GAL/GALRs eliminates the expression of a tumor-suppressive pathway.

A high level of GAL has been detected in HNSCC [120]; GAL/GAL:R blocked human oral tumor cell proliferation [177], and GAL:R inhibited the proliferation of keratinocytes (malignant and immortalized) by blocking the MAPK pathway [123]. Thus, GAL:R acts as a tumor suppressor gene, which is frequently silenced in HNSCC [177,198]; in fact, in some HNSCC cell lines, the expression of GAL:R is absent [198]. GAL:R blocked the proliferation of tumor cells through the activation of ERK1/2 and cyclin-dependent kinase inhibitors, leading to cell-cycle arrest (regulating cell cycle control proteins such as cyclin D1, p57, p27) [123,196,198,200]. Moreover, the re-expression of GAL:R in GAL:R/GAL:R-negative HNSCC cells also suppressed tumor cell proliferation through ERK1/2-mediated actions on cyclin-dependent kinase inhibitors and cyclin D1 [113]. The overexpression of GAL:R in HNSCC cell lines favored the survival and proliferation of these cells by activating respectively the PI3K/Akt and MAPK/ERK-dependent pathways [122].

**Table: Actions/Presence**

| Species | Actions/Presence                                                                 | References |
|---------|----------------------------------------------------------------------------------|------------|
| Human (cell lines) Mouse                                                        | - GAL (released from nerves) activated GAL:R expressed in tumor cells inducing NFATC2-mediated transcription of cyclooxygenase-2 and GAL. GAL released from tumor cells promoted neuritogenesis, favoring perineural invasion | [84]       |
| Mouse                                           | - GAL:R promoted tumor angiogenesis through the p38-MAPK-mediated inhibition of tristetraprolin (TTP), leading to an enhanced secretion of cytokines. GAL:R activated Ras-related protein 1b (Rap1B) favoring a p38-mediated inactivation of TTP, which acted as a destabilize cytokine transcript | [201]      |

Q-MSP: quantitative methylation-specific PCR. RT-PCR: real-time PCR.
Ras-like signaling protein) is involved in HNSCC progression [122], and GAL-R activated rap1B (small-GTP protein) favoring a p38-mediated inactivation of the mRNA binding protein tristetraprolin, which inhibited the production of many pro-inflammatory cytokines. This means that GAL-R p38-mediated cytokine production could be a therapeutic target against HNSCC, since p-38 inhibitors are currently used in clinical practice. In HNSCC, GAL-R promotes tumor angiogenesis by enhancing the secretion of cytokines (vascular endothelial growth factor, interleukin-6) via the p38-MAPK-mediated inhibition of tristetraprolin [201]. By contrast, GAL-R exerted an antitumor effect by inducing cell-cycle arrest and apoptotic mechanisms (caspase 3-dependent) [190] and this means that the activation of these mechanisms could exert a beneficial therapeutic action against HNSCC. The proliferative or antiproliferative actions mediated by GAL-R in HNSCC could be explained by the signaling pathways activated depending on the coupled G protein type. Moreover, GAL-R transfection into human HNSCC cells suppressed cell proliferation [113,197] and the re-expression of GAL-R blocked HNSCC cell proliferation (showing mutant p53) [113]. Importantly, apoptotic mechanisms via the activation of GAL-R by GAL have not been reported, and in HNSCC cells, GAL-R/GAL-R are suppressor tumors in a p53-independent manner [11]. GAL-R mediated apoptotic mechanisms (caspase-independent) in HEp-2 cells by downregulating ERK1/2 and inducing Bim (a pro-apoptotic Bcl-2 protein) [199]. Although the receptors tend to be tumor suppressive, it has recently been reported that GAL released by HNSCC cells exerted a pro-tumoral and immune-suppressive effect and data from the Cancer Genome Atlas have shown that a reduced overall survival of HNSCC patients was correlated with a high expression of GAL [202].

3.5. Galanin and Glioma

The GAL/GALR system has been described in glioma [30,118] in which the most abundant receptor observed was GAL-R, followed by GAL-R; GAL-R was not found (astrocytic/oligodendroglial tumors) [30] (Table 4). A reduced level of GAL-R has been observed in the cerebrospinal fluid of patients with glioblastoma [203], and regarding the expressions of GAL and GAL-R, no correlation with oligodendroglial, astrocytic and mixed neural–glial tumors was reported [30]. Moreover, no correlation was observed between the proliferative activity and GAL/GAL binding levels [118]. However, the high-grade glioma (WHO grade IV) has been related to the expression of GAL-R [30]. GAL-R has been reported in gliosarcoma and glioblastoma multiforme [118]; in the latter, the most abundant receptor found was GAL-R, followed by GAL-R and GAL-R [118]. In glioma, endothelial and immune (e.g., macrophages, neutrophils) cells expressed GAL-R, but GAL-R/GAL-R were not observed around the blood vessels [30]. This means that tumor-associated cells are involved in tumor microenvironment homeostasis. Glioma-associated macrophages (GAMs) are involved in tumor progression; although macrophages produce/secrete GAL, GAMs do not express GAL, but express GAL-R, and this means that GAL could regulate the activity of GAMs [59,204].

Table 4. Involvement of the galaninergic system in glioma.

| Actions/Presence                                      | References |
|------------------------------------------------------|------------|
| - GAL/GAL-R expression: no correlation with oligodendroglial, astrocytic and mixed neural–glial tumors | [30]       |
| - High-grade glioma (WHO grade IV): related to GAL-R expression | [30]       |
| - Endothelial/immune cells: GAL-R expression. Around blood vessels: GAL-R,GAL-R not observed (IH) | [30]       |
| - GAL-R, followed by GAL-R; GAL-R | [30,118]  |
### Actions/Presence

- Absent (astrocytic/oligodendroglia tumors) (IH, autoradiography, reverse transcription-PCR)
- Glioma-associated macrophages: GAL₁R expression (quantitative PCR)
- No correlation between proliferative activity and GAL/GAL binding levels (IH, autoradiography, reverse transcription-PCR)
- Cerebrospinal fluid (glioblastoma): reduced GAL level

| Human Mice | - GAL blocked, via GAL₁R, the proliferation of glioma cells and tumor growth. These effects were mediated through ERK1/2 signal activation. No cytotoxic/apoptotic effect was observed | [205] |

IH: immunohistochemistry.

GAL blocked, through GAL₁R, the proliferation of human glioma cell lines (U251, T98G) and tumor growth in nude mice [205]. The authors reported that GAL did not exert cytotoxic/apoptotic effects and that the blocking actions exerted by GAL were due to the activation of the ERK1/2 signal.

### 3.6. Galanin and Other Cancers

Although the expressions of GAL and pre-pro-GAL mRNA have been reported in breast cancer, it has been suggested that the GALN gene (which encodes the pre-pro-GAL protein) is an unlikely candidate oncogene in breast tumors because an increase in pre-pro-GAL mRNA expression with GALN amplification was not observed [101,206] (Table 5). Many nerve fibers containing GAL have been reported in cardiac and esophageal carcinomas [207]; these fibers contacted closely with cancer cells, including those encircling tumor cells. In this study, GAL favored the extension of processes by dorsal root ganglion neurons, but the action of the peptide on tumor cells is currently unknown [207]. GAL₁R DNA methylation is among the most epigenetic molecular alterations in endometrial cancer; this methylation indicates malignancy with a high degree of sensitivity and specificity [208]. The methylation of the GAL₁R gene in bladder cancer has been involved in the prognosis of the disease, but the role played by the galaninergic system in this cancer is currently unknown [209].

### Table 5. Involvement of the galaninergic system in other cancers.

| Actions/Presence | References |
|------------------|------------|
| Breast cancer Human | - GAL/pre-pro-GAL mRNA level expression. GALN gene: unlike candidate oncogene (Northern blot) [101,206] |
| Carcinoma (cardiac, esophageal) Human | - Fibers containing GAL contacted closely with cancer cells (IH) [207] |
| Endometrial cancer Human | - GAL₁R DNA methylation indicated malignancy (q-PCR) [208] |
| Bladder cancer Human | - GAL₁R gene methylation involved in prognosis [209] |
|                  | Actions/Presence                                                                 | References |
|------------------|----------------------------------------------------------------------------------|------------|
| Salivary duct carcinoma Human | - GAL/R/GAL:R: therapeutic targets/prognostic factors. GAL/R/GAL:R methylation rates correlated with overall survival decrease (IH, Q-MSP) | [210]      |
| Melanoma Human   | - GAL/GAL:R expression (IH)                                                      | [101,119]  |
| Pancreas Human   | - GAL promoted SW1990 cell proliferation                                          | [211]      |
| Pancreas Rat     | - GAL blocked carcinogenesis and decreased norepinephrine level (IH, HPLC)        | [212]      |

HPLC: high-performance liquid chromatography; IH: immunohistochemistry; Q-MSP: quantitative methylation-specific PCR; q-PCR: quantitative real-time PCR.

It has been suggested that GAL:R/GAL:R are therapeutic targets and prognostic factors in salivary duct carcinoma [210]. GAL:R/GAL:R methylation rates were higher in salivary duct carcinomas than in normal tissues, and these rates were correlated with a decrease in overall survival. The expression of GAL has been reported in melanoma, and human Bowes melanoma cells expressed GAL:R [119,213]. In the latter case, a biphasic response (increase of the extracellular acidification rate followed by a decrease below the basal level) was found after the activation of the receptor, being the magnitude of the response depending on the concentration of GAL [213]. GAL blocked pancreatic carcinogenesis in rats, and this was related to the inhibition of the activity of the sympathetic nervous system [212]. The latter study demonstrated that animals treated with GAL showed a lesser number of pancreatic adenocarcinomas than control animals and that GAL decreased the pancreatic level of norepinephrine. By contrast, GAL promoted the proliferation of SW1990 human pancreatic cancer cells in vitro [211]. These contradictory findings may be due to the fact that the former experiment was performed in vivo [212].

4. The Galaninergic System and Cancer Signaling Pathways

Figure 4 shows the main signaling pathways in which the galaninergic system is involved. A GAL/GALR signaling network map focused on the signaling cascades regulated by the galaninergic system has recently been published [87]. GALRs (via PKC) activate the rat sarcoma virus (Ras, a small GTPase)/MAPK/ERK pathway by increasing the intracellular Ca²⁺ concentration [8]. The galaninergic system activates many signal transduction pathways depending on the coupled G protein type: GAL:R/GAL:R, mainly coupled to Gi/o, decrease the cAMP level and inactivate PKA, whereas GAL:R, preferably coupled to Gq/11 mobilizing intracellular Ca²⁺, promotes (via PKC) the activation of cell survival (via Akt or PKB) and MAPK1/MAPK3-dependent cell proliferation pathways [17,30,87]. GAL:R can also be coupled to Gβγ- and/or Gi-signaling pathways and then the activation of MAPKs occurs in a Ras/Raf-dependent manner [17,25,87]. GAL:R activation also favors the Akt/Akt substrate of the 160 kDa (AS160) cascade [87], regulates GIRK channels [4,77] and activates the ERK1/2 signal through the Ga/i subunit and not via the PI3K pathway linked to the Gβγ subunit [196]. GAL:R induces cell-cycle control proteins (p27kip1, p57kip2) and suppresses cyclin D1 in cancer cells [20]. GAL:R, mainly coupled to Gq/11, mediated the activation of PLC and small GTPase proteins in the Rho family [87]. PLC converted phosphatidylinositol, 4, 5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3), which mediated PKC activation and increased the intracellular concentration of Ca²⁺ [77]. GAL:R activated the small GTPase protein Rho A in SCLC cells, suggesting the coupling to G12/13 [20]. GAL:R inhibited the production of cAMP, meaning that the receptor was coupled to Gi protein [83]. GAL:R decreased coflin
activation and Rho and Cdc42 GTPase activity [20]. In tumor cells, GAL-R activated the MAPK/ERK pathway in a PKC manner, meaning that GAL-R was coupled to a Go protein [20]. GAL-R regulated cell-cycle control proteins (p27kip1, p57kip2) and cyclin D1 and promoted apoptosis (caspase 3-dependent) in HNSCC cells [26]. GAL-R decreased the expression of p21cip1, phosphorylated BAD forms (pBad) and phosphorylated Akt (pAkt), downstream of the Gq11/Pi3K pathway [26]. The GAL-mediated Akt pathway blocked the activation of caspases 3 and 9, whereas the GAL-R-mediated apoptosis in tumor cells was induced by the activation of the pro-apoptotic Bcl-2 protein Bim, through a mechanism independent of caspase [20]. GAL-R, involved in inward potassium ion (K⁺) currents, is coupled to the Gi/o signaling pathway and its activation favored the inhibition of cAMP and AC altering CREB phosphorylation [17,87,90]. GAL opened adenosine triphosphate (ATP)-sensitive K⁺ channels and hyperpolarized cell membranes in the rat RINm5F insulinoma cell line [214], and the peptide blocked the activity of AC and the secretion of insulin via the interaction with Gα1, Gα2 and Gα3 proteins [53,215]. C7 peptide (GAL1-13-spantide amide), a GAL receptor antagonist, blocked hepatocellular carcinoma metastasis by targeting the hepatocyte growth factor/c-mesenchymal–epithelial transition receptor axis signaling pathway [216]. C7 inhibited the migration and invasion of tumor cells by blocking the phosphorylation of Akt and ERK1/2 [216].

Figure 4. Main signaling pathways in which the galaninergic system is involved. Black arrows indicate activation pathways, inverted red “T” indicates blockade/suppression, green arrows mean final results. AC, adenylyl cyclase; Akt, Akt serine/threonine kinase family (also called PKB); AS160, Akt substrate of 160 kDa; ATP, adenosine triphosphate; Ca²⁺, calcium ion; cAMP, cyclic adenosine monophosphate; CKI, cyclin-dependent kinase inhibitor 1; CREB, cAMP regulatory element-binding protein; D1, a cyclin protein; DAG, diacylglycerol; ER, endoplasmic reticulum; FORSKOLIN, enzyme that produces cyclic adenosine monophosphate; GAL, galanin; GAL1-15 fragment, galanin 1–15 fragment; GAL1-16, galanin 1–16 fragment; GAL1-29, galanin 1–29 fragment; GAL-R, galanin receptor 1; GAL-R, galanin receptor 2; GAL-R, galanin receptor 3; GALP: GAL-like
peptide; GIRK, G protein-coupled inwardly-rectifying potassium; Ga11, G protein alpha subunit (11); Gai/aro, G protein alpha i/o subunits; Gao, G protein alpha subunit (o); Gβγ, G protein beta-gamma subunit; IL-6, interleukin 6; IP3, inositol triphosphate; K+, potassium ion; MAPK, mitogen-activated protein kinases cascade; p21cip1, a cyclin-dependent kinase inhibitor; p27kip1, cell-cycle control protein; p38, a class of mitogen-activated protein kinase; p57kip2, cell-cycle control protein; pAkt, phosphorylated Akt; pBad, phosphorylated BAD forms (induces apoptosis by inhibiting antiapoptotic BCL-2 family members); PI3K, phosphatidylinositol 3-kinase;PIP2, phosphatidylinositol bisphosphate; PIP2, phosphatidylinositol 4, 5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; Rap1B, Ras-related protein Rap-1b; Ras, rat sarcoma virus (a small GTPase); Rho, a family of small signaling G proteins (a subfamily of the Ras superfamily); TTP, tristetraprolin; VEGF, vascular endothelial growth factor.

The interaction between GALr/GALr-5-hydroxytryptamine 1A receptor heteromer (a macromolecular complex formed by at least two different receptor units) promoted conformational changes in GAL recognition sites, altering the binding affinity of GAL [100]. In this sense, conformational changes in the GALr/GALr complex favored a higher affinity of GALr for GAL1-15 than for GAL, increasing Gi/o-mediated signaling and decreasing AC activity and CREB levels [98]. In addition, GALr heteromerization with other peptide receptors or other GALRs has been suggested [20].

5. Therapeutic Strategies

Peptides play an important role in cancer; the in-depth knowledge of the functions mediated by these substances is an emerging and promising line of research that could lead to new clinical applications on oncology. One line of research could be the use of peptides coupled to cytotoxic agents to exert an antitumor action, and another, the use of peptide receptor antagonists or agonists. In the case of GAL, GALR antagonists or agonists could be used as antitumor treatments according to the different signaling pathways and actions mediated by GALRs. GALR antagonists have been administered for the treatment of food intake disorders, anxiety, depression and Alzheimer's disease, whereas GALR agonists have been used for the treatment of chronic pain [18,93]. It has also been reported that SNAP 37889, a non-peptidergic GALR antagonist, promoted apoptosis in promyelocytic leukemia cells expressing GALR [217].

In vitro and in vivo experiments using human gastric cancer cell lines have been performed to study the antitumor action of a triple treatment with GAL, serotonin and octreotide (an octapeptide that mimics the actions mediated by somatostatin) [178]. Treatment with one compound or with a double/triple combination decreased cell proliferation and viability in vitro, and tumor volume/weight was reduced in vivo after the triple treatment. However, this reduction was not due to apoptosis or cell proliferation inhibition; thus, other unknown mechanisms were involved [178]. In experimental animals, implanted human colon cancer cells were treated with the triple treatment (octreotide, serotonin and GAL were administered subcutaneously or intraperitoneally) [218–220]: tumor volume/weight, number of viable cells, proliferation index and tumor vascularization decreased, whereas the apoptotic index increased. In nude mice implanted with colonic adenocarcinoma cells and treated with the triple treatment, the tumor volume decreased and the apoptotic index and volume density of the tumor necrotic tissue increased [221]. The triple therapy did not show any apparent side effects [222]. Low concentrations of GAL, somatostatin and serotonin have been reported in CRC patients and treatment with GAL alone showed an important decrease in the number of tumor blood vessels [223]. Comparing the administration of one, two or three compounds, the antitumor effect was higher when the three compounds (GAL, serotonin, octreotide) were administered [224]. Importantly, the antitumor effect promoted by the triple therapy was comparable to the treatment with 5-fluorouracil/leucovorin, a chemotherapeutic agent used for CRC treatment [225]. Triple treatment has a better safety profile and, hence, it is a potential therapeutic strategy against CRC [226], but more preclinical and clinical studies are needed to confirm its beneficial use in clinical practice.
Peptide analogs have been used as an antiproliferative strategy and promising results targeting peptide-ergic systems have been reported; accordingly, GAL analogs could be tested in tumors expressing certain GALRs. The half-life of GAL in plasma is about 5 min, but the half-life of synthetic GAL (e.g., GAL1-29, GAL1-16) is 60–120 min. GAL1-16 was synthesized as a free carboxylic acid, whereas GAL1-29 was synthesized with a C-terminal amide corresponding to the endogenous peptide; the data mean that analogs showing an increased half-life are required for a therapeutic application of the peptide [20]. Ligand specificity/selectivity must be understood in-depth at GALRs to understand the molecular interactions that occur in these mechanisms and to develop drug-design studies. It is important to note that, currently, there are few experiments focused on the antitumor activity mediated by GALR antagonists or agonists [217]; this is a promising research field that must be developed immediately, since many in vitro and in vivo experiments are required to fully demonstrate the anticancer properties of GALR antagonists or agonists. Moreover, radiolabeled cytotoxic agents linked to peptides have been used for therapeutic applications (e.g., neurotensinergic system, substance P/neurokinin-1 receptor system) [9,227,228]. Again, this line of research (peptide and non-peptide ligands as radiopharmaceuticals) must be developed as targeted radionuclide cancer therapy in tumors expressing GALRs because it could serve to demonstrate the potential use of GALR agonists or antagonists in nuclear medicine for the diagnosis/treatment of GALR-positive tumors. Thus, it is important to know whether GALRs are molecular targets to radiosensitize cancer cells.

6. Discussion

The potential antitumor clinical application of GALR ligands (GALR antagonists or agonists) has unfortunately been neglected by the scientific community and the pharmaceutical industry. However, the expression of the galaninergic system could be used for the diagnosis, treatment and prognosis of tumors [30,36,39,101–112], and this system has also been correlated with tumor stage/subtypes (Figure 5). Stage/tumor size has been related to the level of GAL mRNA in colon cancer: the higher the GAL expression, the shorter the disease-free survival [30,101,106]. In neuroblastic tumors, a low level of GAL binding sites has been correlated with survival and GAL/GALR expressions have been related to tumor differentiation stages [136,137]. GAL expression has also been related to smaller adenomas and better prognosis [102,105]; the low level of GAL has been suggested for use as a biomarker in gastric cancer, and the level of GAL has been related to tumor size, tumor node metastasis stage and lymph node metastasis in patients suffering from gastric cancer [176]. A correlation between the shorter disease-free survival of early-stage CRC patients and the higher expression of GAL has also been reported [7,106,121]: the expression of GAL has been related to the aggressive behavior of CRC, and a relationship between a high GAL expression and metastasis has been observed in CRC [180].
Figure 5. The galaninergic system has been correlated with survival, metastasis, tumor recurrence and poorer prognosis. M: methylation; M,M,M: hypermethylation.

Some peptidergic systems (e.g., substance P/neurokinin-1 receptor system, neurotensinergic system) exclusively promote the proliferation of tumor cells; however, GAL, via different GALRs, exerts a tumor cell proliferative action, but also the peptide suppresses the development of tumors [8,11–13,102,122,157]. This is an important characteristic of the galaninergic system that opens the door to a double potential therapeutic strategy using GALR agonists or antagonists. For this reason, it is crucial to determine which are the GALRs involved in cancer to develop specific antitumor ligands and drug-design studies; this is a line of research yet to be explored. The expression of GAL1R has been demonstrated in insulinoma cells [32,162]; however, the proliferative or antiproliferative actions mediated by GAL on these cells are currently unknown. Importantly, GAL’s antiproliferative potency was much higher in GAL2R-expressing cells than in those expressing GAL1R, meaning that a high level of GAL2R could block cancer cell proliferation [12]. The expression of GAL3R could be used as a marker for relapsing pituitary tumors and GAL3R antagonists could also be used to treat these tumors [145]; this must be confirmed. The GAL gene expression was blocked in thyrotroph adenomas [147,160]; these inhibitory mechanisms must be studied in-depth, since they could be useful to develop antitumor strategies. Another important point is to understand the role played by GAL in sexual dimorphism in estrogen-induced anterior pituitary tumorigenesis, since female tumors average twice the size of male tumors [168]. GAL, via GAL3R, promoted the proliferation of SCLC cells through an autocrine manner [88,113,150,151,173]; however, it is currently
unknown whether or not GAL-R antagonists exert an antitumor action against SCLC cells. GAL inhibited the proliferation of glioma cells and tumor growth via GAL-R [205]; a reduced level of GAL was observed in the cerebrospinal fluid of patients with glioblastoma [203], and GAL-R expression has been related to high-grade glioma [30]; the line of research on glioma must be developed in the future. In other cancers, the galaninergic system must be better studied, since the current data are fragmentary. Thus, it must be confirmed whether or not GAL plays an important role in breast cancer, and the proliferative/antiproliferative action of the peptide in bladder cancer, melanoma and cardiac and esophageal carcinomas must be investigated in-depth. Confirmation is also required as to whether GAL-R/GAL-R are therapeutic targets and prognostic factors in salivary duct carcinoma, as well as whether GAL-R DNA methylation indicates malignancy or not in endometrial cancer. Finally, the dual role of GAL as a proliferative and antiproliferative agent must be clarified in pancreatic carcinogenesis.

Epigenetic mechanisms regulate the galaninergic system and play a crucial role in tumor development (Figure 6). GAL downregulation favored tumor development in gastric cancer, which was due to an epigenetic inactivation, since the hypermethylation of GAL impaired its tumor-suppressive action [177]. Poor survival has been associated with the methylation of GAL/GAL-R genes in HNSCC and it has been reported that hypermethylation promoted the inactivation of GAL/GAL-R/GAL-R genes [195]. Thus, methylation changes could be a possible molecular marker for HNSCC risk/prognosis, since the methylation of the GAL-R gene promoter has been related to HNSCC carcinogenesis [193], and GAL-R/GAL-R hypermethylation has been associated with higher recurrence rates and reduced disease-free survival [194,200]. The GAL-R methylation status could be a biomarker for predicting HNSCC clinical outcomes. However, more studies must be performed to confirm whether GAL-R/GAL-R are potential therapeutic targets and prognostic factors.

Figure 6. Effects of the epigenetic alterations in the galaninergic system: higher recurrence, malignancy and poor survival. Methylation (M)/hypermethylation (M,M,M) of GAL/GALR genes.
Many of the proliferative and antiproliferative actions mediated by GAL on tumor cells could be explained by the signal transduction pathways depending on the coupled G protein type [17,30,77,87,196]. These actions could be also explained by GALR heteromer complexes, formed by GALRs with each other or with other types of G protein-coupled receptors, that promote conformational changes in GAL recognition sites, altering the binding affinity of GAL and favoring a certain signaling pathway [99]. This line of research must be investigated in-depth and it will serve to identify potential antitumor targets against the galaninergic system. For example, the blockade of signaling pathways common to several peptides could be an effective antitumor strategy as well as the development of broad-spectrum antagonists. Detailed studies on the antitumor effects of GAL agonists or antagonists have yet to be conducted in many types of cancer; thus, to obtain a detailed understanding of the different distribution patterns of GALRs and the different signaling pathways involved in tumor cells will help to identify the antiproliferative or proliferative actions played by these receptors and to develop new antitumor strategies. The use of an antitumor triple therapy (GAL, serotonin, octreotide) has been tested with good results against some tumors [219,220,223], but for unknown reasons, these investigations were not continued. Additional experiments are required to confirm the anticancer actions exerted by the three compounds. Moreover, two important lines of research must be developed: (1) the use of GAL analogs in tumors expressing certain GALRs; and (2) targeted radionuclide cancer therapy: the use of GAL and non-peptide ligands as radiopharmaceuticals for the diagnosis/treatment of GALR-positive tumors. Because GALRs play a crucial role in certain tumors, it is important to determine whether GALRs are involved in the viability of cancer cells, as has previously been demonstrated in tumor cells for the neurokinin-1 receptor [10]. Moreover, the possible tumor and/or antitumor actions mediated by GAL fragments and other members of the GAL family of peptides such as GALP, spexin, alarin, and GMAP must also be studied, since most of the studies in this field have focused on the entire molecule of GAL. Finally, it is important to note that the expression of peptides (e.g., neurotensin) has been reported in fetal tissues, but not in adult organs [9]. The authors suggested that the expression of peptides in these organs could be related to a malignant transformation, probably due to the presence of stem cells expressing peptides. This also suggests that a reversal to the fetal expression pattern occurred. This is an interesting issue that must be studied in the galaninergic system.

7. Conclusions

The galaninergic system is involved in tumorigenesis, invasion and migration and has been correlated with tumor stage/subtypes and metastasis and, in this system, epigenetic mechanisms have been related with carcinogenesis and recurrence rates. GALRs play a crucial role in cancer and their specific actions must be clearly understood in many tumor types because GALRs mediate different signal transduction pathways and actions depending on the tumor cell type and the particular G protein involved. GALRs could be used as a therapeutic target and diagnostic marker for the treatment, prognosis and surgical outcome in certain tumors. Different from other peptidergic systems, the galaninergic system exerts a proliferative action on tumor cells, but GAL also suppresses the development of tumors (Table 6). Thus, in-depth studies using GALRs agonists or antagonists as antitumor agents must be conducted to search for therapeutic strategies (alone or in combination with chemotherapy/radiotherapy) against tumor development. The involvement of the galaninergic system in cancer is a line of research that has been abandoned, but it must be re-opened and developed in the future. Additional studies must be carried out, for example, on the use of GALR agonists/antagonists as antitumor agents, the activation of signaling transduction pathways, the involvement of heteromers, targeted radionuclide cancer therapy and the viability of GALRs. This knowledge is crucial to establish future potential clinical antitumor applications, although unfortunately, the pharmaceutical industry has generally had no interest in this line of research; however, the data reported here suggest that the galaninergic system is a promising target for the treatment of tumors (Figure 7).
Table 6. Proliferative and antiproliferative actions of the galaninergic system in different tumors. +: action mediated by GAL, GAL1R, GAL2R or GAL3R.

| Cancer                          | GAL | GAL1R | GAL2R | GAL3R | References |
|--------------------------------|-----|-------|-------|-------|------------|
| **A. Proliferative action**    |     |       |       |       |            |
| Colorectal                     | +   |       |       |       | [8,30,106,117,185] |
| Glioma                         |     | +     |       |       | [30]       |
| Head and neck squamous cell carcinoma | +   |       |       |       | [84,122,123,201] |
| Neuroblastoma                  | +   |       |       |       | [137,141] |
| Pancreas                       | +   |       |       |       | [211]      |
| Pituitary adenoma              | +   |       |       |       | [38,145]   |
| Prolactinoma                   | +   |       |       |       | [148,160]  |
| Small-cell lung cancer         | +   |       |       |       | [88,113,150,151,173] |
| **B. Antiproliferative action** |     |       |       |       |            |
| Colorectal                     | +   |       |       |       | [182]      |
| Endometrial                    |     | +     |       |       | [208]      |
| Gastric                        | +   |       |       |       | [13,177,178] |
| Gastrointestinal               | +   |       |       |       | [101]      |
| Head and neck squamous cell carcinoma | +   |       |       |       | [13,16,120,170,184,190,192] |
| Neuroblastoma                  | +   |       |       |       | [12]       |
| Pancreas                       | +   |       |       |       | [212]      |
| Phaeochromocytoma              | +   |       |       |       | [141]      |
| Salivary duct carcinoma        | +   |       |       |       | [210]      |
Figure 7. Involvement of the GAL/GALR system in cancer, diagnosis and treatment. GAL1R/GAL3R mediate an antiproliferative effect, whereas GAL2R/GAL4R promote a proliferative action on tumor cells. GAL originates from tumor cells, tumor-infiltrating cells and nerve cells. Circulating GAL can also bind to GALRs. ↑: increase; ↓: decrease; ?: mechanisms that must be investigated (presence/functions of heteromers in tumor cells, involvement of GALRs in the viability of cancer cells and involvement of GAL fragments and other peptides belonging to the GAL family of peptides in cancer). *, biomarkers; M: methylation.
Author Contributions: Conceptualization, M.L.S. and R.C.; resources, M.L.S. and R.C.; writing—original draft preparation, M.L.S. and R.C.; writing—review and editing, M.L.S. and R.C.; supervision, M.L.S. and R.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 2021, 71, 209–249. https://doi.org/10.3322/caac.21660.

2. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The Next Generation. Cell 2011, 144, 646–674. https://doi.org/10.1016/j.cell.2011.02.013.

3. Muñoz, M.; Coveñas, R. Involvement of Substance P and the NK-1 Receptor in Human Pathology. Amino Acids 2014, 46, 1727–1750. https://doi.org/10.1007/s00726-014-1736-9.

4. Kastin, A.J. Handbook of Biologically Active Peptides, 2nd ed.; Academic Press: Amsterdam, The Netherlands, 2013.

5. Zhao, M.; Wang, T.; Liu, Q.; Cummins, S. Copy Number Alteration of Neuropeptides and Receptors in Multiple Cancers. Sci. Rep. 2017, 7, 4598. https://doi.org/10.1038/s41598-017-04832-0.

6. Kasprzak, A.; Adamek, A. The Neuropeptide System and Colorectal Cancer Liver Metastases: Mechanisms and Management. Int. J. Mol. Sci. 2020, 21, 3494. https://doi.org/10.3390/ijms21103494.

7. Colucci, R.; Blandizzi, C.; Ghisu, N.; Florio, T.; Del Tacca, M. Somatostatin Inhibits Colon Cancer Cell Growth through Cyclooxygenase-2 Downregulation: Somatostatin and Cyclooxygenase-2 in Colon Cancer. Br. J. Pharmacol. 2008, 155, 198–209. https://doi.org/10.1038/bjp.2008.268.

8. Godlewski, J.; Kmiec, Z. Colorectal Cancer Invasion and Atrophy of the Enteric Nervous System: Potential Feedback and Impact on Cancer Progression. Int. J. Mol. Sci. 2020, 21, 3391. https://doi.org/10.3390/ijms2103391.

9. Sánchez, M.L.; Coveñas, R. The Neurotensinergic System: A Target for Cancer Treatment. Curr. Med. Chem. 2022, 29, 3231–3260. https://doi.org/10.2174/092986732866621027124328.

10. Muñoz, M.F.; Argüelles, S.; Rosso, M.; Medina, R.; Coveñas, R.; Ayala, A.; Muñoz, M. The Neurokinin-1 Receptor Is Essential for the Viability of Human Glioma Cells: A Possible Target for Treating Glioblastoma. BioMed Res. Int. 2022, 2022, 1–13. https://doi.org/10.1155/2022/629405.

11. Kazanazawa, T.; Misawa, K.; Carey, T.E. Galanin Receptor Subtypes 1 and 2 as Therapeutic Targets in Head and Neck Squamous Cell Carcinoma. Expert Opin. Ther. Targets 2010, 14, 289–302. https://doi.org/10.1517/147282210035989922.

12. Berger, A.; Lang, R.; Moritz, K.; Santic, R.; Hermann, A.; Sperl, W.; Kolfer, B. Galanin Receptor Subtype Gal2Mediates Apoptosis in SH-SYSY Neuroblastoma Cells. Endocrinology 2004, 145, 500–507. https://doi.org/10.1210/en.2003-0649.

13. Ishi, H.; Tatsuta, M.; Baba, M.; Uehara, H.; Nakaizumi, A. Protection by Galanin against Gastric Carcinogenesis Induced by N-Methyl-N’-Nitro-N-Nitrosoguanidine in Wistar Rats. Cancer Res. 1994, 54, 3167–3170.

14. Tatemoto, K.; Rökaeus, Å.; Jörnvall, H.; McDonald, T.J.; Mutt, V. Galanin—a Novel Biologically Active Peptide from Porcine Intestine. FEBS Lett. 1983, 164, 124–128. https://doi.org/10.1016/0014-5793(83)80033-7.

15. Bersani, M.; Johnsen, A.H.; Hoijrup, P.; Dunning, B.E.; Andreasen, J.J.; Holst, J.J. Human Galanin: Primary Structure and Indentification of Two Molecular Forms. FEBS Lett. 1991, 283, 189–194. https://doi.org/10.1016/0014-5793(91)80585-Q.

16. Schmidt, W.E.; Kratzin, H.; Eckart, K.; Drevs, D.; Mundkowski, G.; Clemens, A.; Katsoulis, S.; Schäfer, H.; Gallwitz, B.; Creutzfeldt, W. Isolation and Primary Structure of Pituitary Human Galanin, a 30-Residue Nonamidated Neuropeptide. Proc. Natl. Acad. Sci. USA 1991, 88, 11435–11439. https://doi.org/10.1073/pnas.88.24.11435.

17. Chen, D.; Kodama, Y.; Kulseng, B.; Johannessen, H.; Zhao, C. Galanin in Handbook of Biologically Active Peptides; Kastin, A.J., Ed.; Academic Press: Amsterdam, The Netherlands, 2013; pp. 1210–1218.

18. Maria, E.; Vrontakis, B.S.P. Galanin: A Biologically Active Peptide. Curr. Drug Target-CNS Neurol. Disord. 2002, 1, 531–541. https://doi.org/10.2174/156807020333891.

19. Katsoulis, S.; Clemens, A.; Morrys-Wortmann, C.; Schwörer, H.; Schaube, H.; Klomp, H.J.; Fölsch, U.R.; Schmidt, W.E. Human Galanin Modulates Human Colonic Motility in Vitro Characterization of Structural Requirements. Scand. J. Gastroenterol. 1996, 31, 446–451. https://doi.org/10.3109/00365529609006763.

20. Lang, R.; Gundlach, A.L.; Holmes, F.E.; Hobson, S.A.; Wynnick, D.; Hökfelt, T.; Kolfer, B. Physiology, Signaling, and Pharmacology of Galanin Peptides and Receptors: Three Decades of Emerging Diversity. Pharmacol. Rev. 2015, 67, 118–175. https://doi.org/10.1124/pr.112.006536.

21. Tran, A.; He, W.; Chen, J.T.C.; Belsham, D.D. Spexin: Its Role, Regulation, and Therapeutic Potential in the Hypothalamus. Pharmacol. Ther. 2022, 233, 108033. https://doi.org/10.1016/j.pharmthera.2021.108033.

22. Ohtaki, T.; Kuman, S.; Ishibashi, Y.; Ogi, K.; Matsu, H.; Harada, M.; Kitada, C.; Kurokawa, T.; Onda, H.; Fujino, M. Isolation and CDNA Cloning of a Novel Galanin-like Peptide (GALP) from Porcine Hypothalamus. J. Biol. Chem. 1999, 274, 37041–37045. https://doi.org/10.1074/jbc.274.52.37041.
23. Lawrence, C.; Fraley, G.S. Galanin-like Peptide (GALP) is a Hypothalamic Regulator of Energy Homeostasis and Reproduction. *Front. Neuroendocrinol.* **2011**, *32*, 1–9. https://doi.org/10.1016/j.yfrne.2010.06.001.

24. Santic, R.; Fenninger, K.; Graf, K.; Schneider, R.; Hauser-Kronberger, C.; Schilling, F.H.; Kogner, P.; Ratschek, M.; Jones, N.; Sperl, W.; et al. Gangliocyes in Neuroblastic Tumors Express Alarin, a Novel Peptide Derived by Differential Splicing of the Galanin-Like Peptide Gene. *J. Mol. Neurosci.* **2006**, *29*, 145–152. https://doi.org/10.1385/JMN:29:2:145.

25. Marcos, P.; Coveras, R. Neuropeptidergic Control of Feeding: Focus on the Galanin Family of Peptides. *Int. J. Mol. Sci.* **2021**, *22*, 2544. https://doi.org/10.3390/ijms22052544.

26. Evans, H.; Baumgartner, M.; Shire, J.; Herzog, H. Genomic Organization and Localization of the Gene Encoding Human Preprogalanin. *Genomics* **1993**, *18*, 473–477. https://doi.org/10.1016/1096-0188(93)85431-U.

27. Koffer, B.; Evans, H.F.; Liu, M.L.; Falls, V.; Imsaa, T.P.; Shire, J.; Herzog, H. Characterization of the 5' Flanking Region of the Human Preprogalanin Gene. *DNA Cell Biol.* **1995**, *14*, 321–329. https://doi.org/10.1089/dna.1995.14.321.

28. Cunningham, M.J.; Scarlett, J.M.; Steiner, R.A. Cloning and Distribution of Galanin-Like Peptide MRNA in the Hypothalamus and Pituitary of the Macaque. *Endocrinology* **2002**, *143*, 755–763.

29. Landry, M.; Áman, K.; Dostrovsky, J.; Lozano, A.M.; Carlstedt, T.; Spenger, C.; Josephson, A.; Wiesenfeld-Hallin, Z.; Hökfelt, T. Galanin Expression in Adult Human Dorsal Root Ganglion Neurons: Initial Observations. *Neuroscience* **2003**, *117*, 795–809. https://doi.org/10.1016/S0306-4522(02)00965-X.

30. Falkenstetter, S.; Leitner, J.; Brunner, S.M.; Rieder, T.N.; Koffer, B.; Weis, S. Galanin System in Human Glioma and Pituitary Adenoma. *Front. Endocrinol.* **2020**, *11*, 1–14. https://doi.org/10.3389/fendo.2020.00155.

31. Anselmi, L.; Stella, S.L.; Lakher, A.; Hirano, A.; Tonini, M.; Catia Sternini Galanin Receptors in the Rat Gastrointestinal Tract. *Neuropeptides* **2005**, *39*, 349–352. https://doi.org/10.1016/j.npep.2004.12.023.

32. Parker, E.M.; Izzarelli, D.G.; Nowak, H.P.; Mable, C.D.; Iben, L.G.; Wang, J.; Goldstein, M.E. Cloning and Characterization of the Rat GALR1 Galanin Receptor from RIN14B Islet Cells. *Mol. Brain Res.* **1995**, *34*, 179–189. https://doi.org/10.1016/0169-328X(95)00159-P.

33. Waters, S.M.; Krause, J.E. Distribution of Galanin-1, -2 and -3 Receptor Messenger RNAs in Central and Peripheral Rat Tissues. *Neuroscience* **1999**, *95*, 265–271. https://doi.org/10.1016/S0306-4522(99)00407-8.

34. Gustafsson, E.L.; Smith, K.E.; Durkin, M.M.; Gerald, C.; Branchek, T.A. Distribution of a Rat Galanin Receptor MRNA in Rat Brain: *NeuroReport* **1996**, *7*, 953–957. https://doi.org/10.1097/00002201-199603200-00025.

35. Smith, K.E.; Forray, C.; Walker, M.W.; Jones, K.A.; Tamm, J.A.; Bard, J.; Branchek, T.A.; Linemeyer, D.L.; Gerald, C. Expression Cloning of a Rat Hypothalamic Galanin Receptor Coupled to Phosphoinositide Turnover. *J. Biol. Chem.* **1997**, *272*, 24612–24616. https://doi.org/10.1074/jbc.272.39.24612.

36. Bennet, W.M.; Hill, S.F.; Ghatel, M.A.; Bloom, S.R. Galanin in the Normal Human Pituitary and Brain and in Pituitary Adenomas. *J. Endocrinol.* **1991**, *130*, 463–467. https://doi.org/10.1677/joe.0.1300463.

37. Zhang, X.; Verge, V.M.K.; Wiesenfeld-Hallin, Z.; Pielh, F.; Hökfelt, T. Expression of Neuropeptides and Neuropeptide MRNAs in Spinal Cord after Axotomy in the Rat, with Special Reference to Motoneurons and Galanin. *Exp. Brain Res.* **1993**, *93*, 450–461. https://doi.org/10.1007/BF0029360.

38. Peimeral, P.; Vrontakis, M. Transgenic Mice Over-Expressing Galanin Exhibit Pituitary Adenomas and Increased Secretion of Galanin, Prolactin and Growth Hormone. *J. Endocrinol.* **2003**, *179*, 145–154. https://doi.org/10.1677/joe.0.1790145.

39. Hacker, G.W.; Bishop, A.E.; Terenghi, G.; Varmell, I.M.; Aghahowa, J.; Pollard, K.; Thurner, J.; Polak, J.M. Multiple Peptide Production and Presence of General Neuroendocrine Markers Detected in 12 Cases of Human Phaeochromocytoma and in Mammalian Adrenal Glands. *Virochews Arch. A Pathol. Anat. Histopathol.* **1988**, *412*, 399–411. https://doi.org/10.1007/BF00750574.

40. Melander, T.; Hökfelt, T.; Riikaeus, A. Coexistence of Galanin-like Immunoreactivity with Catecholamines, 5-Hydroxytryptamine, GABA and Neuropeptides in the Rat CNS. *J. Neurosci.* **1986**, *6*, 15.

41. Xu, Z.-Q.D.; Shi, T.-J.S.; Hökfelt, T. Galanin/GMAP- and NPY-like Immunoreactivities in Locus Coeruleus and Noradrenergic Nerve Terminals in the Hippocampal Formation and Cortex with Notes on the Galanin-R1 and -R2 Receptors. *J. Comp. Neurol.* **1998**, *392*, 227–251. https://doi.org/10.1002/(SICI)1096-9861(19980309)392:2<227::AID-CNE6>3.0.CO;2-4.

42. Merchenthaler, I.; Lopez, F.J.; Negro-Vilar, A. Colocalization of Galanin and Luteinizing Hormone-Releasing Hormone in a Subset of Preoptic Hypothalamic Neurons: Anatomical and Functional Correlates. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 6326–6330. https://doi.org/10.1073/pnas.87.16.6326.

43. Zhang, X.; Nicholas, A.P.; Ho’kfelt, T. Ultrastructural Studies on Peptides in the Dorsal Horn of the Spinal Cord—I. Co-Existence of Galanin with Other Peptides in Primary Afferents in Normal Rats. *Neuroscience* **1993**, *57*, 365–384. https://doi.org/10.1016/0306-4522(93)90069-R.

44. Zhang, X.; Nicholas, A.P.; Hökfelt, T. Ultrastructural Studies on Peptides in the Dorsal Horn of the Rat Spinal Cord—II. Co-Existence of Galanin with Other Peptides in Local Neurons. *Neuroscience* **1995**, *64*, 875–891. https://doi.org/10.1016/0306-4522(94)00451-A.

45. Gundlach, A.L.; Ryan, P.J. Galanin and GALP. In *Handbook of Biologically Active Peptides*; Kastin, A.J., Ed.; Academic Press: Amsterdam, The Netherlands, 2013; pp. 766–775.

46. McKnight, G.L.; Karlson, A.E.; Kowalsky, S.; Mathewes, S.L.; Sheppard, P.O.; O’Harra, P.J.; Taborsky, G.J. Sequence of Human Galanin and Its Inhibition of Glucose-Stimulated Insulin Secretion From RIN Cells. *Diabetes* **1992**, *41*, 82–87. https://doi.org/10.2337/diab.41.1.82.
47. Hökfelt, T.; Tatemoto, K. Galanin—25 Years with a Multitalented Neuropeptide. Cell. Mol. Life Sci. 2008, 65, 1791–1795. https://doi.org/10.1007/s00018-008-8152-9.

48. Scott, M.K.; Ross, T.M.; Lee, D.H.S.; Wang, H.-Y.; Shank, R.P.; Wild, K.D.; Davis, C.B.; Crooke, J.J.; Potocki, A.C.; Reitz, A.B. 2,3-Dihydro-Dithien- and -Dithiophene-1,1,4,4-Tetroxides: Small Molecule Non-Peptide Antagonists of the Human Galanin HGAL-1 Receptor. Bioorg. Med. Chem. 2000, 8, 1383–1391. https://doi.org/10.1016/S0968-0896(00)00062-6.

49. Hobson, S.-A.; Bacon, A.; Elliott-Hunt, C.R.; Holmes, F.E.; Kerr, N.C.H.; Pope, R.; Vanderplank, P.; Wynick, D. Galanin Acts as a Trophic Factor to the Central and Peripheral Nervous Systems. Cell. Mol. Life Sci. 2010, 102, 25–38. https://doi.org/10.1007/978-3-0346-0228-0_3.

50. Elliott-Hunt, C.R.; Marsh, B.; Bacon, A.; Pope, R.; Vanderplank, P.; Wynick, D. Galanin Acts as a Neuroprotective Factor to the Hippocampus. Proc. Natl. Acad. Sci. USA 2014, 101, 5105–5110. https://doi.org/10.1073/pnas.0304823101.

51. Quynh, N.T.T.; Islam, S.M.; Florén, A.; Bartfai, T.; Langel, Ü.; Östenson, C.-G. Effects of Galanin, a Non-Peptide Galanin-Receptor Agonist, on Insulin Release from Rat Pancreatic Islets. Biochem. Biophys. Res. Commun. 2005, 328, 213–220. https://doi.org/10.1016/j.bbrc.2004.12.150.

52. Giustina, A.; Schettino, M.; Bodini, C.; Doga, M.; Licini, M.; Giustina, G. Effect of Galanin on the Growth Hormone Response to Growth Hormone-Releasing Hormone in Acromegaly. Metabolism 1992, 41, 1291–1294. https://doi.org/10.1016/0026-0495(92)90098-U.

53. Mazancourt, P.D.; Goldsmith, P.K.; Weinstein, L.S. Inhibition of Adenylate Cyclase Activity by Galanin in Rat Insulinoma Cells Is Mediated by the G-Protein G13. Biochem. J. 1994, 303, 369–375.

54. Guipponi, M.; Chentouf, A.; Webeling, K.E.B.; Freimann, K.; Crespel, A.; Nobile, C.; Lemke, J.R.; Hansen, J.; Dorn, T.; Lesca, G.; et al. Galanin Pathogenic Mutations in Temporal Lobe Epilepsy. Hum. Mol. Genet. 2015, 24, 3082–3091. https://doi.org/10.1093/hmg/ddv060.

55. Webeling, K.E.B.; Runesson, J.; Bartfai, T.; Langel, Ü. Galanin Receptors and Ligands. Front. Endocrinol. 2012, 3, 1–14. https://doi.org/10.3389/fendo.2012.00146.

56. Chan-Palay, V. Galanin Hyperinnervates Surviving Neurons of the Human Basal Nucleus of Meynert in Dementias of Alzheimer’s and Parkinson’s Disease: A Hypothesis for the Role of Galanin in Accentuating Cholinergic Dysfunction in Dementia. J. Comp. Neurol. 1988, 273, 543–557. https://doi.org/10.1002/cne.902730409.

57. Wiesenfeld-Hallin, Z.; Villar, M.J.; Hökfelt, T. The Effects of Intrathecral Galanin and C-Fiber Stimulation on the Flexor Reflex in the Rat. Brain Res. 1989, 486, 205–213. https://doi.org/10.1016/0006-8993(89)90506-4.

58. Wang, P.; Li, H.; Barde, S.; Zhang, M.-D.; Sun, J.; Wang, T.; Zhang, P.; Luo, H.; Wang, Y.; Yang, Y.; et al. Depression-like Behavior in Rat: Involvement of Galanin Receptor Subtype 1 in the Ventral Periaqueductal Gray. Proc. Natl. Acad. Sci. USA 2016, 113, 4726–4735. https://doi.org/10.1073/pnas.1609198113.

59. Koller, A.; Brunner, S.M.; Bianchini, R.; Ramspacher, A.; Emberger, M.; Locker, F.; Schlager, S.; Kofler, B. Galanin Is a Potent Modulator of Cytokine and Chemokine Expression in Human Macrophages. Sci. Rep. 2019, 9, 7237. https://doi.org/10.1038/s41598-019-43704-7.

60. Jurkowski, W.; Yazdi, S.; Elofsson, Å. Ligand Binding Properties of Human Galanin Receptors. Mol. Membr. Biol. 2013, 30, 206–216. https://doi.org/10.3109/09687668.2012.750384.

61. Probst, W.C.; Snyder, L.A.; Schuster, D.I.; Brosius, J.; Sealfon, S.C. Sequence Alignment of the G-Protein Coupled Receptor Superfamily: DNA Cell Biol. 1992, 11, 1–20. https://doi.org/10.1089/dna.1992.11.1.

62. Krishna, A.G.; Menon, S.T.; Terry, T.J.; Sakmar, T.P. Evidence That Helix 8 of Rhodopsin Acts as a Membrane-Dependent Conformational Switch. Biochemistry 2002, 41, 8298–8309. https://doi.org/10.1021/bi012353m.

63. Kolakowski, L.F.; O’Neill, G.P.; Howard, A.D.; Broussard, S.R.; Sullivan, K.A.; Feighner, S.D.; Sawzdargo, M.; Nguyen, T.; Kargman, S.; Shiao, L.-L.; et al. Molecular Characterization and Expression of Cloned Human Galanin Receptors GALR2 and GALR3. J. Neurochem. 2002, 71, 2239–2251. https://doi.org/10.1046/j.1471-4159.1998.71062239.x.

64. Lang, R.; Gundlach, A.; Kofler, B. The Galanin Peptide Family: Receptor Pharmacology, Pleiotropic Biological Actions, and Implications in Health and Disease. Pharmacol. Ther. 2007, 115, 177–207. https://doi.org/10.1016/j.pharmthera.2007.05.009.

65. Fathi, Z.; Battaglini, P.M.; Iben, L.G.; Li, H.; Baker, E.; Zhang, D.; McGovern, R.; Mahle, C.D.; Sutherland, G.R.; Ismaa, T.P.; et al. Molecular Characterization, Pharmacological Properties and Chromosomal Localization of the Human GALR2 Galanin Receptor. Mol. Brain Res. 1998, 58, 156–169. https://doi.org/10.1016/S0169-328X(98)00116-8.

66. Wang, S.; Hashemi, T.; He, C.; Strader, C.; Bayne, M. Molecular Cloning and Pharmacological Characterization of a New Galanin Receptor Subtype. Mol. Pharmacol. 1997, 52, 337–343. https://doi.org/10.1124/mol.52.3.337.

67. Habert-Ortoli, E.; Amiranoff, B.; Loquet, I.; Laburthe, M.; Mayaux, J.F. Molecular Cloning of a Functional Human Galanin Receptor. Proc. Natl. Acad. Sci. USA 1994, 91, 9780–9783. https://doi.org/10.1073/pnas.91.21.9780.

68. Wang, S.; Hashemi, T.; Fried, S.; Clemmons, A.L.; Hawes, B.E. Differential Intracellular Signaling of the GalR1 and GalR2 Galanin Receptor Subtypes. Biochemistry 1998, 37, 6711–6717. https://doi.org/10.1021/bi9728405.

69. Wang, S.; He, C.; Maguire, M.T.; Clemmons, A.L.; Burrier, R.E.; Guzzi, M.F.; Strader, C.D.; Parker, E.M.; Bayne, M.L. Genomic Organization and Functional Characterization of the Mouse GalR1 Galanin Receptor. FEBS Lett. 1997, 411, 225–230. https://doi.org/10.1016/S0014-5793(97)00695-9.
70. Badie-Mahdavi, H.; Lu, X.; Behrens, M.M.; Bartfai, T. Role of Galanin Receptor 1 and Galanin Receptor 2 Activation in Synaptic Plasticity Associated with 3’5’-Cyclic AMP Response Element-Binding Protein Phosphorylation in the Dentine Gyrus: Studies with a Galanin Receptor 2 Agonist and Galanin Receptor 1 Knockout Mice. Neuroscience 2005, 133, 591–604. https://doi.org/10.1016/j.neuroscience.2005.02.042.

71. Zachariou, V.; Georgescu, D.; Kansal, L.; Merriam, P.; Picciotto, M.R. Galanin Receptor 1 Gene Expression Is Regulated by Cyclic AMP through a CREB-Dependent Mechanism: Galanin Receptor I Gene Expression. J. Neurochem. 2008, 76, 191–200. https://doi.org/10.1046/j.1471-4159.2001.00018.x.

72. Haws, J.J.; Brunzell, D.H.; Wynick, D.; Zachariou, V.; Picciotto, M.R. GalR1, but Not GalR2 or GalR3. Levels Are Regulated by Galanin Signaling in the Locus Coeruleus through a Cyclic AMP-Dependent Mechanism: GalR Regulation in the Locus Coeru eles. J. Neurochem. 2005, 93, 1168–1176. https://doi.org/10.1111/j.1471-4159.2005.03105.x.

73. Jacoby, A.S.; Webb, G.C.; Liu, M.L.; Kofler, B.; Hort, Y.J.; Fathi, Z.; Bottema, C.D.K.; Shine, J.; lissma, T.P. Structural Organization of the Mouse and Human GALR1 Galanin Receptor Genes (GnlrandGALNR) and Chromosomal Localization of the Mouse Gene. Genomics 1997, 45, 496–508. https://doi.org/10.1006/geno.1997.4960.

74. Lorimer, D.D.; Benya, R.V. Cloning and Quantification of Galanin-I Receptor Expression by Mucosal Cells Lining the Human Gastrointestinal Tract. Biochem. Biophys. Res. Commun. 1996, 222, 379–385. https://doi.org/10.1006/bbrc.1996.0752.

75. Sullivan, K.A.; Shiao, L.-L.; Cascieri, M.A. Pharmacological Characterization and Tissue Distribution of the Human and Rat GALR1 Receptors. Biochem. Biophys. Res. Commun. 1997, 233, 823–828. https://doi.org/10.1006/bbrc.1997.6542.

76. Howard, A.D.; Tan, C.; Shiao, L.-L.; Palyha, O.C.; McKee, K.K.; Weinberg, D.H.; Feighner, S.D.; Cascieri, M.A.; Smith, R.G.; Van Der Ploeg, L.H.T.; et al. Molecular Cloning and Characterization of a New Receptor for Galanin. FEBS Lett. 1997, 405, 285–290. https://doi.org/10.1016/S0014-5793(97)00196-8.

77. Demsie, D.G.; Altaye, B.M.; Weldedkian, E.; Gebremedhin, H.; Alema, N.M.; Tefera, M.M.; Tilahun, A. Galanin Receptors as Drug Target for Novel Antidepressants: Review. Biol. Targets Ther. 2020, 14, 37–45. https://doi.org/10.2147/BTT.S240715.

78. Bloomquist, B.T.; Beauchamp, M.R.; Zehlin, L.; Brown, S.-E.; Gore-Willse, A.R.; Gregor, P.; Cornfield, L.J. Cloning and Expression of the Human Galanin Receptor GalR2. Biochem. Biophys. Res. Commun. 1998, 243, 474–479. https://doi.org/10.1006/bbrc.1998.8133.

79. Mitchell, V.; Bouret, S.; Howard, A.D.; Beuvillain, J.-C. Expression of the Galanin Receptor Subtype Gal-R2 MRNA in the Rat Hypothalamus. J. Chem. Neuroanat. 1999, 16, 265–277. https://doi.org/10.1016/S0891-0618(99)00011-3.

80. O’Donnell, D.; Ahmad, S.; Wah lestedt, C.; Walker, P. Expression of the Novel Galanin Receptor Subtype GALR2 in the Adult Rat CNS: Distinct Distribution from J. Comp. Neurol. 1999, 409, 2.

81. Mons, N.; Decorte, L.; Jaffard, R.; Cooper, D. Ca2+-Sensitive Adenylyl Cyclases, Key Integrators of Cellular Signalling. Life Sci. 1998, 62, 1647–1652. https://doi.org/10.1016/S0024-3205(98)00122-2.

82. Burazin, T.C.D.; Larr, J.A.; Ryan, M.C.; Gundlach, A.L. Galanin-R1 and -R2 Receptor MRNA Expression during the Development of Rat Brain Suggests Differential Subtype Involvement in Synaptic Transmission and Plasticity: Gal-R1 and Gal-R2 MRNA Localization in Developing Rat Brain. Eur. J. Neurosci. 2000, 12, 2902–2917. https://doi.org/10.1046/j.1460-9568.2000.00184.x.

83. Ding, X.; MacTavish, D.; Kar, S.; Jhamandas, J.H. Galanin Attenuates β-Amyloid (Aβ) Toxicity in Rat Cholinergic Basal Forebrain Neurons. Neurobiol. Dis. 2006, 21, 413–420. https://doi.org/10.1016/j.nbd.2005.08.016.

84. Scanlon, C.S.; Banerjee, R.; Inglehart, R.C.; Liu, M.; Russo, N.; Hariharan, A.; van Tubergen, E.A.; Corson, S.L.; Asangani, I.A.; Mistrretta, C.M.; et al. Galanin Modulates the Niches of Perineural Inversion in Head and Neck Cancer. Nat. Commun. 2015, 6, 6885. https://doi.org/10.1038/ncomms7885.

85. Filhi, Z.; Cunningham, A.M.; Iben, L.G.; Battaglino, P.B.; Ward, S.A.; Nichol, K.A.; Pine, K.A.; Wong, J.; Goldstein, M.E.; lissma, T.P.; et al. Cloning, Pharmacological Characterization and Distribution of a Novel Galanin Receptor. Mol. Brain Res. 1997, 51, 49–59. https://doi.org/10.1016/S0169-328X(97)00210-6.

86. Elliott-Hunt, C.R.; Pope, R.J.P.; Vanderplank, P.; Wynick, D. Activation of the Galanin Receptor 2 (GalR2) Protects the Hippocampus from Neuronal Damage: GalR2 and Hippocampal Neuroprotection. J. Neurochem. 2007, 100, 780–789. https://doi.org/10.1111/j.1471-4159.2006.04239.x.

87. Gopalakrishnan, L.; Chatterjee, O.; Raj, C.; Pullimamidi, D.; Advani, J.; Mahadevan, A.; Keshava Prasad, T.S. An Assembly of Galanin–Galanin Receptor Signaling Network. J. Cell Commun. Signal. 2021, 15, 269–275. https://doi.org/10.1007/s12079-020-00590-3.

88. Wittau, N.; Grosse, R.; Kalkbrenner, F.; Gohla, A.; Schultz, G.; Gudermann, T. The Galanin Receptor Type 2 Initiates Multiple Signaling Pathways in Small Cell Lung Cancer Cells by Coupling to Gq, Gi and G12 Proteins. Oncogene 2000, 19, 4199–4209. https://doi.org/10.1038/sj.onc.1203777.

89. Wang, S.; He, C.; Hashemi, T.; Bayne, M. Cloning and Expression Characterization of a Novel Galanin Receptor. J. Biol. Chem. 1997, 272, 31949–31952. https://doi.org/10.1074/jbc.272.51.31949.

90. Smith, K.E.; Walker, M.W.; Artymsyshyn, R.; Bard, J.; Borowsky, B.; Tamm, J.A.; Yao, W.-J.; Vaysse, P.-J.; Branchek, T.A.; Gerald, C.; et al. Cloned Human and Rat Galanin GALR3 Receptors. J. Biol. Chem. 1998, 273, 23321–23326. https://doi.org/10.1074/jbc.273.36.23321.

91. Mennicken, F.; Hoffert, C.; Pelletier, M.; Ahmad, S.; O’Donnell, D. Restricted Distribution of Galanin Receptor 3 (GalR3) MRNA in the Adult Rat Central Nervous System. J. Chem. Neuroanat. 2002, 24, 257–268. https://doi.org/10.1006/jcne.2002.00568-6.
115. Kepron, C.; Reis, P.; Bharadwaj, R.; Shaw, J.; Kamel-Reid, S.; Ghazarian, D. Identification of Genomic Predictors of Non-Melanoma Skin Cancer in Solid Organ Transplant Recipients. *Eur. J. Dermatol.* 2009, 19, 278–280. https://doi.org/10.1684/ijd.2009.0649.

116. Berger, A.; Santic, R.; Hauser-Kronberger, C.; Schilling, F.H.; Kogner, P.; Ratschek, M.; Gamper, A.; Jones, N.; Sperl, W.; Kofler, B. Galanin and Galanin Receptors in Human Cancers. *Neuropeptides* 2005, 39, 353–359. https://doi.org/10.1016/j.npep.2004.12.016.

117. Stevenson, L.; Allen, W.L.; Turkington, R.; Jithesh, P.V.; Proutski, I.; Stewart, G.; Lenz, H.-J.; Van Schayckbroeck, S.; Longley, D.B.; Johnston, P.G. Identification of Galanin and Its Receptor GalR1 as Novel Determinants of Resistance to Chemotherapy and Potential Biomarkers in Colorectal Cancer. *Clin. Cancer Res.* 2012, 18, 5412–5426. https://doi.org/10.1158/1078-0432.CCR-12-1780.

118. Berger, A.; Santic, R.; Almer, D.; Hauser-Kronberger, C.; Huemer, M.; Humpel, C.; Stockhammer, G.; Sperl, W.; Kofler, B. Galanin and Galanin Receptors in Human Gliomas. *Acta Neuropathol.* 2003, 105, 555–560. https://doi.org/10.1007/s00401-003-0680-7.

119. Gilaberte, Y.; Vera, J.; Coscojuela, C.; Roca, M.J.; Parrado, C.; González, S. Expression of Galanin in Melanocytic Tumors. *Actas Dermosifiliogr.* 2007, 98, 24–34. https://doi.org/10.1016/S1578-2190(07)70386-4.

120. Sugimoto, T.; Soki, N.; Shimizu, S.; Kikkawa, N.; Tsukada, J.; Shimada, H.; Sasaki, K.; Hanazawa, T.; Okamoto, Y.; Hata, A. The Galanin Signaling Cascade Is a Candidate Pathway Regulating Oncogenesis in Human Squamous Cell Carcinoma. *Genes. Chromosomes Cancer* 2009, 48, 132–142. https://doi.org/10.1002/gcc.20626.

121. Godlewski, J.; Pidsudko, Z. Characteristic of Galaninergic Components of the Enteric Nervous System in the Cancer Invasion of Human Large Intestine. *Annu. Rev. Annu. Antiz.* 2012, 194, 368–372. https://doi.org/10.1016/j.anatan.2011.11.009.

122. Banerjee, R.; Henson, B.S.; Russo, N.; Tsidakov, A.; D'Silva, N.J. Rap1 Mediates Galanin Receptor 2-Induced Proliferation and Survival in Squamous Cell Carcinoma. *Cell. Signal.* 2011, 23, 1110–1118. https://doi.org/10.1016/j.cellsig.2011.02.002.

123. Henson, B.S.; Neubig, R.R.; Jang, I.; Ogawa, T.; Zhang, Z.; Carey, T.E.; D'Silva, N.J. Galanin Receptor 1 Has Anti-Proliferative Effects in Oral Squamous Cell Carcinoma. *J. Biol. Chem.* 2005, 280, 22564–22571. https://doi.org/10.1074/jbc.M415489200.

124. Barakat, M.T.; Meeran, K.; Bloom, S.R. Neuroendocrine Tumours. *Endocr. Relat. Cancer* 2004, 11, 1–18. https://doi.org/10.1677/erc.0.0110001.

125. Reubi, J.C. Regulatory Peptide Receptors as Molecular Targets for Cancer Diagnosis and Therapy. *Q. J. Nucl. Med.* 1997, 41, 63–70.

126. Wood, S.M.; Polak, J.M.; Bloom, S.R. Gut Hormone Secretory Tumours. *Scand. J. Gastroenterol.* 1983, 82, 165–179.

127. Kogner, P. Neuropeptides in Neuroblastomas And ganglioneuromas. *Proc. Brain Res.* 1995, 104, 325–338. https://doi.org/10.1016/S0076-1123(08)61798-7.

128. Gregg, D.W.; Galkin, M.; Gorski, J. Effect of Estrogen on the Expression of Galanin mRNA in Pituitary Tumor-Sensitive and Tumor-Resistant Rat Strains. *Stereoids* 1996, 61, 468–472. https://doi.org/10.1016/0039-6878(96)00761-1.

129. Hsu, D.W.; El-Azouzi, M.; Black, P.McL.; Chin, W.W.; Hedley-Whyte, E.T.; Kaplan, L.M. Estrogen Increases Galanin Immunoreactivity in Hyperplastic Prolactin-Secreting Cells in Fisher 344 Rats. *Endocrinology* 1990, 126, 3159–3167. https://doi.org/10.1210/endo-126-6-3159.

130. Hyde, J.F.; Howard, G. Regulation of Galanin Gene Expression in the Rat Anterior Pituitary Gland by the Somatostatin Analog SMS 201-995. *Endocrinology* 1992, 131, 2097–2102. https://doi.org/10.1210/endo.131.5.1385097.

131. Wynick, D.; Hammond, P.J.; Akinsanya, K.O.; Bloom, S.R. Galanin Regulates Basal and Oestrogen-Stimulated Lactotroph Function. *Nature* 1993, 364, 529–532. https://doi.org/10.1038/364529a0.

132. Wynick, D.; Small, C.J.; Bacon, A.; Holmes, F.E.; Norman, M.; Ormandy, C.J.; Kilic, E.; Kerr, N.C.H.; Ghanai, M.; Talamantes, F.; et al. Galanin Regulates Prolactin Release and Lactotroph Proliferation. *Proc. Natl. Acad. Sci. USA* 1998, 95, 12671–12676. https://doi.org/10.1073/pnas.95.21.12671.

133. Lu, X.; Bartfai, T. Analyzing the Validity of GalR1 and GalR2 Antibodies Using Knockout Mice. *Naunyn. Schmiedebergs Arch. Pharmacol.* 2009, 379, 417–420. https://doi.org/10.1007/s00210-009-0394-z.

134. Beermann, S.; Seifert, R.; Neumann, D. Commercially Available Antibodies against Human and Murine Histamine H4-Receptor Lack Specificity. *Naunyn. Schmiedebergs Arch. Pharmacol.* 2012, 385, 125–135. https://doi.org/10.1007/s00210-011-0700-4.

135. Invitti, C.; Giraldi, F.P.; Dubini, A.; Moroni, P.; Lasa, M.; Piccolotti, R.; Cavagnini, F. Galanin Is Released by Adrenocorticotropic-Secreting Pituitary Adenomas In Vivo and In Vitro. *J. Clin. Endocrinol. Metab.* 1999, 84, 1351–1356. https://doi.org/10.1210/jcem.84.4.54629.

136. Berger, A.; Tuchler, C.; Almer, D.; Kogner, P.; Ratschek, M.; Kerbl, R.; Ismama, T.P.; Jones, N.; Sperl, W.; Kofler, B. Elevated Expression of Galanin Receptors in Childhood Neuroblastic Tumors. *Neuroendocrinology* 2002, 75, 130–138. https://doi.org/10.1159/000048229.

137. Perel, Y.; Amrein, L.; Dobremez, E.; Rivel, J.; Daniel, J.Y.; Landry, M. Galanin and Galanin Receptor Expression in Neuroblastic Tumours: Correlation with Their Differentiation Status. *Br. J. Cancer* 2002, 86, 117–122. https://doi.org/10.1038/sj.bjc.660019.

138. Sjöholm, Å. Regulation of Insulinoma Cell Proliferation and Insulin Accumulation by Peptides and Second Messengers. *Upps. J. Med. Sci.* 1995, 100, 201–216. https://doi.org/10.3109/00309739509178906.

139. Fehmann, H.C.; Habener, J.F. Galanin Inhibits Proinsulin Gene Expression Stimulated by the Insulinotropic Hormone Glucagon-like Peptide-1(7-37) in Mouse Insulinoma Beta TC-1 Cells. *Endocrinology* 1992, 130, 2890–2896. https://doi.org/10.1210/endo.130.5.1374016.
140. Toneff, T.; Funkelstein, L.; Mosier, C.; Abagyan, A.; Ziegler, M.; Hook, V. Beta-Amyloid Peptides Undergo Regulated Co-Secretion with Neuropeptide and Catecholamine Neurotransmitters. Peptides 2013, 46, 126–135. https://doi.org/10.1016/j.peptides.2013.04.020.

141. Cheng, S.; Yuan, C.-G. Differential Effect of Galanin on Proliferation of PC12 and B104 Cells. NeuroReport 2007, 18, 1379–1383. https://doi.org/10.1097/WNR.0b013e3282c489ec.

142. Squillaci, S.; Gal, A.A. Galanin Immunoreactivity in a Laryngealparaganglioma: Case Report and Literature Review. Pathologica 2004, 96, 111–116.

143. Tofigli, R.; Joseph, B.; Xia, S.; Xu, Z.-Q.D.; Hamberger, B.; Hökfelt, T.; Ceccatelli, S. Galanin Decreases Proliferation of PC12 Cells and Induces Apoptosis via Its Subtype 2 Receptor (GalR2). Proc. Natl. Acad. Sci. USA 2008, 105, 2717–2722. https://doi.org/10.1073/pnas.0712300105.

144. Bauer, F.E.; Hacker, G.W.; Terenghi, G.; Adrian, T.E.; Polak, J.M.; Bloom, S.R. Localization and Molecular Forms of Galanin in Human Adrenals: Elevated Levels in Pheochromocytomas. J. Clin. Endocrinol. Metab. 1986, 63, 1372–1378. https://doi.org/10.1210/jcem-63-6-1372.

145. Tofigli, R.; Barde, S.; Palkovits, M.; Höög, A.; Hökfelt, T.; Ceccatelli, S.; Hulting, A.-L. Galanin and Its Three Receptors in Human Pituitary Adenoma. Neuropeptides 2012, 46, 195–201. https://doi.org/10.1016/j.nepep.2012.07.003.

146. Hyde, J.F.; Morrison, D.G.; Moore, J.P.; Howard, G. MtTW-10 Pituitary Tumor Cells: Galanin Gene Expression and Peptide Secretion. Endocrinology 1993, 133, 2588–2593. https://doi.org/10.1210/endo.133.6.7694842.

147. Hyde, J.F.; Moore, J.P.; Drake, K.W.; Morrison, D.G. Galanin Gene Expression in Radiothyroidectomy-Induced Thyrotrypin Adenomas. Am. J. Physiol. Endocrinol. Metab. 1996, 271, E24–E30. https://doi.org/10.1152/ajpendo.1996.271.1.E24.

148. Vrontakis, M.E.; Peden, L.M.; Duckworth, M.L.; Friesen, H.G. Isolation and Characterization of a Complementary DNA (Galanin) Clone from Estrogen-Induced Pituitary Tumor Messenger RNA. J. Biol. Chem. 1987, 262, 16755–16758. https://doi.org/10.1016/S0021-9258(18)34546-4.

149. Piroli, G.G.; Cassataro, J.; Pietranera, L.; Grillo, C.A.; Ferrini, M.; Lux-Lantos, V.; De Nicola, A.F. Progestin Regulation of Galanin and Prolactin Gene Expression in Oestrogen-Induced Pituitary Tumours: Progestin Regulation of Galanin Expression in Prolactinomas. J. Neuroendocrinol. 2001, 13, 302–309. https://doi.org/10.1046/j.1365-2826.2001.00663.x.

150. Sethi, T.; Rozengurt, E. Multiple Neuropeptides Stimulate Clonal Growth of Small Cell Lung Cancer: Effects of Bradykinin, Vasopressin, Cholecystokinin, Galanin, and Neurtensin. Cancer Res. 1991, 51, 3621–3623.

151. Sethi, T.; Rozengurt’, E. Galanin Stimulates Ca2+ Mobilization, Inositol Phosphate Accumulation, and Clonal Growth in Small Cell Lung Cancer Cells. Cancer Res. 1991, 51, 1674–1679.

152. Sethi, T.; Langdon, S.; Smyth, J.; Rozengurt, E. Growth of Small Cell Lung Cancer Cells: Stimulation by Multiple Neuropeptides and Inhibition by Broad Spectrum Antagonists In Vitro and In Vivo. Cancer Res. 1992, 25 (Suppl. 9), 2737S–2743S.

153. Seufferlein, T.; Rozengurt, E. Galanin, Neurtensin, and Phorbol Esters Rapidly Stimulate Activation of Mitogen-Activated Protein Kinase in Small Cell Lung Cancer Cells. Cancer Res. 1996, 56, 5785–5786.

154. Guidermann, T.; Roelle, S. Calcium-Dependent Growth Regulation of Small Cell Lung Cancer Cells by Neuropeptides. Endocr. Relat. Cancer 2006, 13, 1069–1084. https://doi.org/10.1677/erc.1.01302.

155. Yamamoto, H.; Ben, S.; Saitoh, S.; Kamata, K.; Iguchi, K.; Hoshino, M. Plasmin: Its Role in the Extracellular Processing of Progalanin in Tumor Tissue. Protein Pept. Lett. 2011, 18, 1204–1211. https://doi.org/10.2174/092986611797642751.

156. Yamamoto, H.; Iguchi, K.; Ohno, S.; Yokogawa, T.; Nishikawa, K.; Hoshino, M. Activation of Large Form Galanin-LI by Extracellular Processing in Small Cell Lung Carcinoma Tissue. Protein Pept. Lett. 2011, 18, 1058–1064. https://doi.org/10.2174/092986611796378693.

157. Hulting, A.-L.; Land, T.; Berthold, M.; Langel, Ü.; Hökfelt, T.; Bartfai, T. Galanin Receptors from Human Pituitary Tumors Assayed with Human Galanin as Ligand. Brain Res. 1993, 625, 173–176. https://doi.org/10.1016/0006-8993(93)90152-D.

158. Giustina, A.; Ragni, G.; Bollati, A.; Cozzi, R.; Licini, M.; Polies, C.; Turazzi, S.; Bonfanti, C. Inhibitory Effects of Galanin on Growth Hormone (GH) Release in Cultured GH-Secreting Adenoma Cells: Comparative Study with Octreotide, GH-Releasing Hormone, and Thryrotrypin-Releasing Hormone. Metabolism 1997, 46, 425–430. https://doi.org/10.1016/S0026-0495(97)90060-3.

159. Giustina, A.; Bonfanti, C.; Licini, M.; De Rango, C.; Milani, G. Inhibitory Effect of Galanin on Growth Hormone Release from Rat Pituitary Tumor Cells (GH1) in Culture. Life Sci. 1994, 55, 1845–1851. https://doi.org/10.1016/0024-3205(94)90095-7.

160. Hyde, J.F.; Moore, J.P.; Cai, A. Galanin in Normal and Hyperplastic Anterior Pituitary Cells: From Pituitary Tumor Cell Lines to Transgenic Mice. Ann. N. Y. Acad. Sci. 1998, 863, 48–55. https://doi.org/10.1111/j.1749-6632.1998.tb06882.x.

161. Mathur, A.; Gorden, P.; Libutti, S.K. Insulinoma. Surg. Clin. N. Am. 2009, 89, 1105–1121. https://doi.org/10.1016/j.suc.2009.06.009.

162. Gallwitz, B.; Schmidt, W.E.; Schwarzhoff, R.; Creutzfeldt, W. Galanin: Structural Requirements for Binding and Signal Transduction in RINm5F Insulinoma Cells. Biochem. Biophys. Res. Commun. 1990, 172, 268–275. https://doi.org/10.1016/S0006-291X(05)80204-9.

163. Fridoll, T.; Ahren, B. Dual Action of the Neuropeptide Galanin on the Cytoplasmic Free Calcium Concentration in RIN M5F Cells. Biochem. Biophys. Res. Commun. 1993, 191, 1224–1229. https://doi.org/10.1006/bbrc.1993.1348.

164. Maris, J.M. Recent Advances in Neuroblastoma. N. Engl. J. Med. 2010, 362, 2202–2211. https://doi.org/10.1056/NEJMra10804577.

165. London, W.B.; Castleberry, R.P.; Matthey, K.K.; Look, A.T.; Seeger, R.C.; Shimada, H.; Thorner, P.; Brodeur, G.; Maris, J.M.; Reynolds, C.P.; et al. Evidence for an Age Cutoff Greater Than 365 Days for Neuroblastoma Risk Group Stratification in the Children’s Oncology Group. J. Clin. Oncol. 2005, 23, 6459–6465. https://doi.org/10.1200/JCO.2005.05.571.
166. US Department of Health and Human Services; National Cancer Institute. Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975–1995; National Cancer Institute: Bethesda, MD, USA, 1999.

167. Hyde, J.F.; Keller, B.K. Galanin secretion from Anterior Pituitary Cells in Vitro Is Regulated by Dopamine, Somatostatin, and Thyrrotropin-Releasing Hormone. Endocrinology 1991, 128, 917–922. https://doi.org/10.1210/endo-128-2-917.

168. Pirolo, G.G.; Pietranera, L.; Grillo, C.A.; De Nicola, A.F. Gender Differences in the Expression of Galanin and Vasoactive Intestinal Peptide in Oestrogen-Induced Prolactinomas of Fischer 344 Rats. J. Neuroendocrinol. 2004, 16, 64–71. https://doi.org/10.1111/j.1365-2826.2004.01130.x.

169. Cai, A.; Hayes, J.D.; Patel, N.; Hyde, J.F. Targeted Overexpression of Galanin in Lactotrophs of Transgenic Mice Induces Hyperprolactinemia and Pituitary Hyperplasia. Endocrinology 1999, 140, 10.

170. Schneider, B.J.; Kalemkerian, G.P. Personalized Therapy of Small Cell Lung Cancer. In Lung Cancer and Personalized Medicine: Novel Therapies and Clinical Management; Ahmad, A., Gadgeel, S.M., Eds.; Advances in Experimental Medicine and Biology; Springer International Publishing: Cham, Switzerland, 2016; Volume 890, pp. 149–174, ISBN 978-3-319-24931-5.

171. Torre, L.A.; Siegel, R.L.; Jemal, A. Lung Cancer Statistics. In Lung Cancer and Personalized Medicine; Ahmad, A., Gadgeel, S., Eds.; Advances in Experimental Medicine and Biology; Springer International Publishing: Cham, Switzerland, 2016; Volume 893, pp. 1–19, ISBN 978-3-319-24221-7.

172. Kalemkerian, G.P.; Akerley, W.; Bogner, P.; Borghaei, H.; Chow, L.Q.; Downey, R.J.; Gandhi, L.; Ganti, A.K.P.; Govindan, R.; Grecula, J.C.; et al. Small Cell Lung Cancer. J. Natl. Compr. Canc. Netw. 2013, 11, 78–98. https://doi.org/10.6004/jnccn.2013.0011.

173. Roelle, S.; Grosse, R.; Buech, T.; Chubanov, V.; Gudermann, T. Essential Role of Pyk2 and Src Kinase Activation in Neuropeptide-Induced Proliferation of Small Cell Lung Cancer Cells. Oncogene 2008, 27, 1737–1748. https://doi.org/10.1038/sj.onc.1210819.

174. Kozłowska, A.; Kozera, P.; Majewski, M.; Godlewski, J. Co-Expression of Caspase-3 or Caspase-8 with Galanin in the Human Stomach Section Affected by Carcinoma. Apoptosis 2018, 23, 484–491. https://doi.org/10.1007/s10495-018-1470-y.

175. Kozłowska, A.; Godlewski, J.; Majewski, M. Distribution Patterns of Cocaine- and Amphetamine-Regulated Transcript- and/or Galanin-Containing Neurons and Nerve Fibers Located in the Human Stomach Wall Affected by Tumor. Int. J. Mol. Sci. 2018, 19, 3357. https://doi.org/10.3390/ijms19113357.

176. Zhang, L.; Fang, P.; Chai, C.; Shao, L.; Mao, H.; Qiao, D.; Kong, G.; Dong, X.; Shi, M.; Zhang, Z.; et al. Galanin Expression Is Down-Regulated in Patients with Gastric Cancer. J. Int. Med. Res. 2019, 47, 1241–1249. https://doi.org/10.1177/0300060518193882.

177. Yoon, D.; Bae, K.; Lee, M.-K.; Kim, J.H.; Yoon, K.-A. Galanin Is an Epigenetically Silenced Tumor Suppressor Gene in Gastric Cancer Cells. PLoS ONE 2013, 8, e6193275. https://doi.org/10.1371/journal.pone.0193275.

178. El-Salhy, M. Effects of Ooctreotide, Galanin and Serotonin on a Human Gastric Cancer Cell Line. Oncol. Rep. 2005, 13, 537. https://doi.org/10.3892/or.13.5.787.

179. Kwiatkowski, P.; Godlewski, J.; Kieżun, J.; Kraziański, B.E.; Kmiec, Z. Colorectal Cancer Patients Exhibit Increased Levels of Galanin in Serum and Colon Tissues. Oncol. Lett. 2016, 12, 3323–3329. https://doi.org/10.3892/ol.2016.5037.

180. Nagayoshi, K.; Ueki, T.; Tashiro, K.; Mizuuchi, Y.; Manabe, T.; Araki, H.; Oda, Y.; Kuhara, S.; Tanaka, M. Galanin Plays an Important Role in Cancer Invasiveness and Is Associated with Poor Prognosis in Stage II Colorectal Cancer. Oncol. Rep. 2015, 33, 539–546. https://doi.org/10.3892/or.2014.3660.

181. Kim, J.C.; Lee, H.C.; Cho, D.H.; Choi, E.Y.; Cho, Y.K.; Ha, Y.J.; Choi, P.W.; Roh, S.A.; Kim, S.Y.; Kim, Y.S. Genome-Wide Identification of Possible Methylation Markers Chemosensitive to Targeted Regimens in Colorectal Cancers. J. Cancer Res. Clin. Oncol. 2011, 137, 1571–1580. https://doi.org/10.1007/s00432-011-1036-7.

182. Ishi, H.; Tatsuta, M.; Baba, M.; Uehara, H.; Yano, H.; Nakaizumi, A. Chemoprevention by Galanin against Colon Carcinogenesis Induced by Azoxymethane in Wistar Rats. Int. J. Cancer 1995, 61, 861–863. https://doi.org/10.1002/ijc.2910610619.

183. Talaat, I.M.; Yakout, N.M.; Soliman, A.S.A.; Venkatachalam, T.; Vinod, A.; Eldohaj, L.; Nair, V.; Hareedy, A.; Kandil, A.; Abdel-Rahman, W.M.; et al. Evaluation of Galanin Expression in Colorectal Cancer: An Immunohistochemical and Transcriptomic Study. Front. Oncol. 2022, 12, 877147. https://doi.org/10.3389/fonc.2022.877147.

184. Kiezun, J.; Godlewski, J.; Kraziański, B.E.; Koziełec, Z.; Kmiec, Z. Galanin Receptors (GalR1, GalR2, and GalR3) Expression in Colorectal Cancer Tissue and Correlations to the Overall Survival and Poor Prognosis of CRC Patients. Int. J. Mol. Sci. 2022, 23, 3735. https://doi.org/10.3390/ijms23073735.

185. Kozłowska, A.; Kwiatkowski, P.; Oponowicz, A.; Majewski, M.; Kmiec, Z.; Godlewski, J. Myenteric Plexuses Atrophy in the Vicinity of Colorectal Cancer Tissue Is Not Caused by Apoptosis or Necrosis. Folia Histochem. Cytobiol. 2016, 54, 99–107. https://doi.org/10.5630/FHCc.2016.0012.

186. Jung, K.; Narwal, M.; Min, S.Y.; Keam, B.; Kang, H. Squamous Cell Carcinoma of Head and Neck: What Internists Should Know. Korean J. Intern. Med. 2020, 35, 1031–1044. https://doi.org/10.3940/kjim.2020.078.

187. Liebig, C.; Ayala, G.; Wilks, J.A.; Berger, D.H.; Albo, D. Perineural Invasion in Cancer: A Review of the Literature. Cancer 2009, 115, 3379–3391. https://doi.org/10.1002/cncr.24396.

188. Ayala, G.E.; Dai, H.; Powell, M.; Li, R.; Ding, Y.; Wheeler, T.M.; Shine, D.; Kadmon, D.; Thompson, T.; Miles, B.J.; et al. Cancer-Related Axonogenesis and Neurogenesis in Prostate Cancer. Clin. Cancer Res. 2008, 14, 7593–7603. https://doi.org/10.1158/1078-0432.CCR-08-1164.

189. Marchesi, F.; Piemonti, L.; Mantovani, A.; Allavena, P. Molecular Mechanisms of Perineural Invasion, a Forgotten Pathway of Dispersion and Metastasis. Cytokine Growth Factor Rev. 2010, 21, 77–82. https://doi.org/10.1016/j.cytogfr.2009.11.001.
211. Tjomsland, V.; El-Salhy, M. Effects of Single, Double or Triple Combinations of Octreotide, Galanin and Serotonin on a Human Pancreatic Cancer Cell Line. Histol. Histopathol. 2005, 537–541. https://doi.org/10.14670/HH-20.537.

212. Iishi, H.; Tatsuta, M.; Baba, M.; Yano, H.; Iseki, K.; Uehara, H.; Nakaizumi, A. Inhibition by Galanin of Experimental Carcinogenesis Induced by Azaserine in Rat Pancreas. Int. J. Cancer 1998, 75, 396–399. https://doi.org/10.1002/(SICI)1097-0215(19980130)75:3<396::AID-IJC12>3.0.CO;2-T.

213. Lang, R.; Berger, A.; Hermann, A.; Kofler, B. Biphasic Response to Human Galanin of Extracellular Acidification in Human Bowes Melanoma Cells. Eur. J. Pharmacol. 2001, 423, 135–141. https://doi.org/10.1016/S0014-2999(01)01359-9.

214. Dunne, M.J.; Bullett, M.J.; Li, G.D.; Wollheim, C.B.; Petersen, O.H. Galanin Activates Nucleotide-Dependent K+ Channels in Insulin-Secreting Cells via a Pertussis Toxin-Sensitive G-Protein. EMBO J. 1989, 8, 413–420. https://doi.org/10.1002/j.1460-2075.1989.tb0392.x.

215. Cormont, M.; Le Marchand-Brustel, Y.; Van Obberghen, E.; Spiegel, A.M.; Sharp, G.W. Identification of G Protein Alpha-Subunits in RINm5F Cells and Their Selective Interaction with Galanin Receptor. Diabetes 1991, 40, 1170–1176. https://doi.org/10.2337/diabetes.40.9.1170.

216. Zhao, M.; Wang, Y.; Liu, Y.; Zhang, W.; Liu, Y.; Yang, X.; Cao, Y.; Wang, S. C7 Peptide Inhibits Hepatocellular Carcinoma Metastasis by Targeting the HGF/c-Met Signaling Pathway. Cancer Biol. Ther. 2019, 20, 1430–1442. https://doi.org/10.1080/15384047.2019.1647051.

217. Koller, A.; Rid, R.; Beyreis, M.; Bianchini, R.; Holub, B.S.; Lang, A.; Locker, F.; Brodowicz, B.; Velickovic, O.; Jakab, M.; et al. In Vitro Toxicity of the Galanin Receptor 3 Antagonist SNAP 37889. Neuroptides 2016, 56, 83–88. https://doi.org/10.1016/j.nepep.2015.12.003.

218. El-Salhy, M. Effects of Triple Therapy with Octreotide, Galanin and Serotonin on a Human Colon Cancer Cell Line Implanted in Mice: Comparison between Different Routes of Administration. Histol. Histopathol. 2005, 20, 19–25. https://doi.org/10.14670/HH-20.19.

219. El-Salhy, M. Comparison between Triple Therapy with Octreotide, Galanin and Serotonin, 5-Fluorouracil/Leucovorin, and Sequential Treatment with Both, on Human Colon Cancer. Oncol. Rep. 2004, 11, 1161161168. https://doi.org/10.3892/or.11.6.1161.

220. El-Salhy, M.; Dennerqvist, V. Effects of Triple Therapy with Octreotide, Galanin and Serotonin on Liver Metastasis of Human Colon Cancer in Xenografts. Oncol. Rep. 2004, 6, 1177–1182. https://doi.org/10.3892/or.11.6.1177.

221. Sitohy, B.; El-Salhy, M. A Comparison between Double and Triple Therapies of Octreotide, Galanin and Serotonin on a Rat Colon Carcinoma. Histol. Histopathol. 2003, 1, 103–110. https://doi.org/10.14670/HH-18.103.

222. El-Salhy, M.; Sitohy, B.; Norrgård, Ö. Triple Therapy with Octreotide, Galanin, and Serotonin Reduces the Size and Blood Vessel Density and Increases Apoptosis of a Rat Colon Carcinoma. Regul. Pept. 2003, 111, 145–152. https://doi.org/10.1016/S0167-0115(02)00280-X.

223. El-Salhy, M. Triple Treatment with Octreotide, Galanin and Serotonin Is a Promising Therapy for Colorectal Cancer. Curr. Pharm. Des. 2005, 11, 2107–2117. https://doi.org/10.2174/1381612054063800.

224. El-Salhy, M.; Starefeldt, A. Direct Effects of Octreotide, Galanin and Serotonin on Human Colon Cancer Cells. Oncol. Rep. 2003, 6, 1723–1728. https://doi.org/10.3892/or.10.6.1723.

225. Tjomsland, V.; El-Salhy, M. Anti-Tumour Effects of Triple Therapy with Octreotide, Galanin and Serotonin in Comparison with Those of 5-Fluorouracil/Leukovorin on Human Colon Cancer. Int. J. Oncol. 2005, 2, 427–432.https://doi.org/10.3892/ijo.27.2.427.

226. El-Salhy, M.; Hilding, L.; Royson, H.; Tjomsland, V. Comparison between Triple Therapy with Octreotide, Galanin and Serotonin vs. Irinotecan or Oxaliplatin in Combination with 5-Fluorouracil/Leukovorin in Human Colon Cancer. Int. J. Oncol. 2005, 27, 1.

227. Muñoz, M.; Berger, M.; Rosso, M.; Gonzalez-Ortega, A.; Carranza, A.; Coveñas, R. Antitumor Activity of Neurokinin-1 Receptor Antagonists in MG-63 Human Osteosarcoma Xenografts. Int. J. Oncol. 2014, 44, 137–146. https://doi.org/10.3892/ijo.2013.2164.

228. Muñoz, M.; Gonzalez-Ortega, A.; Salinas-Martín, M.V.; Carranza, A.; García-Recio, S.; Almendro, V.; Coveñas, R. The Neurokinin-1 Receptor Antagonist Aprepitant Is a Promising Candidate for the Treatment of Breast Cancer. Int. J. Oncol. 2014, 45, 1658–1672. https://doi.org/10.3892/ijo.2014.2565.