Neurotransmission and cancer: implications for prevention and therapy

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Published evidence compiled in this review supports the hypothesis that the development, progression, and responsiveness to prevention and therapy of the most common human cancers is strongly influenced, if not entirely orchestrated, by an imbalance in stimulatory and inhibitory neurotransmission. The neurotransmitters acetylcholine, adrenaline, and noradrenaline of the autonomic nervous system act as powerful upstream regulators that orchestrate numerous cell and tissue functions, by releasing growth factors, angiogenesis factors and metastasis factors, arachidonic acid, proinflammatory cytokines, and local neurotransmitters from cancer cells and their microenvironment. In addition, they modulate proliferation, apoptosis, angiogenesis, and metastasis of cancer directly by intracellular signaling downstream of neurotransmitter receptors. Nicotine and the tobacco-specific nitrosamines have the documented ability to hyperstimulate neurotransmission by both branches of the autonomic nervous system. The expression and function of these neurotransmitter pathways are cell type specific. Lifestyle, diet, diseases, stress, and pharmacological treatments modulate the expression and responsiveness of neurotransmitter pathways. Current preclinical testing systems fail to incorporate the modulating effects of neurotransmission on the responsiveness to anticancer agents and should be amended accordingly. The neurotransmitter γ-aminobutyric acid has a strong inhibitory function on sympathicus-driven cancers whereas stimulators of cyclic adenosine monophosphate/protein kinase A signaling have strong inhibitory function on parasympathicus-driven cancers. Marker-guided restoration of the physiological balance in stimulatory and inhibitory neurotransmission represents a promising and hitherto neglected strategy for the prevention and therapy of neurotransmitter-responsive cancers. *Anti-Cancer Drugs* 19:655–671 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2008, **19**:655–671

Keywords: acetylcholine receptors, autonomic nervous system, β-adrenergic receptors, cell type-specific signaling, regulation of cancer cell by neurotransmitters

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Received 18 February 2008 **Revised form accepted** 27 March 2008

Introduction

Our current understanding of the mechanisms responsible for cancer initiation and progression was to a great extent molded by experiments on the effects of chemical carcinogens in laboratory rodents. This research has culminated in the widely accepted paradigm that most chemical carcinogens are metabolically activated to reactive intermediates that form DNA adducts associated with gene mutations, including activating point mutations in k-ras and inactivating mutations in p53, and that these mutated genes trigger the development of cancer [1–3]. Past and present efforts to develop effective cancer intervention strategies have been based on this paradigm, with cancer prevention focusing on inhibition of carcinogen metabolism and oxygen radical formation [4–7] whereas conventional cancer therapy aims to kill cancer cells by chemotherapy and radiation [8]. Owing to the short life span of laboratory animals, very large doses of chemical carcinogens that represent the equivalent of lifetime exposures in people are commonly used. Although the gene mutations observed in such studies are commonly seen in human cancers, mechanistic insights gained by these experiments should be considered with caution. The mega doses of chemical carcinogens used in animal experiments may artificially override intracellular regulatory pathways that are modulated in people by much lower systemic and organ concentrations caused by environmental and occupational exposure to such agents. For the same reason, in-vitro studies that use concentrations of carcinogens well above the systemic levels of these agents in people should be viewed with skepticism.

Smoking is a documented risk factor for the development of most cancers, an association that is particularly strong for cancer of the lungs and pancreas. Tobacco smoke contains several thousand different irritants, carcinogens, and toxicants. Among these, nicotine is generally believed to be responsible for cardiovascular disease and addiction associated with smoking, effects mediated by binding of nicotine to nicotinic acetylcholine receptors (nAChRs). On the other hand, tobacco-specific nitrosamines (TSNs) as well as polycyclic aromatic hydrocarbons (PAHs) are

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thought to be primarily responsible for the development of smoking-associated cancer, and their carcinogenic actions are thought to require the interaction of reactive metabolites with DNA, resulting in activating point mutations in k-ras and inactivating mutations in p53 [3]. The PAHs are contained in the tar found in the particulate matter fraction of smoke, whereas the TSNs are additionally formed from nicotine in the mammalian organism. Since the introduction of cigarette filters that trap the tar, squamous cell carcinoma of the lungs, which is reproducibly induced in laboratory animals by PAHs, has declined in incidence. On the other hand, pulmonary adenocarcinoma (PAC), which is induced in all animal species tested by the TSNs n-nitroso-nornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), has dramatically increased in incidence during that time frame and is the leading type of lung cancer today, particularly in women [9].

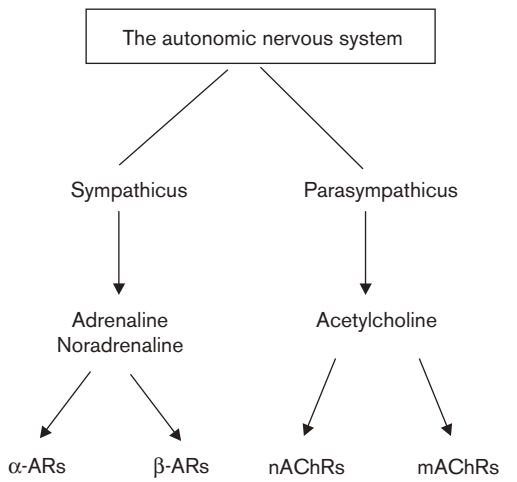
Research on the biology, prevention, and therapy of cancer typically uses studies in human cancer tissues and cells and in animal models for human cancer. The problem with this strategy is that investigations of fully developed cancers in a standardized environment exclude the detection of potentially altered regulatory factors outside of cancer cells that may well be the driving forces for cancer development and progression. Such factors may reside in the immediate tumor microenvironment or even in distant organs. It is thus common knowledge that the clinical behavior of breast cancer is often significantly influenced by the interaction of estrogen with estrogen receptors (ERs), even though this hormone is primarily produced in the ovary. In fact, this led to the classification of breast cancers into ER+ versus ER– malignancies, resulting in the treatment of ER+ cases with antiestrogens [10]. Similarly, the interaction of androgens with androgen receptors has a significant impact on the therapy of androgen-responsive prostate cancer [10].

Although the scientific community has readily accepted the concept that hormones play an important role in the regulation of hormone-responsive cancers, a potential role of neurotransmitters and their associated receptor-initiated signaling pathways in the development, progression, and responsiveness of cancer to therapy has only recently attracted attention.

Classic neuroscience has taught us that the autonomic nervous system (Fig. 1) with its two antagonistic branches, the sympathicus and the parasympathicus, regulates all involuntary organ functions. The catecholamines adrenaline and noradrenaline (also termed epinephrine and norepinephrine) are the neurotransmitters of the sympathicus. They are released from the adrenal medulla and from sympathicus nerve endings, plexuses, and ganglia into the systemic and local organ circulation to bind as agonists to the families of α-adrenergic receptors (ARs) and β-ARs expressed in all mammalian cells. Acetylcholine is the neurotransmitter of the parasympathicus that is released from parasympathetic nerve endings, plexuses, and ganglia into local organ circulations. Acetylcholine exerts its biological effects by binding to the families of nicotinic and muscarinic acetylcholine receptors. The sympathicus is considered the 'accelerator' of the mammalian body because it increases most organ functions whereas the parasympathicus is considered the physiological 'brake' that slows down most organ functions. The neurotransmitters of the autonomic nervous system typically regulate organ functions in a coordinated manner by initiating multiple functions that either stimulate or inhibit a given organ as a whole. This is best illustrated by the effects of adrenaline and noradrenaline on the cardiovascular system. Binding of these catecholamines to ARs accelerates heart rate, increases cardiac muscle contractility, regulates the release of arachidonic acid (AA) from endothelial cells, and increases blood pressure by constricting blood vessels while increasing blood oxygen levels by widening airway diameters [11].

The first reports suggestive of a potential role of the autonomic nervous system in the regulation of cancer

Fig. 1



The autonomic nervous system with its two branches, the sympathicus and the parasympathicus, regulates autonomic functions of organs and tissues that are not under conscious control. The catecholamine neurotransmitters of the sympathicus, adrenaline, and noradrenaline (synonyms: epinephrine and norepinephrine) and the neurotransmitter of the parasympathicus, acetylcholine, are released from the central nervous system and from peripheral nerves, ganglia, and plexuses. The catecholamines are additionally released from the adrenal medulla. Adrenaline and noradrenaline communicate with their effector cells in organs and tissues by binding to the families of α-adrenergic receptors and β-adrenergic receptors (α-ARs and β-ARs) expressed in the cell membrane of effector cells whereas acetylcholine binds to the families of nicotinic (nAChR) and muscarinic (mAChR) receptors. The sympathicus and parasympathicus have frequently antagonistic effects, with the sympathicus mostly acting as the 'accelerator' of cell and organ functions whereas the parasympathicus acts as the 'brake'.

cells were published by our laboratory in 1989 when we showed that nicotine as well as NNK stimulated the proliferation of human small cell lung cancer cells via binding to nAChRs [12], whereas the selective agonist for β-ARs, isoproterenol, stimulated the growth of human lung adenocarcinoma cells [13]. These two initial reports prompted additional investigations by us and a small number of other laboratories that culminated in the novel concept of cholinergic and ARs and their physiological ligands as key regulators of the most common human cancers [14,15]. In turn, these findings led to the discovery that the continuous growth of cancer is not only supported by neoangiogenesis (the formation of new blood vessels that vascularize the tumors) but also by neoneurogenesis (the formation of new nerve endings that provide the tumors with neurotransmitters) [16,17]. This review summarizes our current knowledge on the role of neurotransmission in the initiation and progression of cancer and their implications for cancer prevention and therapy.

The role of cholinergic neurotransmission in the regulation of cancer

Regulatory functions of nicotinic acetylcholine receptors in small cell lung cancer and pulmonary neuroendocrine cells

The parasympathetic neurotransmitter acetylcholine exerts its biological effects by binding to acetylcholine receptors that are expressed on the cell membrane of most mammalian cells. These receptors are comprised of two families, the nAChRs, to which nicotine binds with high affinity, and the muscarinic acetylcholine receptors (mAChRs), to which nicotine does not bind. All nAChRs are ion channels whereas the mAChRs are G-protein coupled receptors (GPCRs). The nAChRs are comprised of a central ion channel surrounded by subunits termed α through δ. Neuronal nAChRs are further subclassified based on the expression of α_2 through α_9 and β_2 through β_4 subunits, whereas the muscle nAChRs contain in addition γ and δ subunits [18–20]. The terminology 'neuronal' versus 'muscle' nAChRs was prompted by the original discovery and characterization of these receptors in the central and peripheral nervous system as opposed to striated and smooth muscle. However, both families of nAChRs were later discovered in numerous non-neuronal and nonmuscle cells. The chronic interaction of nicotine with neuronal nAChRs in the brain comprised of α_4/β_2 subunits has long been recognized as a major cause for nicotine addiction in smokers [21,22]. On the other hand, nAChRs with the subunit compositions α_2–α_5 and β_2–β_4 regulate the release of adrenaline and noradrenaline from the adrenal medulla and their chronic stimulation by nicotine contributes to the development of smoking-associated cardiovascular disease [23].

A potential role of neuronal nAChRs for smoking-associated lung carcinogenesis was first suggested by us in 1989 when we showed that nicotine and NNK stimulated the proliferation of small cell lung carcinoma (SCLC) cells *in vitro* and that this response was blocked by antagonists for nAChRs but not by an antagonist for mAChRs [12,24]. These reports were followed by publications from another laboratory in 1990 and 1994 that documented a nicotine-induced reversal of apoptosis in response to opioids in a large panel of SCLC and non-small cell lung cancer (NSCLC) cell lines [25,26]. A third independent laboratory described in 1993 that nicotine stimulated the proliferation of human SCLC cell lines by stimulating the release of serotonin, which acts as an autocrine growth factor for these cells [27]. The initial findings from these three laboratories suggested that nicotine itself may contribute to the development of smoking-associated lung cancer by interaction with nAChR-mediated proliferative and apoptotic signaling pathways. In addition, the data with NNK [12,24] indicated that the extreme potency of NNK as a pulmonary carcinogen might be linked to its ability to function as an agonist for nAChRs. Radio-receptor assays with site-selective ligands revealed high levels of the α_7nAChR in human SCLC cell lines as opposed to lung adenocarcinoma cell lines which demonstrated low or nondetectable levels of this receptor [28]. These findings were extended by recent investigations that showed expression of mRNA for the α_7nAChR in a large panel of cell lines derived from different types of human lung cancers and in immortalized human small airway epithelial cells whereas significant amounts of receptor protein were only detected in SCLC cell lines [29]. Taken together, these findings indicate that the α_7nAChr is expressed in many lung cell types but is upregulated in SCLC cells.

Radio-receptor assays assessing the relative binding affinities of nicotine, NNN, and NNK in competition with a selective ligand for the α_7nAChR identified NNK as a ligand with high affinity (1300 × higher affinity than nicotine) for the α_7nAChR [28]. By contrast, NNN bound with significantly lower affinity to this receptor than nicotine. On the other hand, saturation binding assays with the selective ligand for nAChRs containing α_2–α_6 and β_2–β_4 nAChR subunits, epibatidine (EB), revealed high expression levels of these receptors in cell lines from human PACs, whereas binding of EB was below detectable levels in SCLC cell lines [28]. Radio-receptor assays in which nicotine, NNK, or NNN competed for nicotinic binding sites with EB revealed an exceptionally high affinity of NNN for this receptor (about 5100 times higher than nicotine) whereas the affinity of NNK was very low (about 12 times lower than nicotine). Similar binding characteristics of nicotine, NNN, and NNK to the α_7nAChR and α_4nAChR were recently reported in oral keratinozytes [30]. Stimulation of the α_7nAChR in SCLC cells or their putative cells of origin, pulmonary neuroendocrine cells, by NNK (100 pmol/l to 30 nmol/l) significantly increased cell number and DNA synthesis

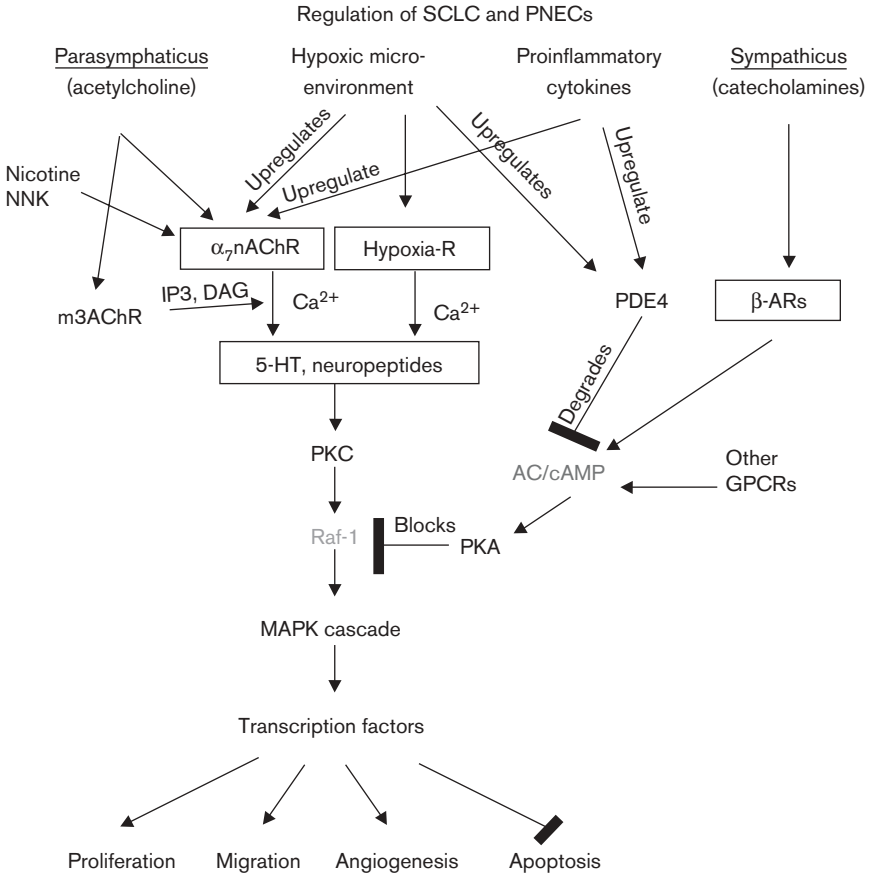
while inducing the phosphorylation of Raf-1, extracellular signal-regulated protein kinase (ERK)1/2, and c-myc [28]. These findings are in accord with the frequent expression of amplified c-myc in SCLC [31]. Flow cytometric analysis showed a significant increase in intracellular Ca^{2+} in response to 1 nmol/l NNK and this effect was blocked by the selective α_7nAChR antagonist α-bungarotoxin [32,33]. In conjunction with earlier reports, these findings clearly established an important regulatory role of the α_7nAChR in SCLC and pulmonary neuroendocrine cells. On the other hand, binding of nicotine or NNN to the EB-sensitive nAChRs expressed in PAC cells did not modulate cell proliferation under the assay conditions used and the function of this receptor in PAC cells remains to be elucidated [28].

Recent investigations additionally reported α_7nAChR-mediated, NNK-induced functional cooperation between Bcl2 and c-Myc that inhibited apoptosis while stimulating cell proliferation of human SCLC cell lines [34]. In addition, NNK phosphorylated μ-calpains and m-calpains in human SCLC cells in an ERK1/2 and Ca^{2+}-dependent manner, resulting in the induction of cell migration and invasion and these effects were abrogated by the α_7nAChR antagonist α-bungarotoxin [35]. It is of note that the concentration of NNK required to elicit these effects by all three laboratories was extremely low (100 pmol/l), thus underlining the very high affinity of NNK for the α_7nAChR, which is upregulated in SCLC cells. In addition, it has been shown that the PKC/Raf-1/ERK1/2 signaling cascade is also stimulated by autocrine growth factors for SCLC, including the neuropeptide growth factors bradykinin, vasopressin, bombesin (MB), neurotensin, and galananine as well as serotonin and acetylcholine [27,36–38]. In turn, it was shown that the release of MB, serotonin, or acetylcholine from SCLC cells was triggered by the influx of Ca^{2+} in response to stimulation of the α_7nAChR [27,36]. Collectively, these data suggest that the major signaling pathways that regulate SCLC growth, apoptosis, and invasiveness as well as the release of autocrine growth factors from these cells are controlled by the α_7nAChR (Fig. 2).

Although the in-vitro evidence for a critical role of α_7nAChR-mediated signaling in the genesis of smoking-associated SCLC is strong, proof for this hypothesis should come from animal experiments. However, nicotine does not cause cancer in healthy laboratory animals and NNK reproducibly induces PAC when administered to healthy rats, mice, or hamsters [2,39]. The physiological role of pulmonary neuroendocrine cells (PNECs), which are thought to give rise to SCLC, is to function as receptors with sensitivity to changes in pulmonary oxygen concentrations, particularly hypoxia [40,41]. It has thus been shown that PNECs express a receptor protein that senses hypoxia and triggers the release of serotonin (5-hydroxytryptamine, 5-HT) and MB via the influx of Ca^{2+} [42–44]. In addition to modulating bronchial smooth muscle tone and respiration, 5-HT and MB act as autocrine growth factors for PNECs and SCLC. Chronic obstructive pulmonary disease (COPD) is a chronic inflammation of the lungs associated with increased pulmonary CO_2 owing to obstruction of expiratory airflow. COPD is an independent risk factor in addition to smoking for the development of SCLC [45]. We therefore hypothesized that COPD would facilitate the development of a neuroendocrine type of lung cancer in animals exposed to the nicotinic agonists nicotine or NNK as well as diethylnitrosamine (DEN), which has structural similarities with acetylcholine [46]. In support of this hypothesis, hamsters with hyperoxia-induced COPD developed a high incidence of SCLC-like lung tumors when treated with NNK [39] or DEN [47]. Similar to most human SCLCs, these tumors expressed the neuroendocrine markers 5-HT, calcitonin, MB, and neuron-specific enolase, and they lacked activating point mutations in k-ras whereas overexpressing c-myc [48]. Hamsters with hyperoxia-induced COPD and treated with multiple subcutaneous injections of nicotine developed a low but significant incidence of lung tumors with focal areas of positive immunoreactivity to the neuroendocrine markers 5-HT and neuron-specific enolase [49]. In-vitro experiments demonstrated that SCLC or PNEC cells maintained in an environment of high CO_2 similar to that in the COPD lung showed upregulation of the α_7nAChR, induction of ERK1/2 activation [50] as well as increased proliferation responses to nicotine or NNK [51,52]. In addition, the proinflammatory mediators, tumor necrosis factor α and interleukin-1-β, each upregulated α_7nAChR protein in SCLC cells [53]. Collectively, these data suggest that COPD provides a selective growth advantage to SCLC cells by upregulating and sensitizing the α_7nAChR, resulting in excessive stimulation of autocrine mitogenic signaling pathways in the presence of nicotinic agonists, such as nicotine or NNK, in concert with increased angiogenesis and migration and a concomitant inhibition of apoptosis (Fig. 2).

Unlike GPCRs and peptide receptors, which are downregulated by chronic exposure to agonists, the nAChRs are upregulated by chronic exposure to nicotine [22]. Whereas this has been well documented for all nAChR types in the brain, similar effects on nAChRs expressed in lung cells have only been recently documented. It has thus been shown that treatment of pregnant monkeys from day 26 through day 134 of gestation with nicotine (1 mg/kg/day) upregulated the expression of α_7nAChRs in the lungs of their offspring as assessed by immunohistochemistry and real-time-PCR [54]. This was associated with an increase in the number of clustered PNECs. In accord with this report, we found a significant upregulation of the α_7nAChR by relative competitive real-time-PCR in the lungs of hamster fetuses harvested 1 day before birth whose mothers had been given NNK

Fig. 2



Simplified scheme illustrating the regulation of small cell lung carcinoma (SCLC) and one of several possible precursor cells, pulmonary neuroendocrine cell (PNECs). In these cells, the parasympathicus has stimulatory function via binding of acetylcholine to α7 nicotinic acetylcholine receptors (α_7nAChRs) and muscarinic-3 acetylcholine receptors (m3AChRs). The resulting increase in intracellular Ca^{2+} is intensified by an ion channel receptor with sensitivity to hypoxia or elevated CO_2 (hypoxia-R). The increase in intracellular Ca^{2+} triggers the release of autocrine growth factors of the neuropeptide and serotonin families, all of which signal primarily via PKC, Raf-1, and the mitogen-activated protein kinase cascade (MAPK cascade). The sympathicus has inhibitory function in these cells via inhibition of Raf-1 by PKA downstream of β-adrenergic receptor-mediated cAMP signaling. The hypoxic microenvironment and proinflammatory cytokines in the COPD lung upregulate the stimulatory α_7nAChR while at the same time upregulating phosphodiesterase 4 (PDEA), which degrades the inhibitory cAMP. Exposure to nicotine and NNK in tobacco smoke additionally activates and upregulates the α_7nAChR. Conditions or pharmacological agents that upregulate the α_7nAChr sensitize the receptor owing to increased intracellular Ca^{2+}, or reduce its physiological inhibitor PKA, render the stimulatory pathway more responsive while conditions or pharmacological agents that increase intracellular cAMP/PKA or inhibit Ca^{2+} or PKC reduce the stimulatory responses. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; GPCRs, G-protein coupled receptors; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PKA, protein kinase A; PKC, protein kinase C; Raf, v-raf-1 murine leukemia viral oncogene homolog 1.

(100 pmol/l) in their drinking water throughout their pregnancy [55]. These findings may have important implications for the risk assessment of individuals whose mothers smoked during pregnancy because the unregulated α_7nAChR may convey an increased susceptibility to the development of SCLC. However, epidemiological studies that have assessed cancer risk associated with maternal smoking have focused on childhood cancers, such as leukemia and brain cancer. No information is currently available on the modulation of lung cancer risk in adults whose mothers smoked during pregnancy. Animal experiments have shown that DEN or NNK when administered during the last trimester to pregnant hamsters caused the development of lung tumors in the offspring [56,57]. However, these experiments were conducted before research that implicated nicotinic receptors in lung carcinogenesis, and no information is available at this time on the expression levels of nAChRs in the transplacentally induced tumors and their potential role in the carcinogenic process.

Regulatory functions of nicotinic acetylcholine receptors in non-small cell lung cancer cells and non-neuroendocrine airway epithelial cells

Recent studies have revealed the expression of the EB-sensitive nAChRs as well as α_7nAChRs in a wide variety of cell types in the monkey lung [54,58]. In addition, a comprehensive analysis of nAChR subunit gene expres-

sion in NSCLC tissues from smokers and nonsmokers revealed significantly reduced gene expression for subunits α_4 and α_6 in adenocarcinomas of smokers, whereas the gene expression for subunit β_4 was increased [59]. The investigators also reported a significant reversible upregulation of subunits α_1, α_5, and α_7 in human bronchial epithelial cells exposed for 72 h to nicotine (100 nmol/l) *in vitro*. Studies by another laboratory with immortalized human bronchial epithelial cells and human small airway epithelial cells have shown that both receptor families participate in the activation of the serine/threonine kinase AKT in response to nicotine or NNK, an effect resulting in the attenuation of apoptosis induced by etoposide, radiation or hydrogen peroxide as well as the induction of a transformed phenotype [60]. However, the concentrations of nicotine (10–100 μmol/l) or NNK (1 μmol/l) required to elicit these effects were significantly higher than those reported in studies with PNECs or SCLC cell lines. Another laboratory additionally reported the activation of NFkappaB and upregulation of cyclin D1 in normal human bronchial epithelial cells and human small airway epithelial cells exposed to NNK at concentrations ranging from 0.5–10 μmol/l [61]. Again, these concentrations are considerably higher than those required to stimulate mitogenic signaling in SCLC cells. Studies in a variety of NSCLC cells have identified α_7nAChR-mediated induction of fibronectin associated with the activation of phosphoinositide 3-kinase and ERK1/2 and cell proliferation [62], β-arrestin-dependent activation of Src, induction of Rb-Raf-1 interaction, and phosphorylation of Rb [63]. In addition, it has been shown that nicotine inhibits apoptosis induced by cancer therapeutic agents in these cells via induction of survivin [64]. These antiapoptotic effects of nicotine were mediated by dihydro β-erythroidine-sensitive, α3-containing nAChRs and required the Akt pathway. As all of these pathways can also be activated by the epidermal growth factor (EGF) in NSCLC cells and non-neuroendocrine airway epithelial cells, it remains to be determined if the reported effects of nicotine on these cells were perhaps the result of nAChR-mediated EGF release or transactivation of the (EGFR).

In addition to the summarized effects of nicotinic agonists on lung cancer cells and airway epithelial cells, it has been recently discovered that nicotine stimulates angiogenesis and enhances the neovascularization of lung tumors [65]. In fact, studies in xenografts from Lewis lung cancer cells even showed that exposure to side stream smoke increased tumor size and angiogenesis and that this effect was inhibited by the broad-spectrum antagonist for neuronal nAChRs, mecamylamine [66].

Regulatory functions of nicotinic acetylcholine receptors in other cancers

In addition to their role in the initiation and progression of cancers derived from epithelial lung cells, nAChRs have been identified as important regulators of several other cancer types. It has thus been shown that signaling via the α_7nAChR stimulates the growth of mesothelioma cells while having antiapoptotic effects [67]. A recent report additionally showed that nicotine induced the proliferation of colon cancer cells via the α_7nAChR by stimulating the synthesis and release of adrenaline that in turn exerted mitogenic activities by binding to β_1 and β_2-ARs [68]. The same laboratory also described stimulation of proliferation, angiogenesis, and cell migration of gastric cancer cells in response to nicotine-induced upregulation of prostaglandin E_2 activity, cyclooxygenase-2 (COX-2), and vascular endothelial growth factor (VEGF), both *in vitro* and in an orthotopic mouse model [69,70]. Though the investigators reported involvement of β-ARs in the observed responses to nicotine, adrenaline levels were not determined. It is therefore not clear if nicotine interacted directly with the β-ARs, as discussed by the authors, or if nicotine acted indirectly via the nAChR-mediated release of adrenaline, as described in colon cancer cells [68]. An important physiological function of nAChRs is the regulation of catecholamine release and binding of these neurotransmitters to β-ARs stimulates the release of AA and VEGF in a host of different cell types [71–74]. It is therefore logical to assume that the observed responses of gastric cancer cells to nicotine were also cause by indirect mechanisms involving adrenaline release.

Upregulation of the α_3, α_5, and α_7nAChRs indicative of potential regulatory roles was recently reported in squamous cell carcinomas of the head and neck [75]. Mechanistic studies in oral keratinocytes revealed mitogenic activites of nicotine, NNK, or NNN in these cells that were primarily mediated by the α_7nAChR and involved signaling via ras/raf/ERK1/2, NF-κB, and the JAK-2/STAT-3 pathway [30,76,77]. These signaling cascades are the major downstream effectors of the EGFR, which is frequently overexpressed in squamous cell carcinoma of the head and neck [78,79]. It remains to be elucidated if the observed responses of oral keratinozytes and squamous cell carcinomas to nAChR stimulation involved indirect mechanisms via the release of EGF or if, perhaps, the EGFR was transactivated by nAChR signaling.

In addition to the catecholamines, nAChRs regulate the release of other neurotransmitters, including glutamate/aspartate and γ-aminobutyric acid (GABA) [80,81]. Both α_7nAChRs and non-α_7nAChRs of the subunit compositions $\alpha_3\beta_4$ and $\alpha_4\beta_2$ participate in these regulatory functions [82]. Interestingly, the N-methyl-D-aspartate receptor type 2B (NMDAR2B), for which glutamate and aspartate are the physiological agonists, stimulates apoptosis in esophageal cancer cells [83]. The expression of this receptor was suppressed by hypermethylation in 95% of primary esophageal cancer specimens [83]. Similar

findings were reported in gastric cancer cells, with 61% of fresh tumor tissues demonstrating hypermethylation of the NMDAR2B [84]. Underexpression of cellular receptors is often caused by chronic exposure to agonists [85]. It is therefore intriguing to speculate that chronic nicotinic receptor-mediated stimulation of glutamate/aspartate release in smokers may lead to the reported suppression of NMDARs accompanied by depletion of their physiological agonists. In analogy to this interpretation, the expression of GABA was suppressed in smokers [86] and in NNK-induced PACs in hamsters [87], whereas in-vitro studies in human PAC and pancreatic ductal adenocarcinoma (PDAC) cells suggested the tumor suppressor function of GABA via activation of the $GABA_BR$ [87,88].

Targeting of nicotinic acetylcholine receptors signaling for cancer prevention and therapy

In-vitro studies have shown that the influx of Ca^{2+} in response to agonists for the α_7nAChR triggers the release of autocrine growth factors and resulting activation of PKC, Raf-1 ERK1/2, and c-myc and cell proliferation in SCLC and PNECs. In accordance with these findings, the inhibitor of Ca^{2+} channels and PKC, dexniguldipine, completely blocked the development of neuroendocrine lung tumors when administered to hamsters that had been initiated for the development of such tumors by hyperoxia-induced COPD and DEN [89]. Dexniguldipine also completely abrogated the proliferation of SCLC and PNECs *in vitro* at very low concentrations in the picomolar range [90,91]. By comparison, much higher concentrations (100 nmol/l) of verapamil, which only blocks L-type Ca^{2+} channels, were required to yield a similar response [90]. Collectively, these in-vivo and in-vitro findings suggest that Ca^{2+} channel blockers and inhibitors of PKC may have protective effects against the development of SCLC and may be of value as adjuvant therapy of this cancer.

Another key protein in the signaling cascades that regulate SCLC and PNECs is the serine threonine kinase Raf-1 (Fig. 2). Cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) acts in many cell types as the physiological inhibitor of Raf-1 [92]. In turn, activation of cAMP/PKA signaling is the major downstream effector of GPCRs that induce cAMP via activation of the stimulatory G-protein $G\alpha_s$ [93]. Beta-ARs, which are activated by the neurotransmitters of the sympathicus, adrenaline and noradrenaline, are members of this GPCR family and should therefore have inhibitory effects on nAChR-mediated signaling in SCLC cells and their cells of origin. In support of this hypothesis, the selective β-adrenergic agonist isoproterenol suppressed NNK-induced ERK1/2 activation and proliferation of SCLC cells [94]. An addition to GPCR-mediated signaling, intracellular cAMP is also increased by inhibitors of phosphodiesterases that catalyze the enzymatic breakdown of cAMP. Accordingly, it has been shown that the phosphodiesterase inhibitor theopylline inhibits the proliferation of SCLC cells [95] and prevents the development of NNK-induced SCLC in hamsters with experimentally induced COPD [96]. In addition, it has been shown that green tea, which contains significant amounts of theophylline, has significant cancer preventive effects in this animal model [96]. These findings suggest that the upregulation of phosphodiesterases associated with COPD may contribute to the prevalence of SCLC in COPD patients because it results in reduced intracellular cAMP levels. In addition, these findings indicate that phosphodiesterase inhibitors that are currently in clinical trials for COPD may significantly reduce the risk of development of SCLC associated with this disease. These agents may also be useful as adjuvants to established cancer therapeutics for SCLC.

Role of muscarinic acetylcholine receptors in the regulation of cancer

The neurotransmitter of the parasympathicus, acetylcholine, is an agonist for both nAChRs and mAChRs. The mAChRs are GPCRs with receptors M1, M3, and M5 coupled to GQ that mobilizes Ca^{2+} from intracellular stores via activation of the inositol 1,4,5-trisphosphate (IP3)/diacylglycerol pathway. The resulting increase in intracellular Ca^{2+} triggers the release of autocrine growth factors in SCLC cells, thus cooperating with the nAChRs, which have the same effect via the influx of Ca^{2+} through their ion channel pore. A recent study has shown that agonists for the M3 mAChR induced ERK1/2, AKT, and cell proliferation in SCLC cell lines [97]. Antagonists for M3 receptors or silencing of the M3 mAChR significantly reduced base level and acetylcholine-induced mitogenic signaling. These findings are in accord with earlier observations by the same laboratory that acetylcholine acts as an autocrine growth factor for SCLC cells [98]. In nonsmokers, M3 antagonists may therefore be useful adjuvants for therapy of SCLC, whereas they would be ineffective in patients who smoke because nicotine and the TSNs would still activate nAChR-mediated release of autocrine growth factors and their associated mitogenic signaling. Unfortunately, not all SCLCs are stimulated in their growth by M3 receptors. Inhibitory actions of this receptor have been described in several SCLC cell lines, underlining the heterogeneity of human lung cancers within the family of SCLC and the need for marker-guided therapy and prevention. M3 receptors have also been shown to stimulate growth and angiogenesis of mammary cancer cells [99] and the proliferation of mesotheliomas [100], colon, and prostate cancer cells [101,102].

The role of sympathicus neurotransmission in the regulation of cancer

Regulatory functions of beta-adrenergic receptors in pulmonary adenocarcinomas

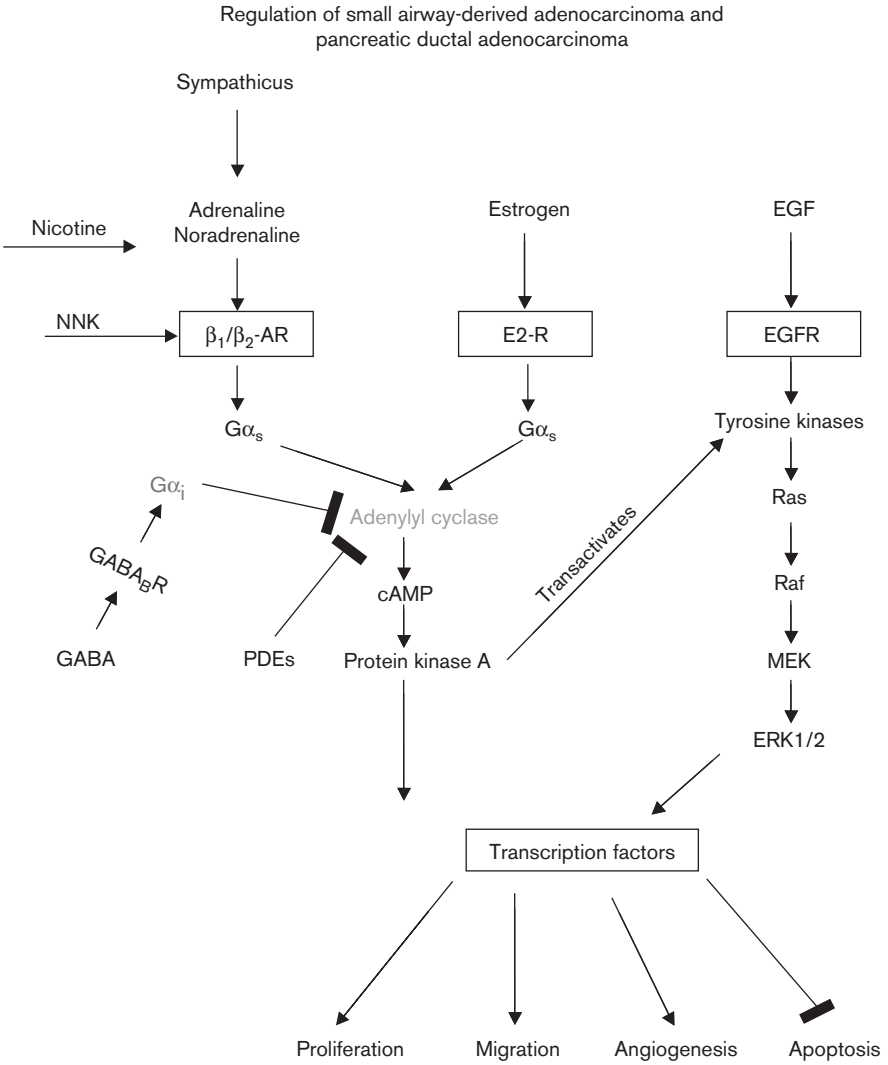
PAC is an aggressive cancer with mortality near 100% within 5 years of diagnosis. The majority of PACs are

thought to arise from nonciliated Clara cells of small airway epithelia, whereas alveolar type II cells and mucin-producing cells may be the origin of small subsets of PAC [103,104]. The incidence of PAC continues to rise in smokers and nonsmokers [9] and this malignancy predominates in women. The majority of human PACs harbors activating point mutations in k-ras [105] and overexpresses the EGFR and COX-2 [106,107], suggesting important regulatory functions of EGFR signaling via ras and an associated activation of the AA cascade. The TSNs NNK and NNN as well as several nitrosamines that are nontobacco-specific induce the development of small airway-derived PAC in Syrian golden hamsters [39,108,109] whereas all of these agents cause alveolar type II cell-derived PAC in mice and rats [110]. In addition, spontaneous [111,112] and genetically engineered PACs in mice and rats are also of alveolar type II cell origin [113]. Similar to human PAC, the experimentally induced PACs in all three animal models harbor activating point mutations in k-ras and overexpress the EGFR and COX-2. However, in accord with the highly cell-specific function of neurotransmitters, the responses of PACs, derived from small airway epithelia, to β-adrenergic signaling are the opposite of responses observed in the PACS of alveolar type II cell origin [71,114]. Data generated in mouse or rat models of PAC should therefore only be extrapolated to the small subset of human alveolar type II cell PACs, whereas data generated in the hamster model should be extrapolated to the larger population of human small airway-derived PAC.

An important role of β-adrenergic signaling in the regulation of small airway-derived PAC (Fig. 3) was first suggested by a report from our laboratory that the selective β-adrenergic agonist isoproterenol as well as the TSN NNK stimulated the proliferation of human PAC cell lines with features of bronchiolar Clara cells via binding of these agents to β-ARs, causing an increase in intracellular cAMP [13,115]. Radioreceptor assays with Chinese hamster ovary cells transfected with the human β-1-AR or β-2-AR genes and conducted in the presence of inhibitors for the oxidative enzymes required for NNK metabolism, subsequently, identified the unmetabolized NNK as a high affinity ligand for both receptors [71]. Similar assays conducted in human lung adenocarcinoma cells NCI-H322 and NCI-H441 identified the presence of β-1 and β-2 ARs, with β-1 predominating and NNK binding to both receptor types. NNK as well as isoproterenol stimulated the release of AA from these cells and increased their proliferation by enhancing DNA synthesis. These effects were inhibited by the β-blocker, propranolol. In addition, cell proliferation was partially inhibited by COX-2 inhibitors or by an inhibitor of the mitogen-activated kinases kinase [71]. Subsequent experiments with NCI-H322 cells and immortalized human small airway epithelial cells, HPL1D, showed that NNK increased intracellular cAMP, resulting in the activation of PKA and the transcription factor CREB while simultaneously transactivating the EGFR and its downstream effectors ERK1/2 in a manner dependent on the binding of NNK to β-1 adrenoreceptors and activation of PKA [116]. The resulting stimulation of cell proliferation was completely blocked by PKA inhibitors whereas equimolar concentrations of EGFR tyrosine kinases yielded partial inhibition. These in-vitro data identified the tobacco-specific carcinogenic nitrosamine NNK as a high affinity agonist for β-1 and β-2 ARs and implicated NNK-induced β-adrenergic signaling in the genesis and progression of lung adenocarcinoma. This interpretation was supported by experiments with NNK-induced lung adenocarcinomas in hamsters that showed a strong inhibition of adenocarcinoma development in animals that were given the β-blocker propranolol immediately before each NNK injection [117]. Treatment of hamsters with the β-adrenergic agonist, epinephrine, the cAMP activator, forskolin, or the phosphodiesterase inhibitor, theophylline, after discontinuation of NNK treatments demonstrated strong tumor promoting effects [96,117]. Furthermore, the dual signaling of NNK via β-adrenoreceptor and EGFR pathways suggested by the in-vitro studies were supported by the simultaneous overexpression of members of signaling proteins of both pathways in NNK-induced adenocarcinomas in hamsters [118].

PAC is more common in women than men, and an association between high expression levels of ER-β and occurrence of this cancer type has been reported [119]. The classic estrogen pathway involves interaction of estradiol (E2) with nuclear ERs α and β (ER-α, ER-β), resulting in the regulation of gene transcription of specific estrogen-responsive elements. However, recent studies have shown rapid activation of signaling pathways in response to estrogen resembling the actions of G-protein-coupled receptor ligands that transactivate the EGFR pathway [120,121]. These observations led to the concept that the ERs are proteins that shuttle between the nucleus and the cell membrane. Cooperative signaling of the ER-β with the β-1-AR was recently discovered by our laboratory in the human small airway epithelial cells, HPL1D [122]. These studies showed that transient overexpression of the ER-β significantly enhanced NNK-induced stimulation of cAMP as well as activation of ERK1/2 and cell proliferation. In addition, NNK rapidly phosphorylated the ER-β, an effect completely blocked by the antagonist for β-1-adrenoreceptors, atenolol. In transiently transfected cells, β-1-AR coprecipitated with ER-β, which increased with NNK treatment. ER-β gene knockdown, as well as coexpression of the dominant negative Ras and Raf, reduced stimulation of ERK1/2 by NNK. Whereas NNK phosphorylated Akt at Thr(308) and Ser(473), ER-β had no effect on this activity. These findings suggest direct interactions of NNK with non-genomic ER-β signaling (Fig. 3) and potential tumor

Fig. 3



Simplified scheme illustrating the regulation of small airway-derived adenocarcinomas of the lung (PAC) and small airway epithelia as well as pancreatic ductal adenocarcinomas (PDACs) and pancreatic ductal epithelia. In these cells, the sympathicus has stimulatory function via activation of the adenylyl cyclase/cAMP/PKA cascade downstream of $G\alpha_s$ activation in response to binding of catecholamines to β-adrenergic receptors (β-ARs). The action of the catecholamines is mimicked by NNK, which is a high-affinity agonist for β-ARs. Nongenomic membrane estrogen receptor β (ER-β) intensifies the effects of β-AR signaling via stimulation of $G\alpha_s$. Activated PKA downstream of β-adrenergic and ER-β signaling transactivates the EGFR pathway. In addition, β-ARs stimulate the release of EGF. The inhibitory neurotransmitter γ-aminubutyric acid (GABA) inhibits all of these pathways by $G\alpha_i$-mediated inhibition of adenylyl cyclase downstream of the GABA-B receptor ($GABA_BR$). Conditions, stress, diet, and lifestyle factors or pharmacological agents that stimulate the sympathicus, or otherwise increase and activate adenylyl cylase/cAMP, or reduce $GABA_BR$ signaling stimulate and sensitize this pathway whereas increased GABA signaling, blockage of β-ARs or E2-Rs, or reductions in catecholamine neurotransmitters or estrogen reduce the responsiveness of this pathway. The β_2-AR and EGFR each may stimulate multiple additional signaling cascades such as the Src, JAK/STAT, and Akt pathways and the AA-cascade. cAMP, cyclic adenosine monophosphate; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated protein kinase; MEK, mitogen activated kinase kinase; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PDE, phosphodiesterase; Raf, v-raf-1 murine leukemia viral oncogene homolog 1; Ras, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog.

promoting effects of estrogen as well as agents that upregulate the ER-β on the development of smoking-associated lung adenocarcinoma. This kind of cross-talk between β-adrenergic, EGFR, and ER-β signaling may well contribute to the documented prevalence of lung adenocarcinoma in women.

In contrast to the stimulatory signaling of β-ARs observed in small airway-derived PACs and their cells of origin, studies in human PAC cell lines of alveolar type II cell features have shown a lack of response to β-adrenergic agonists whereas cAMP even inhibited cell proliferation while inducing apoptosis [114,123,124]. In accord with

these in-vitro findings, studies in mouse and rat models of alveolar type II cell-derived PAC have shown strong cancer preventive effects of agents that stimulate cAMP-dependent signaling. Green or black tea that contains the phosphodiesterase inhibitors theophylline and caffeine thus showed strong cancer preventive effects on NNK-induced PAC in both species, an effect reduced by decaffeination [6,125]. By contrast, green tea or theophylline had strong tumor promoting effects on NNK-induced, small airway-derived PAC in hamsters [96]. Collectively, these data emphasize that β-adrenergic and cAMP signaling can exert stimulatory as well as inhibitory effects on lung cancer cells and that these responses are highly cell type specific. In addition, the responsiveness of this signaling pathway can be modulated by a host of environmental and lifestyle factors as well as diet, preexisting non-neoplastic diseases, and chronic drug treatments. The resulting interindividual variations in expression and responsiveness of cAMP signaling pathways therefore render the histopathology diagnosis of PAC useless as a predictor of responsiveness to cAMP modulating drugs.

Regulatory functions of beta-adrenergic receptors in pancreatic ductal adenocarcinomas

PDAC is the most common histological type of pancreatic cancer and demonstrates a mortality of $>90\%$ within 1 year of diagnosis [126]. Similar to lung adenocarcinoma, the majority of these tumors harbor activating point mutations in k-ras and overexpress the EGFR and COX-2 [14]. Smoking and alcohol consumption, pancreatitis, and diabetes have been identified as risk factors for this malignancy [127,128]. The tobacco carcinogen NNK is a weak pancreatic carcinogen when administered to adult laboratory rodents [129]. However, when female hamsters were given 10% ethanol in their drinking water throughout their pregnancy while receiving one injection of NNK on the last day of their gestation period, about 60% of the offspring developed pancreatitis-associated PDAC when they were between 8 and 12 months old [130]. The development of these tumors was significantly inhibited when the offspring were subjected to cancer preventive treatments with the COX-2 inhibitor ibuprofen [131] or the β-blocker propranolol (unpublished), suggesting both β-adrenergic signaling and the AA cascade as factors in the development and/or progression of this malignancy. In support of this interpretation, studies with the human PDAC cell lines Panc-1, BXPC-3 showed that NNK induced the release of AA from these cells as well as DNA synthesis via stimulation of the β-2-adrenoreceptor [132]. Isoproterenol and NNK also stimulated the migration of Panc-1 and BXPC-3 cells [133], suggesting that β-adrenergic signaling also regulates invasiveness and metastasis of PDAC. Experiments with immortalized human pancreatic duct epithelial cells, the putative origin of this cancer type, further extended these findings to show a concentration-dependent increase in intracellular cAMP in response to NNK or the classic β-adrenergic agonist, isoproterenol [134]. Antagonists for β-1- adrenoreceptors and β-2-adrenoreceptors inhibited this response. In addition, these studies revealed phosphorylation of EGFR-specific tyrosine kinases and ERK1/2 in response to NNK or isoproterenol, effects completely blocked by the β-blocker propranolol, suggesting transactivation of the EGFR pathway via β-adrenergic signaling. The inhibitor of EGFR-specific tyrosine kinases, AG1478, or the MEK inhibitor, PD98059, also significantly reduced the observed induction of ERK1/2 activation. Treatment of the cells with ethanol caused a concentration-dependent increase in intracellular cAMP and reduced the concentration of NNK from 1 nmol/l to 100 pmol/l required to induce significant activation of PKA, P-CREB, and P-ERK1/2 [135]. This effect is consistent with the concept that agents that increase intracellular cAMP sensitize β-adrenoreceptors, resulting in a requirement of lower concentrations of agonists to elicit responses.

Collectively, these data suggest that β-adrenergic signaling, including transactivation of the EGFR pathway (Fig. 3), is critically involved in the development and/or progression of PDAC and that the tobacco carcinogen NNK utilizes these signaling pathways. In addition, ethanol appears to promote carcinogenic signaling of NNK by sensitizing β-adrenoreceptors, an effect that may contribute to the increased risk of alcoholics, who also smoke, for the development of PDAC.

Regulatory functions of alpha-adrenergic and beta-adrenergic receptors in other cancers

Current knowledge of the role of neurotransmitters and their receptors in the regulation of breast cancer is only rudimentary. Studies with the ER positive (ER+) human breast cancer cell lines ZR-75, MCF-7, and MDA-MB-361 and the three ER negative (ER−) cell lines MDA-MB-435, MDA-MB-453, and MDA-MB-468 have shown that the β-blocker propranolol significantly inhibited DNA synthesis in all cell lines, suggesting an important role of β-adrenoreceptors in the regulation of cell proliferation regardless of the ER status [136]. The antagonist for β-1-adrenoreceptors, atenolol, and the antagonist for β-2-adrenoreceptors, ICI118,551, both significantly reduced the proliferation of all six cell lines, with ICI118,551 having the greater effects. Exposure of ER-cell lines MDA-MB-435 or MDA-MB-453 to isoproterenol additionally stimulated the release of AA from these cells and increased DNA synthesis while having no effect on the ER+ cell lines or the ER− cell line, MDA-MB-468 [136]. Furthermore, it has been shown that NNK or the selective agonist for β-2-adrenoreceptors, formoterol, stimulated influx of potassium via G-protein inwardly rectifying potassium channels, phosphorylation of ERK1/2, and cell proliferation in MDA-MB-453 cells [137]. These findings are in accord with a report that breast cancers that overexpress G-protein inwardly

rectifying potassium channels are more aggressive and less responsive to therapy [138]. Studies with the ER– cell line MDA-MB-468 showed that the physiological agonist for β-adrenoreceptors, norepinephrine, stimulated the migration of these cells and that this effect was primarily mediated by β-2-adrenoreceptors [139]. In addition, MDA-MB-468 cells demonstrated positive chemotaxis toward this neurotransmitter [139]. A recent report that nerve growth factor (NGF) is often overexpressed in breast cancer and that NGF induces tumor growth and angiogenesis while inhibiting apoptosis in breast cancer xenografts [140] introduces yet another potential aspect of β-adrenergic breast cancer regulation: the regulation of neurotrophin synthesis by β-2-ARs. It has thus been shown that agonists for the β-2-AR stimulate the synthesis of NGF in the central and peripheral nervous system [141,142]. The observed high levels of NGF in breast cancer cells may therefore be caused by increased NGF release from nerve endings in the tumor microenvironment in response to β-adrenergic stimulation. In contrast to these stimulatory actions of β-adrenergic signaling on several ER+ and ER– breast cancer cell lines, the opposite effect has also been reported. Studies in MDA-MB-231 cells thus showed significant inhibition of DNA synthesis in response to isoproterenol and this effect was mediated by cAMP [143].

Antagonists for α-1-ARs are widely used for the therapy of benign prostate hyperplasia because of their growth inhibiting effects on prostate cells and some of these agents also induce apoptosis in prostate cancer cells [144]. In addition, it has been shown that norepinephrine stimulates the migration of prostate cancer cells and promotes the development of metastases from prostate cancer xenografts, effects primarily mediated by β-ARs [145,146]. In accord with these experimental findings, β-blockers and α-1-AR antagonists have been shown to reduce the risk of development of prostate cancer [147,148]. Norepinephrine also stimulated the migration of colon cancer cells via β-ARs [149]. In addition, the in-vitro and in-vivo growth of colon cancer cells and gastric cancer cells was stimulated by nicotine-induced release of epinephrine and norepinephrine, resulting in β-adrenergic-receptor-mediated transactivation of the EGFR pathway, including the downstream activation of c-src [68,101,150]. Norepinephrine also stimulated the progression of ovarian and nasopharyngeal cancer cell growth via β-AR-mediated release of VEGF and induction of metalloproteinases [73,151,152]. In addition, chronic stress, which enhances systemic levels of the stress hormones epinephrine and norepinephrine, significantly enhanced the progression of ovarian cancer xenografts [152]. Similarly, psychological stress stimulated the development of fibrosarcomas implanted in the footpads of mice, and this effect was inhibited by the β-blocker propranolol [153].

Collectively, these data identify the sympathicus with its stress-hormone-activated α-ARs and β-ARs as powerful driving forces in the development and progression of some of the most common human cancers. In turn, nicotine and NNK hyperstimulate sympathicus neurotransmission by the release of catecholamines and by the direct activation of ARs, respectively. However, as exemplified with small cell lung cancer earlier in this review, some cancers are inhibited in their growth by β-adrenergic signaling. It has thus been shown that melanoma cells are inhibited in their growth by agonists of α-1-ARs, an effect that involved cAMP-mediated decrease in ERK1/2 activation [154]. Cholangiocarcinoma cells were also inhibited in their growth by agonists for α-1-ARs via cAMP-mediated inhibition of Raf-ERK1/2 signaling [155], whereas S49 lymphoma cells showed induction of apoptosis in response to β-adrenergic cAMP signaling [156].

Gamma-amino butyric acid receptors as a target for the prevention and therapy of cancers stimulated by cyclic adenosine monophosphate signaling

The neurotransmitter GABA is the major inhibitory neurotransmitter in the central nervous system and controls the excitatory effects of cAMP signaling by inhibiting adenylyl cyclase via activation of the inhibitory G-protein ($G\alpha_i$) coupled $GABA_B$ receptor, whereas $GABA_A$ receptors are ion channels with predominantly excitatory functions [157]. GABA and its receptors are also expressed in most non-neuronal tissues. It has been shown that β-blockers inhibit the in-vitro and in-vivo growth of NNK-induced adenocarcinomas of the lung [71,117] and pancreas [132], gastric [69,158] colon [149,159], prostate [146], and breast [136] cancer cells. However, the long-term use of such agents for the prevention or therapy of cancer is problematic. Not only do β-blockers have significant cardiovascular effects, but their chronic use sensitizes β-ARs, a response that would ultimately render these receptors more sensitive to the cancer promoting effects of their physiological agonists. Moreover, most cancers that are stimulated in their growth, metastasis, and progression by cAMP signaling will continue to progress under treatment with β-blockers because a host of other $G\alpha_s$-coupled receptors will continue to stimulate cAMP. By contrast, the use of GABA-ergic agents that reduce the formation of cAMP by inhibiting $G\alpha_s$-mediated activation of adenylyl cyclase seems to be a more promising strategy because these agents have weaker cardiovascular activity and are less likely to sensitize GPCRs. Studies with cell lines from adenocarcinomas of the breast [139] and colon [160] have first shown that GABA inhibits the norepinephrine-induced migration of these cells.

However, the potential exploitation of inhibitory $GABA_B$-R signaling for the prevention or adjuvant therapy of certain cancers will require the careful monitoring of

patients for GABA levels and perhaps systemic cAMP levels. Environmental and lifestyle factors as well as diet, dietary supplements, drug treatments, stress, and pre-existing non-neoplastic diseases can profoundly modulate cell and tissue levels of GABA and cAMP, the number and sensitivity of GABA receptors as well as $G\alpha_s$-coupled receptors, including the β-ARs, that stimulate cAMP signaling. Large intraindividual differences in tissue GABA levels and responsiveness to agonists for $GABA_B$-receptors are therefore to be expected. The literature on the expression and function of GABA and its receptors in neoplastic diseases reflects this diversity. A recent publication from our laboratory thus reported underexpression of GABA in 29 of 30 investigated tissue arrays from human PDACs and $GABA_BR$-mediated inhibition of cAMP-dependent transactivation of the EGFR pathway, cell proliferation, and cell migration in immortalized human pancreatic duct epithelial cells and in human PDAC cell lines, Panc-1 and BXPC-3 [88]. The observed underexpression of GABA is in accord with the increased risk for pancreatic cancer in people with diabetes and pancreatitis because both diseases reduce the number of islet β-cells that are the primary source of GABA in the pancreas [161]. Similar inhibitory actions of GABA and the $GABA_BR$ agonist baclophen on cell proliferation and migration were observed in human small airway-derived PAC cells and NNK-induced, small airway-derived PACs in hamsters underexpressed GABA [87]. By contrast, a Japanese laboratory described overexpression of GABA in five of 15 investigated tissue samples of human PDACs that was associated with the overexpression of the π subunit of the $GABA_A$ receptor [162]. Moreover, these investigators showed that two of seven investigated human PDAC cell lines overexpressed this $GABA_AR$ subunit and were stimulated in their growth by GABA whereas the remaining cell lines were not. Unfortunately, the responses to GABA of the five PDAC cell lines without over-expressed $GABA_A$-π (including Panc-1 used in our study) were not shown although it was mentioned that they were not stimulated by GABA [162]. Differences in GABA contents in a typical Western diet as opposed to a typical Asian diet owing to the high GABA contents in rice [163] may have contributed to the high GABA levels observed in PDACs by the Japanese group. In addition, the described overexpression of the $GABA_AR$, which unlike the inhibitory $GABA_BR$ mediates excitatory responses to GABA, may have reversed the effects of GABA from inhibitory to stimulatory. Similar discrepancies have been reported for colon cancer, with GABA overexpression reported as an indication that GABA signaling may contribute to the carcinogenic process and GABAergic agonists may be of use for cancer intervention [164], whereas others have reported inhibition of colon cancer cell migration by GABA [160]. In conjunction with our current data, these findings emphasize the need for marker-guided cancer intervention as the histopathology classification of cancers fails to provide reliable information on the presence or absence of hyperactive or hypoactive regulatory pathways that may be suitable drug targets in individual cases.

Implications for cancer prevention and therapy

The development of agents for the prevention and therapy of cancer typically involves testing of the drug under study in human cancer cells *in vitro* followed by in-vivo tests in mouse xenografts or experimental tumor models in laboratory rodents. If the drug shows significant anticancer activity in these systems, it moves on into toxicity testing in animals and ultimately into clinical trials in cancer patients. The problem with this approach is that the in-vitro and in-vivo cancer systems are maintained under standardized laboratory conditions devoid of crucial factors that drive the development and progression of cancer in the patient and have profound effects on the responsiveness of cancers to preventive and therapeutic treatments. Data compiled in this review suggest that neurotransmitters of the autonomic nervous system released from the brain, peripheral plexuses, ganglia, and nerves, and the adrenal medulla act as powerful upstream regulators, which orchestrate numerous cell and tissue functions, that are essential for the development and progression of the most common human cancers. Nicotine and the TSNs have the documented ability to hyperstimulate neurotransmission by both branches of the autonomic nervous system. The normal cells of origin of cancers, cancer cells as well as their micro environment are the recipients of these signals and respond with a wide range of adaptative changes in cellular signaling pathways and gene expression. Although some of these responses are caused by direct intracellular signaling downstream of cholinergic and ARs, including the direct transactivation of growth factor pathways, a host of these effects are additionally triggered by the neurotransmitter-mediated release of growth factors, angiogenesis factors, and locally produced stimulatory or inhibitory neurotransmitters from the cancer cells and their microenvironment. Many of the abnormalities that we find in fully developed cancer cells are thus the mere 'footprints' of regulatory disturbances in neurotransmission that may originate in noncancerous organs and tissues. Cancer preventive and treatment strategies that inhibit individual molecules or individual pathways identified in cancer cells, therefore, often work beautifully in our standard testing systems, although disappointing in clinical trials. The inhibitor of EGFR-specific tyrosine kinases gefitinib thus has strong antitumor effects *in vitro* on PAC and PDAC cells without EGFR mutations that render cells susceptible to this class of agents, because the only cancer stimulating agents in the in-vitro environment are EGFR agonists contained in fetal bovine serum. When the same cells are additionally provided with epinephrine, norepinephrine,

isoproterenol, or NNK in the culture medium, their responsiveness to gefitinib is greatly diminished [165]. Similarly, any agents that prevent the development of cancer in laboratory animals maintained in a carefully controlled and stress-free environment will be considerably less effective in people who have elevated levels of stress hormones or are exposed to other agents that continuously hyperstimulate cAMP-responsive cancers. Even conventional cytotoxic chemotherapy and radiation as well as surgical resection will be unlikely to cure the cancer when neurotransmitters released from outside of the cancer tissue continue to stimulate cancerous growth and progression. To add to the complexity of this problem, the expression and function of neurotransmitters is not only cell type specific, but the number and sensitivity of their receptors and downstream effectors is significantly modulated by a host of environmental and lifestyle factors, diet, stress, preexisting chronic diseases, and pharmaceutical treatments. Neither the histopathology diagnosis nor a subclassification by cell type will therefore provide useful information on the potential responsiveness of individual cancers to agents that target neurotransmission and/or signal transduction. Given this level of complexity, it is highly unlikely that any given agent will ever be found that prevents or cures all cancers. It seems to be similarly futile to attempt to block cellular signaling molecules such as tyrosine kinase, ras, or the MAPK cascade that are overexpressed in some cancer cells. First of all, these pathways have essential functions in normal cells, and second, the cancer cells will eventually switch to a different signaling pathway under neurotransmitter control. It is particularly worrisome that numerous agents that are widely used on a daily basis for the prevention of cancer stimulate cAMP signaling and are therefore likely to promote the development of cAMP-responsive cancers. In analogy to the successful approach taken with hormone-responsive cancers, it is necessary to develop tests for the identification of hyperactive and hypoactive neurotransmission in individual patients. In addition, the preclinical development of anticancer agents needs to include in-vitro and in-vivo tests in the presence of elevated epinephrine/norepinephrine as well as acetylcholine. Published evidence compiled in this review supports the hypothesis that the development, progression, and responsiveness to prevention and therapy of the most common human cancers is strongly influenced, if not entirely orchestrated, by an imbalance in stimulatory and inhibitory neurotransmission. The restoration of the physiological balance in neurotransmitters guided by a careful monitoring of the patient before, during, and after treatment should therefore be the key element of successful cancer prevention and therapy. Moreover, analysis and adjustment of factors that may modulate neurotransmission in the individual patient, including lifestyle, diet, disease, pharmacological treatments, and stress, should be routinely included in cancer prevention and treatment regimens. Lessons learned from the clinical management of incurable non-neoplastic conditions, such as diabetes and cardiovascular disease, should assist in developing marker-guided individualized strategies for the successful long-term management of cancer and the prevention of cancer in individuals at risk.

Acknowledgement

This work was supported in part by grants RO1 CA096128 and RO1CA42829 from the National Cancer Institute.

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