Tumor progression: the neuronal input

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Abstract: One of the challenges of cancer is its heterogeneity and rapid capacity to adapt. Notwithstanding significant progress in the last decades in genomics and precision medicine, new molecular targets and therapies appear highly necessary. One way to approach this complex problem is to consider cancer in the context of its cellular and molecular microenvironment, which includes nerves. The peripheral nerves, the topic of this review, modulate the biological behavior of the cancer cells and influence tumor progression, including the events related to the metastatic spread of the disease. This mechanism involves the release of neurotransmitters directly into the microenvironment and the activation of the corresponding membrane receptors. While this fact appears to complicate further the molecular landscape of cancer, the neurotransmitters are highly investigated molecules, and often are already targeted by well-developed drugs, a fact that can help finding new therapies at a fraction of the cost and time needed for new medicines (through the so-called drug repurposing). Moreover, the modulation of tumor progression by neurotransmitters can probably explain the long-recognized effects of psychological factors on the burden of cancer. We begin with an introduction on the tumor-nervous-connections and a description of the perineural invasion and neoneurogenesis, the two most important interaction patterns of cancer and nerves. Next, we discuss the most recent data that unequivocally demonstrate the necessity of the nervous system for tumor onset and growth. We introduce the molecular players of the tumor-nervous-connections by citing the role of three main families: neurotropic factors, axon guidance molecules, and neurotransmitters. Finally, we review the role the most important neurotransmitters in tumor biology and we conclude by analyzing the significance of the presented data for cancer therapy, with all the potential advantages and caveats.

Keywords: Tumor-nervous connections (TNCs); tumor microenvironment; neurotransmitters; drug repurposing

Introduction

Nerves along with blood vessels, immune cells, fibroblasts, and the extracellular matrix are part of the tumor microenvironment, an established contributor to tumor progression.

A discussion of the nervous system in the tumor environment must at least cite the vascular biology field for two reasons. The first is that blood vessels and nerves have strong evolutionary relations, display both anatomical and molecular similarities, and the fields of vascular biology and neurobiology are linked at different levels both in basic and translational research. The second is that the formation of new blood vessels in tumors, or angiogenesis, is considered one of the hallmarks of cancer (1). The so-called neurovascular connections have been exhaustively reviewed from different angles in the recent years, for example in by Quaegebeur and colleagues (2), and will not be treated further here.

This review is dedicated to the crosstalk between tumors and nerves, which we generally call tumor-nervous connections (TNCs), and in particular on the role that the soluble signals produced by neurons, or neurotransmitters, have in cancer. The aim is to attract the interest of both basic researchers and clinicians, because neurotransmitters have long established functions in the nervous system and are potential oncological targets of already used drugs (a process called drug repurposing).

Tumor nervous connections: anatomical aspects

As any two cell systems within an organ, tumor cells and nerves make up a complex array of cellular and molecular interactions that can be approached from a broad spectrum of perspectives, both clinical and experimental.

For historical reasons the discussion on the TNCs starts by citing two main patterns of physical/humoral interaction: (I) the invasion of nerves by cancer cells, called perineural invasion, and (II) the growth of new axons into the tumor tissue, in a way similar to angiogenesis, called neoneurogenesis. It should be mentioned that, in a reciprocal manner, numerous types of cancers (e.g., breast, colorectal, kidney, lung and melanoma) frequently give rise to intracranial metastases, infiltrating the central nervous tissues (3). This type of TNCs, which obviously represent a unique clinical challenge by themselves, will not be treated further here.

Perineural invasion is a process that has been observed for centuries and is routinely evaluated by the pathologist (4,5), and occurs as peripheral cancers infiltrate the surrounding nerve arborescence, wrapping around these structures. Virtually all peripheral cancer types have been observed to contract interactions with neuronal structures at least at advanced stages of the disease, and in particular cancer of the bladder (6), prostate (7), pancreas (8), colon (9) lung (10), head and neck (11) and bile duct (12). The process has been mostly studied for prostate, head and neck, and pancreatic carcinomas, and the invasion takes place by the destruction of the perineurium and invasion via the perforating vessels of the perineurium (13).

Neoneurogenesis is the stimulation of axon growth and tumor innervation, by producing soluble or cell-to-cell signaling components (14). It is well known that peripheral nerve cells, in contrast to cells of the central nervous system, can regenerate during wound healing (15) and the reconstitution of lost body parts in animals. This last process and its common ground with nerve outgrowth in cancer is of high biological interest and has been reviewed recently (16). Because of its nature and similarities to angiogenesis, neoneurogenesis has offered the most fertile ground for the discovery of molecules and pharmacological targets that regulate the TNCs. Nevertheless, PNI and neoneurogenesis share, at least at certain stages, cellular and molecular events, and will be treated both as TNCs from here on, unless differently stated. Along with the axonal ingrowth into the tumor as neoneurogenesis, the possibility of neogenesis of neuronal cells should be mentioned. Indeed, mesenchymal stem cells (MSC), a bone-marrow derived population usually recruited by tumor cells, can differentiate into neurons under the right conditions (17).

TNCs: more than casual interactions

Nerves invading cancers were initially thought as "easy paths" that allow the spread of tumors cells. This simplified version of the facts, as a recurring event in science, has now been dismissed. We can now definitely state that the nervous system is functionally relevant, modulating a complex network of interactions that promote tumor progression. Moreover, the interaction between the nervous system and the tumors seems to be reciprocal, as cancer cells also produce factors that promote nervous system development.

Throughout the rest of this review, we will list a long series of relevant descriptive, epidemiological, as well as *in vitro* and *in vivo* studies that sustain the existence of functional TNCs. However, we should begin by describing the first proofs, which have been accumulating only in the last few years, that the nervous system is, in fact, indispensable for tumor onset and growth. These proofs consist of the demonstration that ablation of different portions of the Peripheral Nervous System prevents cancer development in rodent models of prostate (18) and gastric (19) cancer, basal cell carcinoma (BCC) (20), pancreatic ductal adenocarcinoma (21), melanoma (22), and fibrosarcoma (23). The most compelling finding across all the reports demonstrated that adrenergic fibers from the Sympathetic Nervous System are involved in the initial phases of tumor progression, while tumor-infiltrating cholinergic fibers from the Parasympathetic Nervous System are fundamental in tumor cell invasion, and migration (18). Sympathetic and parasympathetic nerves were found to be necessary throughout all phases of prostate cancer development in the mouse. Sympathectomy or genetic deletion of β-adrenergic receptors (βAR)

prevented the early stages of tumor development, while tumors were infiltrated by parasympathetic cholinergic fibers that promoted cancer dissemination. Catecholamines and acetylcholine (see below) secreted by sympathetic and parasympathetic nerves, were responsible for the stimulation of prostate tumor growth and metastasis, respectively. Notably, catecholamines and acetylcholine, secreted by nerves, targeted stromal cells expressing βAR and muscarinic receptors. This observation reinforces the concept that microenvironment impacts tumor biology. Comparable results demonstrated that denervation suppresses gastric tumorigenesis (19). In a mouse model of gastric cancer, pharmacologic or surgical denervation of the stomach, by local injection of neurotoxic agents or vagotomy, strongly reduced tumor progression. Importantly, denervation also enhanced the therapeutic effect of systemic chemotherapy.

While a role for sensory nerves could be hypothesized from these last results (the vagus nerve contains both sensory and autonomic axons), a recent study proved this concept (21). In a mouse model of pancreatic ductal adenocarcinoma, sensory denervation of the pancreas was achieved by specific ablation of sensory neuron by capsaicin. In this genetically engineered mouse model, a Kras gain-of-function mutation and deletion of p53 is targeted to the pancreas, resulting in tumors in >95% of mice within 4 months. Capsaicin-mediated denervation of the pancreas was found to correlate with increased survival; mice with the greatest sensory neuron loss also had little or no pancreatic disease detectable up to 19 months of age.

Tumor nervous connections: an intricate network of signaling

Even though the actual implication of the nervous system in cancer progression has begun to be elucidated in the past few years by studies such as the above, we are only starting to understand the mechanisms of TNCs. A better understanding of how the reciprocal systems operate will provide new targets for therapeutic intervention against cancer progression.

Which molecules or molecular families mediate the TNCs? On a broad perspective, the molecular cues involved in TNCs can be separated into three families: (I) neurotropic factors e.g., NGF, BDNF, FGF or IGF-II; (II) axon guidance molecules, e.g., netrins and their receptors, Eph/Ephrin, Plexins/Semaphorins, Slit-Robo and (III) neurotransmitters/neuropeptides. The first two families,

and especially the axon guidance molecules, have been the object of enormous interest in the last years, and have been exhaustively reviewed (24-28).

This review will instead concentrate on the neurotransmitter family. Neurotransmitters are some of the oldest known cellular communicators (29) and are "historically" defined as molecules that (I) are present in the pre-synaptic neuron; (II) have specific receptors on the post-synaptic neuron; (III) are secreted in a Ca^{2+}-dependent manner, following pre- synaptic depolarization. Because of these specific properties, even though these molecules can be produced outside the nervous system, including cancer cells themselves, we refer to them as the "neuronal input" to tumor progression.

Neurotransmitters are evolutionarily old and secreted by a very conserved machinery. In this context, it is worth mentioning the widespread expression of neuronal markers (especially members of the synaptic machinery, such as synaptophysin) in non-neural cancer tissues. This applies in particular to small cell lung carcinoma, a particularly aggressive form of lung cancer, which shows several features of neuronal cells (30) but also to some types of prostate and colon cancers (31). Besides representing a fascinating biological phenomenon, this could reflect the selection of cancer cells presenting a particular phenotype that allows them to interact with the nervous system. Moreover, this sustains the existence of the so-called neuro-neoplastic synapse, which is not an anatomically discrete structure but a recently proposed functional entity (14).

Neurotransmitters: the neuronal input to tumor progression

Tumor innervations can release neurotransmitters directly into the neuro-neoplastic synapse and interact with cancer cells to influence tumor development and progression (32).

The neuroendocrine system, and in particular the autonomic nervous system (ANS) branch as the regulatory organ in the body, governs the regulation of tumor cell functions and consequently cancer progression. This direct influence may also contribute to the molecular explanation for the long-standing theory of an effect of psychosocial factors on the course of a cancer disease, in addition to the downregulation of the immune system by the neuroendocrine system (33).

In the following part, we will describe the role of the main members of neurotransmitters family in tumor progression, and we will analyze the potential therapeutical

Table 1 A schematic presentation of the neurotransmitters treated in this review, with their receptors and examples of targeting drugs with references

Class	Family	Member	Signaling receptor	Example of targeting drug	Ref.
Neuropeptides	Neuropeptide Y	Neuropeptide Y	Neuropeptide Y receptors NPY1R, NPY2R, PPYR1, NPY5R	MK-0557, CGP71683A	(34,35)
	Tachykinins	Substance P	NK1 NNK2 NNK3	L-733,060, L-732,138 aprepitant, fosaprepitant dimeglumine	(36,37)
Neurotransmitters	Monoamine (Phe/Tyr)	Norepinephrine (noradrenaline) Epinephrine (adrenaline)	α–β-adrenergic receptors	Propranolol, atenolol	(38-42)
		Dopamine	Dopamine 1–5 (D1–5) receptors	Butaclamol, apomorphine flupenthixol, thioridazine aripiprazole	(43-46)
	Monoamine (Trp)	Serotonin (5-hydroxytryptamine)	5-HT1-7 receptors	Various SSRI, paliperidone, pimozide, risperidone	(47-52)
	Acetylcholine	Acetylcholine	Muscarinic acetylcholine receptor, Nicotinic acetylcholine receptor	Darifenacin, 4-DAMP, p-F-HHSiD	(53-56)
	Amino acids	Glutamate	Metabotropic glutamate receptor, NMDA receptor, Kainate receptor, AMPA receptor	Riluzole and BAY 36-7620, quinoxalinediones, willardiines	(57,58)
		Gamma-aminobutyric acid	GABAB receptor, GABAA, GABAA-ρ receptor	Baclofen, nembutal	(59-62)

implications whenever it is possible. *Table 1* is meant to provide a quick reference to the neurotransmitterslisted below, with their receptors and examples of targeting drugs.

The activity of neurotransmitters, as pointed out in the very comprehensive review by Mancino and colleagues (24), is often subtle and multifaceted. Nevertheless they do modulate cancer growth and affect the response to chemotherapy. These mediators can be divided into two classes: (I) neuropeptides, consisting of bombesin, substance P (SP), cholecystokinin (CCK), gastrin, vasoactive intestinal peptide (VIP), bradykinin, neurotensin, calcitonin gene-related peptide (CGRP), adrenomedullin, and the opioid peptides such as dynorphin, enkephalin, and endorphin, and (II) classic, which consists of epinephrine, norepinephrine, dopamine, serotonin, histamine, aspartate, glutamate, glycine, gamma-aminobutyric acid (GABA), and acetylcholine.

Neuropeptides

Neuropeptides are short polypeptides synthesized by several cell types that function both as neurotransmitters or neuromodulators in the nervous system or peripherally, including cancer, as paracrine and endocrine factors to regulate diverse physiopathologic processes (63). While several neuropeptides have been implicated in tumor progression, for space constraints, we will concentrate on neuropeptide Y (NPY), and SP, a member of the tachikinins family.

NPY

NPY is a sympathetic 36 aminoacid neurotransmitter with pleiotropic actions that is widely expressed in the CNS, peripheral nervous system and other organs. As such it modulates many physiological functions ranging from stress responses, food intake, reproduction, circadian rhythms, anxiety and depression and pain processing (64). It exerts its principal activities through neuropeptide receptors 1, 2 and 5 and in the context of cancer (65), much information has been learned from two pediatric diseases with high endogenous NPY production—neuroblastoma and Ewing sarcoma—that have become models to investigate the role of NPY in tumor growth and progression (66). However, NPY and its receptor have been found to be expressed in cells and vasculatures from a variety of tumors (65) where they exert complex effects. It is generally accepted that NPY is an angiogenic factor, which strongly induces the endothelial cell proliferation, differentiation and migration, and promotes vascularization (66). Aside from being

synthesized in tumor cells, NPY is also released systemically from sympathetic neurons (67). Thus, the growth-promoting actions of NPY may also be relevant to tumor types that only express its receptors. Importantly, systemic release of NPY may be triggered by common conditions in cancer patients, such as chronic psychological stress or local hypoxia (67), and in this context the synergistic interactions of NPY with the adrenergic system are particularly attractive (68).

All these findings make NPY a potential target for cancer diagnosis and therapy. A variety of potent and specific NPY receptor antagonists is available, and many of them have been used in animal models and clinical trials. While there are no significant reported side effects associated with the administration of NPY receptor antagonists in animals and people (34), the potential anorexigenic effects of NPY receptor blockage in cancer patients would have to be carefully monitored. Moreover, the use of a brain-impenetrable receptor antagonist could prevent such adverse central effects, while preserving peripheral activities on tumor growth and metastasis (66).

SP

SP is a peptide of 11 aminoacids that belongs to the tachykinin family and is widely expressed in the central, peripheral and enteric nervous system of vertebrates. SP and its high-affinity receptor (the neurokinin-1 receptor, NK1) have been demonstrated to be involved in a many physiological and pathophysiological processes such as respiration, thermoregulation, cardiovascular control, pain transmission, immunomodulation, depression, inflammation and oncogenesis (69). Both SP and NK1 are overexpressed in several cancers including ovarian, breast, prostate, pancreas and thyroid (70). It has been indicated that SP exerts anti-cancer function through T cells and NK cells-inducing immune reactions (71). However, in breast cancer, SP and its receptors are implicated in oncogenic transformation, and bone marrow metastasis formation (72) The antagonists drugs of the NK1 receptor can block the mitogenic effects of SP and produce a pro-apoptotic action both *in vitro* and *in vivo*. Hence neurokinin-1 receptor is a good candidate therapeutic target for cancer therapy and neurokinin-1 receptor antagonists may be useful for cancer treatment (73).

Classic neurotransmitters

Epinephrine and norepinephrine

Epinephrine and norepinephrine are catecholamines derived from the amino acid tyrosine that are secreted mainly by the adrenal medulla and the sympathetic nerves, respectively. These mediators can directly modulate several mechanisms related to tumor progressions, such as cell proliferation, survival, and migration. In addition to their effect on tumor cells, catecholamines are also known to stimulate endothelial cells, immune cells, and fibroblasts and therefore have a broad impact on tumor progression

Most tumor promoting activities of catecholamines depend on the activation of the βAR. In ovarian cancer cells, epinephrine and norepinephrine can modulate cell proliferation, survival, and tumor angiogenesis through the activation of the βAR–cyclic AMP–protein kinase A pathway (74). βAR activation promotes cell migration and metastasis formation in the breast (75), lung (76), colon (77) pancreas (78) and facilitates the angiogenic switch and the secretion of VEGF and IL-6 by various cancer cell lines (79). βAR modulate carcinogenesis induced by a tobacco-specific nitrosamine in the lungs of hamsters (80).

From the clinical point of view, it is interesting to consider the potential role of catecholamines in stress-induced cancer development. The circulation levels of epinephrine and norepinephrine increases during psychological stress and depression favoring the initiation of cancer (39), while β-blockers have preventive effects in the development of cancer in different models, for example ovarian cancer and pancreatic ductal adenocarcinoma in mice (74,81). Likewise, surgical stress also promotes ovarian cancer growth in a mouse model while a β-blocker abrogates this effect (82).

Several population-based studies show that patients taking adrenergic antagonists for the treatment of cardiovascular conditions may have significantly lower rates of cancer, including melanoma, lung, breast, prostate, and colorectal cancer [reviewed in (40)]. Nevertheless, the data are far from conclusive, probably because of many confounding factors, such as comorbidities and interaction with other drugs (especially aspirin), to name a few. More corroborative retrospective studies are needed (83).

Dopamine

Dopamine is a catecholamines family members and a precursor of norepinephrine and epinephrine synthesis. It is also an important neurotransmitter in the brain (84) In peripheral tissues there are at least three primary sources of dopamine. These include sympathetic neurons, neuroendocrine cells and adrenal medulla (84).

Dopamine release causes a "negative feedback" towards stress via two types of receptors (D_1-like or D_2-like receptor families) rather than promoting the 'fight or flight' response of norepinephrine or epinephrine (84). Growing evidence suggests that dopamine or dopamine receptor agonists act in synergy with anticancer drugs [like doxorubicin or 5-fluorouracil (5-FU)] (85) and exert an inhibitory effect on cancer growth, for example in breast, colon, gastric cancers and sarcoma (86-89). Nonetheless, dopamine itself does not affect cancer growth. The main anti-cancer effect is associated with the inhibitory effect on proliferation and migration of tumor endothelial cells by inhibiting the phosphorylation of VEGF receptor-2 and preventing the activation of mitogen-activated protein kinase (MAPK) (86-89). These effects are mainly mediated by dopamine receptor 2 which is expressed in the tumor endothelial cells. Despite the reported anti-tumor effects of dopamine, there is no agreement on the role of the dopaminergic system in cancer development, and more epidemiological data is needed to reach a consensus.

Serotonin

Serotonin (5-hydroxytryptamine or 5-HT) is a monoamine neurotransmitter, that plays cognitive and behavioral functions in humans, with numerous significant peripheral roles in the gut, vasculature and immune system (90). 5-HT is a potent mitogen for many types of tumor cells such as pancreas, lung, bladder, colon and prostate (48,50,91). The multifaceted roles of 5-HT are mediated by 5-HT receptors (5-HT_{1-7}) widely distributed in the human body. It has been shown that 5-HT_{1A} and 5-HT_{1B} receptors are overexpressed in prostate cancer tissues, especially in high-grade tumor cells (49). Antagonists of 5-HT_{1A} and 5-HT_{1B} inhibit the 5-HT effect to different extents and induce apoptosis (48-50). Therapeutic approaches targeting 5-HT are aimed in two directions. The first is to analyze the role of established drugs (drug repurposing): three drugs in wide use to treat mental disorders (paliperidone, pimozide, and risperidone) that are established inhibitors at serotonin receptor 7, are under investigation in an adjunctive role in glioblastoma treatment (52,92). Furthermore, common therapies for stress and anxiety disorders, such as benzodiazepines and SSRIs, also inhibit the proliferation and migration of cancer cells *in vitro* (52,93). The second approach is the search for new modulators of serotonin signaling. For example, a copper-containing respiratory pigment found in a mollusk [keyhole limpet hemocyanin (KLH)] that targets the serotonergic signaling pathway, inhibits cellular proliferation in various human cell cancer lines (94).

Acetylcholine

Acetylcholine is the neurotransmitter of the parasympathetic system, and its biological activity is mediated by nicotinic (n-AchR) and muscarinic (m-AchR) acetylcholine receptors in the central and peripheral nervous system (95,96). AchR are strongly related to cancer because the chemicals contained in cigarette smoke bind these receptors. Cigarette smoking is known to cause cancers of the lung, mouth, pharynx, larynx, esophagus, pancreas, kidney, and bladder (97). Nicotine is the most studied component in tobacco, and while it is not a carcinogen by itself, it promotes proliferation, tumor growth and invasion by binding to n-AChRs (98,99). A nicotine derivative, the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) binds to n-AchR with higher affinity than their natural ligand acetylcholine (99). In recent years, n-AChRs have been identified as important regulators of several other cancer types besides lung. In fact, activation of n-AChRs induces growth of mesothelioma and colon cancer cells (100). Moreover n-AChRs mediate angiogenesis through nicotine-induced prostaglandin E_2 activity, cyclooxigenase-2 (COX-2), and VEGF increase (101).

n-AChRs are also capable of regulating the synthesis and release of catecholamines from the adrenal medulla and sympathetic nervous endings. Hence, combined cigarette smoke exposure with stress stimulation could accelerate tumor growth more than smoking or stress alone (102). In parallel, nicotine stimulates colon cancer cells proliferation through epinephrine production and β-adrenergic activation, while β-blockers reverse this effect (103). A recent study reports that nicotine and NNK could not only elevate the level of norepinephrine and epinephrine but also simultaneously decrease the production of inhibitory neurotransmitter GABA in the pancreatic ductal adenocarcinoma. Conversely, GABA administration abolished the tumor promoting effect of nicotine (104). These studies demonstrate the importance of the crosstalk between neurotransmitters in tumor progression.

The m-AChRs, the other family of acetylcholine receptors, are expressed in most small cell lung carcinomas and many non-small cell lung carcinomas (105), while a growth-promoting effect of m-AChRs, mediated by transactivation of the EGFR/ERK pathway, has been demonstrated in colon cancer cells (106). Finally, activation of the MAP kinase by m-AChRs induces cell proliferation

and protein synthesis in human breast cancer cells (107). The potential therapeutic effects of both the n-AchR and m-AchR receptor antagonists are under investigation (99,108). Interestingly, a muscarinic antagonist currently used in the treatment of overactive bladder (e.g., darifenacin) can arrest tumor progression including small cell lung carcinoma (56).

Glutamate

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. Its functions are mediated by two classes of receptors: ionotropic and metabotropic. The ionotropic glutamate receptors are subdivided into three groups: AMPA, NMDA, and Kainate receptors. On the other hand, metabotropic (mGlu) receptors are G-protein coupled receptors (GPCRs) making up a large family divided into many functional subgroups. In addition to the well-established role of glutamate in the CNS, several reports indicate a function for glutamate and its receptors in peripheral tissues (109) and various cancer types (110): colorectal (111), glioma (112), gastric (113), oral squamous cell carcinoma (114), melanoma (115).

The expression of NMDAR could be used for prognostic purposes because in oral squamous cell carcinoma the up-regulation of NMDAR1 is well-correlated with the TN stage, tumor size, the presence of lymph node metastases and poorer survival (116).

The other receptor subtype (mGlu) is also involved in cancer. In colorectal carcinoma, overexpression of mGluR4 is associated with poor prognosis (117). In colon cancer cell lines, activation of mGluR4 appears to protect cells from 5-FU toxicity, whereas decreased mGluR4 expression or inactivation by antagonists leads to cell death (111). On the contrary, in medulloblastoma (118) the expression of mGluR4 has been shown to be inversely correlated with tumor aggressiveness and recurrence. Thus, the clinical significance of glutamate receptor expression may be different among the various tumors.

GABA

GABA is the main inhibitory neurotransmitter in the central nervous system. GABA functions through two classes of cellular receptors: (I) the ionotropic $GABA_A$ and $GABA_C$ receptors, which are oligomeric chloride channels and (II) the metabotropic $GABA_B$ receptor. The $GABA_B$ receptor is a seven-helix receptor and is related to the catecholaminergic receptors, which are involved in the regulation of tumor cell migration (see above). Different

reports indicate that GABA or GABAB agonists, reduce gastric carcinogenesis in rats (119), inhibit cell migration and metastasis formation of colon cancer in mice (62,120), and decrease the human hepatocellular carcinoma cell growth both *in vitro* and *in vivo* (121). In prostate cancer (122), however, GABA or $GABA_B$ agonist display opposite effects. Hence, it seems that a complicated connection between GABA/GABA receptors and cancer exist as GABA activation can result in different effects in a cancer-type or GABA receptor-type dependent manner.

Conclusions and future directions

We have written this review with two main aims: (I) provide the most compelling data proving the role of the nervous system in tumor biology and (II) analyze the role of a somewhat forgotten class of molecules mediating tumor progression, the neurotransmitters.

Regarding the first point, the last years have brought the most important data existing in the field. The fact that removal of nerves from the tumor microenvironment is sufficient to slow or halt disease progression is particularly remarkable because of the strength of the used models (mostly genetically modified mouse models), where the development of cancer in the absence of intervention is almost guaranteed. While these developments are exciting, the downside is that their translation into a treatment for cancer presents obstacles and conceptual caveats, as discussed by Saloman and co-authors (123). First of all the manner in which denervation was obtained either chemically or surgically in the models is unlikely to be of clinical use (124). Only with the ongoing development of tools that allow 'molecular surgery' on specific populations of neurons in the PNS (e.g., neurotoxins that are population specific), it may be possible to replicate these data. In any case, there is also a staging problem: the most effective impact on disease progression occurred early during the disease process or even before transformation occurred. Thus, at the time of diagnosis, denervation is unlikely to benefit the patient by limiting primary tumor growth, although, for some cancers, it could slow metastasis (e.g., for prostate or pancreatic cancer). One group for which denervation could be valuable would be patients who, because of family history, are at particular risk of developing a type of cancer (e.g., familial chronic pancreatitis).

Regarding the role of neurotransmitters in cancers, which have also been reviewed by Zhi and colleagues (100) there are few considerations to make. The first is the role

of neurotransmitters as factors released by the nervous system in consequence of the psychological pressure that the disease produces. Intriguingly, besides acting directly on the tumor and its environment, neurotransmitters can change the efficiency of the drugs used for the treatment of cancer (111). In the future, this parameter (e.g., the "level" of neurotransmission) should be taken into account while targeting cancer cells with chemotherapy, or even with precision drugs that block critical cellular signaling pathways. The approach should eventually bring to a profiling of the patient, similar to the one used for the hormone-responsiveness of certain cancer types. Finally, following this line of thought, because of the stress that experimental conditions produce, it might be essential to reevaluate the method of testing the drugs in animal models, as it has been proposed (125).

The second point is related to the possible role of psychoactive drugs in oncology (repurposing). The ongoing approaches in this direction have been described for each neurotransmitter when feasible. Drug repurposing has obvious advantages: the novel and most promising anti-cancer drugs have prohibitive cost (126), so considering established non-cancer drugs for anticancer activity provides an opportunity to rapidly advance therapeutic strategies into clinical trials, with benefits for the patient and economic advantages. On the flip side, although it is evident that the neurotransmitter /receptor system has a role in cancer biology, it also regulates normal physiological functions in the human body. The use of neurotransmitter modulators could produce significant side effects with clinical relevance in humans. Targeted delivery of drugs at tumor sites represents a very appealing direction in cancer therapy, but it obviously needs much more development (127). In our opinion it is likely that an advantageous exploitation of the TNCs in cancer therapy will come from a combination of biotechnological improvements, drug development, patient stratification and careful clinical evaluation of the risk to benefits ratio.

Finally, we should consider the (many) open questions that remain in this field, and the steps that our laboratory is taking to contribute data to the field. Starting again from the excellent synthesis made by Saloman and co-authors (123), the outstanding questions are: (I) is there a difference in the impact of different branches of the PNS for the development of various tumor types? For example, the microenvironment of colorectal cancer, the third most common cancer in both men and women (American Cancer Society, *Cancer Facts and figures*, *2017*. Atlanta,

American Cancer Society; 2017), is highly innervated by the autonomic fibers, and the presence PNI is associated with shortened patient survival in colon cancer (128). However, a direct proof impact of nerves on colorectal cancer progression has not been reported; (II) is inflammation, with its links to neurotransmission, only protumorigenic or does it contribute to anticancer conditions? (III) Does deregulated nerve–nerve communication contributes to cancer pathology? (IV) Do and how other cell types (e.g., endothelial, fibroblast or immune) modulate nerve–tumor interactions? (V) Are there molecules in nerve–tumor signaling that can be used as biomarkers? Can we improve prognosis by modulating the signaling of molecules identified as participants in nerve-tumor signaling?

The ongoing projects in our laboratory are aimed at answering the last question. We have focused for about a decade on the role in blood vessels of a new family of neuronal proteins, Neurexins and Neuroligins, which are transmembrane synaptic proteins that modulate pre-post synaptic communication. We have found that these proteins are expressed throughout the vascular system and modulate diverse functions, including vessel tone and morphogenesis, by affecting various forms of cell-to-cell communication (129-137). Thanks to the accumulated experience and reagents, more recently we concentrated on the role of Neuroligins in tumor progression, and we have found that these proteins are indeed expressed by cancers cells of different origin (unpublished data). Hence, in the context of TNCs and more in general in tumor-stroma relations, Neuroligins are uniquely expressed by cancer cells, nerves and endothelial cells. Our actual working hypotheses, that tackle the possible role of Neuroligin isoforms in neurotransmitter activity in the tumor microenvironment, are the following. The first is that one isoform of Neuroligin (NLGN2) that is involved in insulin granules exocytosis from pancreatic beta cells (138) and in the secretion of granules called Weibel Palade Bodies from endothelial cells (our own unpublished data), also controls the release of catecholamines from sympathetic nerves. This is because the β cell insulin secretory apparatus is remarkably similar to the presynaptic secretory machinery (139). A positive answer to this question would make NLGN2 a key protein for the modulation of catecholamines activity in cancer. The second working hypothesis is that another isoform of Neuroligin (NLGN1), that modulates the exposure of NMDAR on the surface of neurons (140), also modulates the exposure of the receptor on cancer cells, thus affecting their response to glutamate.

In conclusion, we realize that this review has posed many questions along with the presented data, and that the role of neurotransmitters in cancer is particularly complex and multifaceted. However, we think that if the crucial necessity of the nervous system for tumor growth is taken into account, this will attract the interest of scientist from multiple disciplines, thus opening new prospects for cancer treatment.

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Footnote

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References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
2. Quaegebeur A, Lange C, Carmeliet P. The neurovascular link in health and disease: molecular mechanisms and therapeutic implications. Neuron 2011;71:406-24.
3. Gavrilovic IT, Posner JB. Brain metastases: epidemiology and pathophysiology. J Neurooncol 2005;75:5-14.
4. Cruveilheir, J. Maladies des Nerfs Anatomie Pathologique du Corps Humain. 2nd ed. Bailliere JB. Paris, France, 1835.
5. Marchesi F, Piemonti L, Mantovani A, et al. Molecular mechanisms of perineural invasion, a forgotten pathway of dissemination and metastasis. Cytokine Growth Factor Rev 2010;21:77-82.
6. Seifert P, Benedic M, Effert P. Nerve fibers in tumors of the human urinary bladder. Virchows Archiv 2002;440:291-7.
7. Rodin AE, Larson DL, Roberts DK. Nature of the perineural space invaded by prostatic carcinoma. Cancer 1967;20:1772-9.
8. Hirai I, Kimura W, Ozawa K, et al. Perineural invasion in pancreatic cancer. Pancreas 2002;24:15-25.
9. Huh JW, Kim HR, Kim YJ. Prognostic value of perineural invasion in patients with stage II colorectal cancer. Ann Surg Oncol 2010;17:2066-72.
10. Yilmaz A, Duyar SS, Cakir E, et al. Clinical impact of visceral pleural, lymphovascular and perineural invasion in completely resected non-small cell lung cancer. Eur J Cardiothorac Surg 2011;40:664-70.
11. Scanlon CS, Banerjee R, Inglehart RC, et al. Galanin modulates the neural niche to favour perineural invasion in head and neck cancer. Nat Commun 2015;6:6885-97.
12. He P, Shi JS, Chen WK, et al. Multivariate statistical analysis of clinicopathologic factors influencing survival of patients with bile duct carcinoma. World J Gastroenterol 2002;8:943.
13. Kayahara M, Nakagawara H, Kitagawa H, et al. The nature of neural invasion by pancreatic cancer. Pancreas 2007;35:218.
14. Palm D, Entschladen F. Neoneurogenesis and the neuro-neoplastic synapse. Prog Exp Tumor Res 2007;39:91.
15. Makwana M, Raivich G. Molecular mechanisms in successful peripheral regeneration. FEBS J 2005;272:2628-38.
16. Boilly B, Faulkner S, Jobling P, et al. Nerve Dependence: From Regeneration to Cancer. Cancer Cell 2017;31:342-54.
17. Scuteri A, Miloso M, Foudah D, et al. Mesenchymal stem cells neuronal differentiation ability: A real perspective for nervous system repair? Curr Stem Cell Res Ther 2011;6:82-92.
18. Magnon C, Hall SJ, Lin J, et al. Autonomic Nerve Development Contributes to Prostate Cancer Progression. Science 2013;341:1236361.
19. Zhao CM, Hayakawa Y, Kodama Y, et al. Denervation suppresses gastric tumorigenesis. Sci Transl Med 2014;6:250ra115.
20. Peterson SC, Eberl M, Vagnozzi AN, et al. Basal cell carcinoma preferentially arises from stem cells within hair follicle and mechanosensory niches. Cell Stem Cell 2015;16:400-12.
21. Saloman JL, Albers KM, Li D, et al. Ablation of sensory neurons in a genetic model of pancreatic ductal adenocarcinoma slows initiation and progression of cancer. Proc Natl Acad Sci U S A 2016;113: 3078-83.
22. Horvathova L, Padova A, Tillinger A, et al. Sympathectomy reduces tumor weight and affects expression of tumor-related genes in melanoma tissue in the mouse. Stress 2016;19:528-34.
23. Lackovicova L, Banovska L, Bundzikova J, et al. Chemical sympathectomy suppresses fibrosarcoma development and improves survival of tumor-bearing rats. Neoplasma 2011;58:424-9.

24. Mancino M, Ametller E, Gascón P, et al. The neuronal influence on tumor progression. Biochim Biophys Acta 2011;1816:105-18.

25. Ylivinkka I, Keski-Oja J, Hyytiäinen M. Netrin-1: A regulator of cancer cell motility? Eur J Cell Biol 2016;95:513-20.

26. Barquilla A, Pasquale EB. Eph receptors and ephrins: therapeutic opportunities. Annual review of pharmacology and toxicology. Annu Rev Pharmacol Toxicol 2015;55:465-87.

27. Rehman M, Tamagnone L. Semaphorins in cancer: biological mechanisms and therapeutic approaches. Semin Cell Dev Biol 2013;24:179-89.

28. Gara RK, Kumari S, Ganju A, Y et al. Slit/Robo pathway: a promising therapeutic target for cancer. Drug Discov Today 2015;20:156-64.

29. Roshchina VV. Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. Microbial Endocrinology 2010:17-52.

30. Onganer PU, Seckl MJ, Djamgoz MB. Neuronal characteristics of small-cell lung cancer. Br J Cancer 2005;93:1197-201.

31. Hagel C, Stavrou D. Neuronal markers in non-neuronal tissues. In Neuronal Activity in Tumor Tissue. Karger Publishers, 2007;39:64-77.

32. Entschladen F, Drell TL, Lang K, et al. Tumour-cell migration, invasion, and metastasis: navigation by neurotransmitters. Lancet Oncol 2004;5:254-8.

33. Reiche EM Nunes SO, Morimoto HK. Stress, depression, the immune system, and cancer. Lancet Oncol 2004;5:617-25.

34. Erondu N, Gantz I, Musser B, et al. Neuropeptide Y5 receptor antagonism does not induce clinically meaningful weight loss in overweight and obese adults. Cell Metab 2006;4:275-82.

35. Zhang L, Bijker MS, Herzog H. The neuropeptide Y system: pathophysiological and therapeutic implications in obesity and cancer. Pharmacol Ther 2011;131:91-113.

36. Muñoz M, González-Ortega A, Rosso M, et al. The substance P/neurokinin-1 receptor system in lung cancer: focus on the antitumor action of neurokinin-1 receptor antagonists. Peptides 2012;38:318-25.

37. Huang SC, Korlipara VL. Neurokinin-1 receptor antagonists: a comprehensive patent survey. Expert Opin Ther Pat 2010;20:1019-45.

38. Banerjee J, John P, Arokya M, et al. Regulation of nonsmall-cell lung cancer stem cell like cells by neurotransmitters and opioid peptides. Int J Cancer 2015;137:2815-24.

39. Krizanova O, Babula P, Pacak K. Stress, catecholaminergic system and cancer. Stress 2016;19:419-28.

40. Akbar S, Alsharidah MS. Are beta blockers new potential anticancer agents? Asian Pac J Cancer Prev 2014;15:9567-74.

41. Entschladen F, Drell TL 4th, Lang K, et al. Neurotransmitters and chemokines regulate tumor cell migration: potential for a new pharmacological approach to inhibit invasion and metastasis development. Curr Pharm Des 2005;11:403-11.

42. Nagaraja AS, Sadaoui NC, Lutgendorf SK, et al. β-blockers: a new role in cancer chemotherapy? Expert Opin Investig Drugs 2013;22:1359-63.

43. Shaikhpoor M, Ahangari G, Sadeghizadeh M, et al. Significant changes in D2-like dopamine gene receptors expression associated with non- small -cell lung cancer: Could it be of potential use in the design of future therapeutic strategies? Current Cancer Therapy Reviews 2012;8:304-10.

44. Wang PS, Walker AM, Tsuang MT, et al. Dopamine antagonists and the development of breast cancer. Arch Gen Psychiatry 2002;59:1147-54.

45. Suzuki S, Okada M, Kuramoto K, et al. Aripiprazole, an antipsychotic and partial dopamine agonist, inhibits cancer stem cells and reverses chemoresistance. Anticancer Res 2016;36:5153-61.

46. Sachlos E, Risueño RM, Laronde S, et al. Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells. Cell 2012;149:1284-97.

47. Lee JK, Nam DH, Lee J. Repurposing antipsychotics as glioblastoma therapeutics: Potentials and challenges (Review). Oncol Lett 2016;11:1281-6.

48. Siddiqui EJ, Shabbir MA, Mikhailidis DP, et al. The effect of serotonin and serotonin antagonists on bladder cancer cell proliferation. BJU Int 2006;97:634-9.

49. Dizeyi N, Bjartell A, Nilsson E, et al. Expression of Serotonin Receptors and Role of Serotonin in Human Prostate Cancer Tissue and Cell Lines. Prostate 2004;59:328-36.

50. Siddiqui EJ, Shabbir M, Mikhailidis DP, et al. The Role of Serotonin (5-Hydroxytryptamine1A and 1B) Receptors in Prostate Cancer Cell Proliferation. J Urol 2006;176:1648-53.

51. Peters MA, Walenkamp AM, Kema IP, et al. Dopamine and serotonin regulate tumor behavior by affecting angiogenesis. Drug Resist Updat 2014;17:96-104.

52. Radin DP, Patel P. A current perspective on the

oncopreventive and oncolytic properties of selective serotonin reuptake inhibitors. Biomed Pharmacother 2017;87:636-9.

53. Brown DA. Acetylcholine. Br J Pharmacol 2006;147 Suppl 1:S120-6.

54. Thunnissen FB. Acetylcholine receptor pathway and lung cancer. J Thorac Oncol 2009;4:943-6.

55. Jia Y, Zu S, Ma X. Nicotinic acetylcholine receptor in tobacco-related cancer. Chin J Clin Oncol 2013;40:236-8.

56. Song P, Sekhon HS, Lu A, et al. M3 muscarinic receptor antagonists inhibit small cell lung carcinoma growth and mitogen-activated protein kinase phosphorylation induced by acetylcholine secretion. Cancer Res 2007;67:3936-44.

57. Speyer CL, Smith JS, Banda M, et al. Metabotropic glutamate receptor-1: a potential therapeutic target for the treatment of breast cancer. Breast Cancer Res Treat 2012;132:565-73.

58. Shou X, Chamberlin AR. Ligands for kainate subtype glutamate receptors. Expert Opin Ther Pat 2004;14:471-86.

59. Miao YF, Wang FY, Chen LB. Relationship of γ-aminobutyric acid and its receptors with proliferation and invasion of tumor. Chinese Journal of Cancer Biotherapy 2009;16:93-6.

60. Herner A, Sauliunaite D, Michalski CW, et al. Glutamate increases pancreatic cancer cell invasion and migration via AMPA receptor activation and Kras-MAPK signaling. Int J Cancer 2011;129:2349-59.

61. Schuller HM, Al-Wadei HA, Majidi M. GABAB receptor is a novel drug target for pancreatic cancer. Cancer 2008;112:767-78.

62. Thaker PH, Yokoi K, Jennings NB, et al. Inhibition of experimental colon cancer metastasis by the GABA-receptor agonist nembutal. Cancer Biol Ther 2005;4:753-8.

63. Heasley LE. Autocrine and paracrine signaling through neuropeptide receptors in human cancer. Oncogene 2001;20:1563-9.

64. Benarroch EE. Neuropeptide Y: Its multiple effects in the CNS and potential clinical significance. Neurology 2009;72:1016-20.

65. Ruscica M, Dozio E, Motta M, et al. Relevance of the neuropeptide Y system in the biology of cancer progression. Curr Top Med Chem 2007;7:1682-91.

66. Tilan J, Kitlinska J. Neuropeptide Y (NPY) in tumor growth and progression: Lessons learned from pediatric oncology. Neuropeptides 2016;55:55-66.

67. Zukowska-Grojec Z. Neuropeptide Y: A novel sympathetic stress hormone and more. New York Academy of Sciences; 1995.

68. Pellieux C, Sauthier T, Domenighetti A, et al. Neuropeptide Y (NPY) potentiates phenylephrine-induced mitogen-activated protein kinase activation in primary cardiomyocytes via NPY Y5 receptors. Proc Natl Acad Sci U S A 2000;97:1595-600.

69. Ebner K, Singewald N. The role of substance P in stress and anxiety responses. Amino Acids 2006;31:251-72.

70. Palma C. Tachykinins and their receptors in human malignancies. Current Drug Targets 2006;7:1043-52.

71. Koon HW, Pothoulakis C. Immunomodulatory properties of substance P: The gastrointestinal system as a model. Ann N Y Acad Sci 2006;1088:23-40.

72. Rao G, Patel PS, Idler SP, et al. Facilitating Role of Preprotachykinin-I Gene in the Integration of Breast Cancer Cells within the Stromal Compartment of the Bone Marrow: A Model of Early Cancer Progression. Cancer Res 2004;64:2874-81.

73. Muñoz M, Coveñas R. Neurokinin-1 receptor: a new promising target in the treatment of cancer. Discov Med 2010;10:305-13.

74. Thaker PH, Han LY, Kamat AA, et al. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat Med 2006;12:939-44.

75. Sloan EK, Priceman SJ, Cox BF, et al. The sympathetic nervous system induces a metastatic switch in primary breast cancer. Cancer Res 2010;70:7042-52.

76. Melamed R, Rosenne E, Shakhar K, et al. Marginating pulmonary-NK activity and resistance to experimental tumor metastasis: Suppression by surgery and the prophylactic use of a β-adrenergic antagonist and a prostaglandin synthesis inhibitor. Brain Behav Immun 2005;19:114-26.

77. Masur K, Niggemann B, Zanker KS, et al. Norepinephrine-induced migration of SW 480 colon carcinoma cells is inhibited by β-blockers. Cancer Res 2001;61:2866-9.

78. Kim-Fuchs C, Le CP, Pimentel MA, et al. Chronic stress accelerates pancreatic cancer growth and invasion: A critical role for beta-adrenergic signaling in the pancreatic microenvironment. Brain Behav Immun 2014;40:40-7.

79. Park SY, Kang JH, Jeong KJ, et al. Norepinephrine induces VEGF expression and angiogenesis by a hypoxia-inducible factor-1α protein-dependent mechanism. Int J Cancer 2011;128:2306-16.

80. Schuller HM, Porter B, Riechert A. Beta-adrenergic modulation of NNK-induced lung carcinogenesis in hamsters. J Cancer Res Clin Oncol 2000;126:624-30.

81. Al-Wadei HA, Al-Wadei MH, Schuller HM. Prevention

of pancreatic cancer by the beta-blocker propranolol. Anticancer Drugs 2009;20:477-82.

82. Lee JW, Shahzad MM, Lin YG, et al. Surgical stress promotes tumor growth in ovarian carcinoma. Clin Cancer Res 2009;15:2695-702.

83. Weberpals J, Jansen L, Carr PR, et al. Beta blockers and cancer prognosis-The role of immortal time bias: A systematic review and meta-analysis. Cancer Treat Rev 2016;47:1-11.

84. Rubí B, Maechler P. Minireview: new roles for peripheral dopamine on metabolic control and tumor growth: let's seek the balance. Endocrinology 2010;151:5570-81.

85. Sarkar C, Chakroborty D, Chowdhury UR, et al. Dopamine increases the efficacy of anticancer drugs in breast and colon cancer preclinical models. Clin Cancer Res 2008;14:2502-10.

86. Asada M, Ebihara S, Numachi Y, et al. Reduced tumor growth in a mouse model of schizophrenia, lacking the dopamine transporter. Int J Cancer 2008;123:511-8.

87. Chakroborty D, Sarkar C, Mitra RB, et al. Depleted dopamine in gastric cancer tissues: Dopamine treatment retards growth of gastric cancer by inhibiting angiogenesis. Clin Cancer Res 2004;10:4349-56.

88. Chakroborty D, Chowdhury UR, Sarkar C, et al. Dopamine regulates endothelial progenitor cell mobilization from mouse bone marrow in tumor vascularization. J Clin Invest 2008;118:1380-9.

89. Ganguly S, Basu B, Shome S, et al. Dopamine, by acting through its D 2 receptor, inhibits insulin-like growth factor-I (IGF-I)-induced gastric cancer cell proliferation via up-regulation of Krüppel-like factor 4 through down-regulation of IGF-IR and AKT phosphorylation. Am J Pathol 2010;177:2701-7.

90. Mohammad-Zadeh LF, Moses L, Gwaltney-Brant SM. Serotonin: a review. J Vet Pharmacol Ther 2008;31:187-99.

91. Drozdov I, Kidd M, Gustafsson BI, et al. Autoregulatory effects of serotonin on proliferation and signaling pathways in lung and small intestine neuroendocrine tumor cell lines. Cancer 2009;115:4934-45.

92. Kast RE. Glioblastoma chemotherapy adjunct via potent serotonin receptor-7 inhibition using currently marketed high-affinity antipsychotic medicines. Br J Pharmacol 2010;161:481-7.

93. Kuwahara J, Yamada T, Egashira N, et al. Comparison of the anti-tumor effects of selective serotonin reuptake inhibitors as well as serotonin and norepinephrine reuptake inhibitors in human hepatocellular carcinoma cells. Biological and Pharmaceutical Bulletin 2015;38:1410-4.

94. Huggins LG, Davis LV. Adjuvant and novel anti-cancer drug, keyhole limpet hemocyanin, targets serotonin signaling pathway by inhibiting feeding, locomotion and egg laying in C. elegans. FASEB J 2008;22:13.

95. Gotti C, Fornasari D, Clementi F. Human neuronal nicotinic receptors. Prog Neurobiol 1997;53:199-237.

96. Shah N, Khurana S, Cheng K, et al. Muscarinic receptors and ligands in cancer. Am J Physiol Cell Physiol 2009;296:C221-32.

97. Hecht SS. Cigarette smoking: cancer risks, carcinogens, and mechanisms. Langenbecks Arch Surg 2006;391:603-13.

98. Dasgupta P, Rizwani W, Pillai S, et al. Nicotine induces cell proliferation, invasion and epithelial-mesenchymal transition in a variety of human cancer cell lines. Int J Cancer 2009;124:36-45.

99. Improgo MR, Tapper AR, Gardner PD. Nicotinic acetylcholine receptor-mediated mechanisms in lung cancer. Biochem Pharmacol 2011;82:1015-21.

100. Li ZJ, Cho CH. Neurotransmitters, more than meets the eye-neurotransmitters and their perspectives in cancer development and therapy. Eur J Pharmacol 2011;667:17-22.

101. Arias HR, Richards VE, Ng D, et al. Role of non-neuronal nicotinic acetylcholine receptors in angiogenesis. Int J Biochem Cell Biol 2009;41:1441-51.

102. Wong HP, Li ZJ, Shin VY, et al. Effects of cigarette smoking and restraint stress on human colon tumor growth in mice. Digestion 2009;80:209-14.

103. Wong HP, Yu L, Lam EK, et al. Nicotine promotes cell proliferation via α7-nicotinic acetylcholine receptor and catecholamine-synthesizing enzymes-mediated pathway in human colon adenocarcinoma HT-29 cells. Toxicol Appl Pharmacol 2007;221:261-7.

104. Al-Wadei HA, Plummer HK, Schuller HM. Nicotine stimulates pancreatic cancer xenografts by systemic increase in stress neurotransmitters and suppression of the inhibitory neurotransmitter γ-aminobutyric acid. Carcinogenesis 2009;30:506-11.

105. Song P, Sekhon HS, Xiao WF, et al. Activated cholinergic signaling provides a target in squamous cell lung carcinoma. Cancer Res 2008;68:4693-700.

106. Ukegawa JI, Takeuchi Y, Kusayanagi S, et al. Growth-promoting effect of muscarinic acetylcholine receptors in colon cancer cells. J Cancer Res Clin Oncol 2003;129:272-8.

107. Jiménez E, Montiel M. Activation of MAP kinase by muscarinic cholinergic receptors induces cell proliferation and protein synthesis in human breast cancer cells. J Cell Physiol 2005;204:678-86.

108. Tata AM. Muscarinic acetylcholine receptors: New potential therapeutic targets in antinociception and in cancer therapy. Recent Pat CNS Drug Discov 2008;3:94-103.

109. Hinoi E, Takarada T, Ueshima T, et al. Glutamate signaling in peripheral tissues. Eur J Biochem 2004;271:1-13.

110. Kalariti N, Pissimissis N, Koutsilieris M. The glutamatergic system outside the CNS and in cancer biology. Expert Opin Investig Drugs 2005;14:1487-96.

111. Yoo BC, Jeon E, Hong SH, et al. Metabotropic glutamate receptor 4-mediated 5-fluorouracil resistance in a human colon cancer cell line. Clin Cancer Res 2004;10:4176-84.

112. de Groot JF, Piao Y, Lu L, et al. Knockdown of GluR1 expression by RNA interference inhibits glioma proliferation. J Neurooncol 2008;88:121-33.

113. Liu JW, Myoung SK, Nagpal J, et al. Quantitative hypermethylation of NMDAR2B in human gastric cancer. Int J Cancer 2007;121:1994-2000.

114. Park SY, Lee SA, Han IH, et al. Clinical significance of metabotropic glutamate receptor 5 expression in oral squamous cell carcinoma. Oncol Rep 2007;17:81-7.

115. Pollock PM, Cohen-Solal K, Sood R, et al. Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia. Nat Genet 2003;34:108-12.

116. Choi SW, Park SY, Hong SP, et al. The expression of NMDA receptor 1 is associated with clinicopathological parameters and prognosis in the oral squamos cell carcinoma. J Oral Pathol Med 2004;33:533-7.

117. Chang HJ, Yoo BC, Lim SB, et al. Metabotropic glutamate receptor 4 expression in colorectal carcinoma and its prognostic significance. Clin Cancer Res 2005;11:3288-95.

118. Iacovelli L, Arcella A, Battaglia G, et al. Pharmacological activation of mGlu4 metabotropic glutamate receptors inhibits the growth of medulloblastomas. J Neurosci 2006;26:8388-97.

119. Tatsuta M, Iishi H, Baba M, et al. Inhibition by gamma-amino-n-butyric acid and baclofen of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. Cancer Res 1990;50:4931-4.

120. Joseph J, Niggemann B, Zaenker KS, et al. The neurotransmitter γ-aminobutyric acid is an inhibitory regulator for the migration of SW 480 colon carcinoma cells. Cancer Res 2002;62:6467-9.

121. Wang T, Huang W, Chen F. Baclofen, a GABAB receptor agonist, inhibits human hepatocellular carcinoma cell growth in vitro and in vivo. Life Sci 2008;82:536-41.

122. Azuma H, Inamoto T, Sakamoto T, et al. Gamma-aminobutyric acid as a promoting factor of cancer metastasis; induction of matrix metalloproteinase production is potentially its underlying mechanism. Cancer Res 2003;63:8090-6.

123. Saloman JL, Albers KM, Rhim AD, et al. Can Stopping Nerves, Stop Cancer? Trends Neurosci 2016;39:880-9.

124. Jobling P, Pundavela J, Oliveira SM, et al. Nerve-Cancer Cell Cross-talk: A Novel Promoter of Tumor Progression. Cancer Res 2015;75:1777-81.

125. Eng JW, Reed CB, Kokolus KM, et al. Housing temperature-induced stress drives therapeutic resistance in murine tumour models through β2-adrenergic receptor activation. Nat Commun 2015;6:6426

126. Gupta SC, Sung B, Prasad S, et al. Cancer drug discovery by repurposing: teaching new tricks to old dogs. Trends Pharmacol Sci 2013;34:508-17.

127. Mitra AK, Agrahari V, Mandal A, et al. Novel delivery approaches for cancer therapeutics. J Control Release 2015;219:248-68.

128. Liebl F, Demir IE, Rosenberg R, et al. The severity of neural invasion is associated with shortened survival in colon cancer. Clin Cancer Res 2013;19:50-61.

129. Samarelli AV, Riccitelli E, Bizzozero L, et al. Neuroligin 1 induces blood vessel maturation by cooperating with the α6 integrin. J Biol Chem 2014;289:19466-76.

130. Graziano S, Marchiò S, Bussolino F, et al. A peptide from the extracellular region of the synaptic protein α Neurexin stimulates angiogenesis and the vascular specific tyrosine kinase Tie2. Biochem Biophys Res Commun 2013;432:574-9.

131. Daugherty A. Recipients of the 2013 ATVB Early Career Awards. Arterioscler Thromb Vasc Biol 2013;33:881

132. Bedell VM, Ekker SC. Nervy Vasculature. Arterioscler Thromb Vasc Biol 2012;32:1546-7.

133. Rissone A, Foglia E, Sangiorgio L, et al. The synaptic proteins β-neurexin and neuroligin synergize with extracellular matrix-binding vascular endothelial growth factor a during zebrafish vascular development. Arterioscler Thromb Vasc Biol 2012;32:1563-72.

134. Arese M, Serini G, Bussolino F. Nervous vascular parallels: axon guidance and beyond. Int J Dev Biol 2011;55:439.

135. Bottos A, Rissone A, Bussolino F, et al. Neurexins and neuroligins: synapses look out of the nervous system. Cell Mol Life Sci 2011;68:2655-66.

136. Rissone A, Sangiorgio L, Monopoli M, et al. Characterization of the neuroligin gene family expression and evolution in zebrafish. Dev Dyn 2010;239:688-702.

137. Bottos A, Destro E, Rissone A, et al. The synaptic proteins neurexins and neuroligins are widely expressed in the

vascular system and contribute to its functions. Proc Natl Acad Sci USA 2009;106:20782-7.

138. Suckow AT, Comoletti D, Waldrop MA, et al. Expression of neurexin, neuroligin, and their cytoplasmic binding partners in the pancreatic beta-cells and the involvement of neuroligin in insulin secretion. Endocrinology 2008;149:6006-17.

139. Burgoyne RD, Morgan A. Secretory granule exocytosis. Physiol Rev 2003;83:581-632.

140. Budreck EC, Kwon OB, Jung JH, et al. Neuroligin-1 controls synaptic abundance of NMDA-type glutamate receptors through extracellular coupling. Proc Natl Acad Sci USA 2013;110:725-30.