Molecular Pathways

Nicotinic Acetylcholine Receptor-Based Blockade: Applications of Molecular Targets for Cancer Therapy

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Abstract

The nicotinic acetylcholine receptor (nAChR) was first characterized in 1970 as a membrane receptor of a neurotransmitter and an ion channel. nAChRs have been shown to be involved in smoking-induced cancer formation in multiple types of human cancer cells. In vitro and in vivo animal studies have shown that homopentameric nAChR inhibitors, such as methyllycaconitine and β-Bgtx, can attenuate nicotine-induced proliferative, angiogenic, and metastatic effects in lung, colon, and bladder cancer cells. Recent publications have shown that α9-nAChR is important for breast cancer formation, and in many in vivo studies, α9-nAChR-specific antagonists (e.g., α-Iml, α-Iml, Vc1.1, RglA, and It14a) produced an analgesic effect. Vc1.1 functions in a variety of animal pain models and currently has entered phase II clinical trials. For cancer therapy, natural compounds such as ginsenoside EGCG have been found to block nicotine- and estrogen-induced breast cancer cell proliferation through inhibition of the α9-nAChR signaling pathway. A detailed investigation of the carcinogenic effects of nAChRs and their specific antagonists would enhance our understanding of their value as targets for clinical translation. Clin Cancer Res; 17(11); 3533–41. ©2011 AACR.

Background

Biological functions of nicotinic acetylcholine receptors

On the basis of their ligand-binding properties, nicotinic acetylcholine receptor (nAChRs) are divided into two classes: (1) α-bungarotoxin (α-Bgtx)-binding nAChRs containing α7 or α9 subunits, which form homopentamers, and (2) α-Bgtx nonbinding nAChRs containing α2–α6 and β2–β4 subunits, which form heteromeric receptors with high affinities for receptor agonists such as acetylcholine and nicotine (1, 2). Each receptor subtype has distinct electrophysiological and pharmacological properties. For example, the αβ2-containing nAChRs have the highest nicotine-binding affinity in mammalian cells (3). Once αβ2 nAChRs are stimulated, dopamine is released in the brain reward pathway, resulting in smoking addiction. In addition, α7- and α9-nAChR receptors have unique properties pertaining to the regulation of signaling mechanisms found in sensory epithelia (α7-nAChR) and other non-neuronal (α9-nAChR) cell types. Homopentamers of α7-nAChR are the most investigated subtype and serve as important channels for Ca2+/Na permeability ratio (12) is 4-fold higher than those of other nAChRs. nAChRs are ligand-gated cation channels, and different subtypes are known to be differentially permeable to calcium ions (Ca2+) (13). However, the role of nAChR-mediated calcium entry in nonneuronal cells is less understood. The calcium permeability of homomeric receptors is significantly higher than that of heteromeric nAChRs. In particular, the α7-nAChR subtype has one of the highest calcium permeabilities. The activation of this receptor can raise cytoplasmic calcium levels and trigger a series of calcium-dependent intracellular processes (14). nAChRs have been observed in various nonneuronal, nonexcitable cells, including cells from the bronchial epithelium, endothelial cells (ECs), keratinocytes, immune cells, vascular smooth muscle cells, and cells from other tissue types. The presence of these receptors in nonneuronal cells seems to suggest that they have distinct functions beyond neurotransmission (15). Several studies have indicated that α7-nAChRs primarily mediate EC proliferation, invasion, and

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angiogenesis (16–19). In addition, it was found that the presence of α7-nAChR inhibitors, such as methyllycaconitine (MLA) and α-Bgtx, can reverse the proangiogenic effects of nicotine (16, 17). However, it is important to note that both α-Bgtx and MLA bind with high affinity to α9-nAChR. Therefore, α9-nAChR may be partially involved in nicotine-induced proangiogenic effects (20). In particular, α7-nAChRs have been found to activate the MAP kinase, PI3-kinase/Akt, and NF-κB pathways, thereby mediating angiogenesis (16–18).

Molecular structure of nAChRs
AChRs are integral membrane proteins that respond to binding of acetylcholine (ACh), which is synthesized, stored, and finally released by cholinergic neurons (21). Like many other ligand-activated neurotransmitter receptors, AChRs are classified according to their pharmacological properties or their relative affinities for various molecules. Therefore, they can be divided into two major subtypes: (1) metabotropic muscarinic AChRs (mAChRs), which are found in vertebrate skeletal muscles, mediate

**Figure 1. nAChR signal transduction.** The nicotinic agonists at the top of the signaling cascades are listed in the order of their affinity for nAChRs. NNK and nicotine bind with highest affinity to the homopentameric nAChRs such as the α7 and α9 subunits, whereas acetylcholine and NNN seem to preferentially bind to heteropentameric nAChRs (α4/β2, α3/β2, or α3/δ4). In lung cancer cells and neuronal cells, the signaling pathways downstream of α7 and heteropentameric nAChRs promote proliferation and angiogenesis by mediating Ca2+-dependent activation of PKA, PKC, PI3K/Akt, and MAPK through the induction of the transcription factors NF-κB, AP1, CREB, and mTOR. On the other hand, nicotine inhibits apoptosis by activating survival factors such as Myc, XIAP, and survivin proteins. In this context, nicotine is also plays a proangiogenic role by releasing MMPs, VEGF, and βFGF from ECs and in the tumor microenvironment. However, in breast tumors, the α9 subunit is the most abundant nAChR, and downstream signaling pathways such as MAPK and PI3K/Akt can be activated through nicotine and E2 stimulation. The downstream transcription factors AP1 and VDR can bind the promoter region of α9-nAChR by activating the ER at Ser-167 and Ser-118, or at Ser-104/106 sites through the PI3K/Akt and MAPK pathways. These signal transduction cascades eventually form a strong positive feedback loop and result in tumor progression. N: nicotine; E2: estrogen.
neuromuscular transmission at the neuromuscular junction, and are particularly responsive to muscarine (22), and (2) ionotropic nAChRs, which are particularly responsive to nicotine and are found not only throughout the peripheral and central nervous systems but also in nonneural tissues (23). Both of these subtypes are activated by the endogenous neurotransmitter ACh and are expressed by both neuronal and nonneuronal cells throughout the body (24).

Previous studies showed that mAChRs are ligand-gated ion channel receptors that are primarily expressed in skeletal neuromuscular junctions and are composed of five subunits: two α1 subunits and one each of β1, δ, and γ (or ε, depending on the stage of development) (25). Only two types of mAChRs are constructed from this complex subunit pool: one is composed of α1, β1, δ, and γ subunits, and the other consists of α1, β1, δ, and ε subunits, both at ratios of 2:1:1:1. In contrast, nAChRs were originally cloned from a neuronal-like cell line and brain cDNA libraries. They are expressed throughout the nervous system and functionally increase neuronal excitability and facilitate synaptic transmission (26). nAChRs can form either homopentamers or heteropentamers containing 10 α-subunits (α1–α10), 4 non-α subunits (β1–β4), δ, γ, and ε. Of note, α8 has only been identified in avian libraries and has not been found in mammals (24). Expression of these nAChR subunits has been observed in many other cell types, including ECs, glial cells, immune cells, and keratinocytes, as well as in gastrointestinal, lung, bladder, colon, and breast tissues.

**nAChR-mediated signaling pathways**

The first evidence that nAChRs regulate cancer growth was reported by Dr. H. M. Schuller in 1989 (27). Since then, various studies have described nAChRs as key molecules that act as central regulators of a complex network governing growth (5, 28), angiogenesis (29), metastasis (7), and apoptosis (30) during carcinogenesis in response to the tumor microenvironment. In addition, nAChRs stimulate intracellular signaling pathways in a cell-type–specific manner. Fig. 1 shows that nicotine-induced upregulation of growth factors (e.g., VEGF and βFGF) and their receptors is one of the major molecular mechanisms underlying the proangiogenic effects of nAChRs in several types of cancer cells (18, 29, 31). Most intriguingly, studies have shown that VEGF and βFGF-induced human microvascular EC migration requires nAChR activation, suggesting that nAChR and VEGF mediate distinct but interdependent pathways of angiogenesis (32). Furthermore, nicotine has been shown to induce activation of NF-kB through the MAP kinase p38 and PI3K/Akt signaling pathways, promoting the survival, proliferation, and angiogenesis of ECs (32). Another study showed that Akt survival signals play an important role in the nicotine-mediated carcinogenic process in human breast cancer cells (10).

A recent study showed that estrogen- and nicotine-induced α9-nAChR expression is transcriptionally regulated by the estrogen receptor (ER) through the PI3K/Akt or MAPK signaling machinery, which mediates phosphorylation of the ER at multiple sites (Fig. 1) (9). Phosphorylated ERs can also act indirectly by altering the activities of other transcription factors (e.g., Sp1, AP1, and NF-kB) at their cognate sites on DNA (33). Fig. 1 shows the potential transcription factor response elements in the promoter regions of α9-nAChR, including two activating protein 1 (AP1) sites and one vitamin D receptor (VDR) site, both of which are responsive to ER binding (34). Thus, the signaling pathways induced by nicotine and estrogen explain the effects of hormones and smoking on human breast cancer formation.

**Smoking-induced cancer formation**

Cigarette smoking has a strong etiological association with the development and progression of several types of cancers. In cigarette smoking, the nicotine-derived metabolic derivatives 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosornicotine (NNN) have been proven to act as initiators of carcinogenesis, especially in lung, breast, and bladder cancers (5, 35–38). In contrast, nicotine is a demonstrated cocarcinogenic factor that promotes carcinogenesis in tobacco replacement therapies (39). However, the mechanisms of nicotine-mediated cell transformation are still uncertain.

**Cell transformation induced by nicotine and its metabolic derivatives.** The average plasma nicotine concentration of active smokers is 100 nM to 1 mM (40). The most potent cigarette smoke carcinogens are polycyclic aromatic hydrocarbons and nicotine-specific metabolites such as NNK and NNN (41). Nicotine and NNK are considered to be carcinogens that react with DNA, and most reports have suggested that the chemical properties of the resulting DNA adducts can cause the diverse genetic changes known to exist in human cancers (35). Accordingly, nicotine and its derivatives can both promote (nicotine itself) and initiate (NNN and NNK) smoking-induced carcinogenesis. In a recent study, we showed that nicotine rapidly (in <30 min) binds to α9-nAChR in normal human breast epithelial (MCF-10A) cells at a physiologically relevant concentration (8 nM) that is below the average plasma concentration of nicotine in active smokers. Similar results were also observed in human small-cell lung cancer cells and pulmonary adenocarcinoma cells (42). These results suggest that apart from active cigarette smoking, passive exposure to smoke is still a hazardous source of nicotine. Several studies have shown NNK-triggered human breast epithelial cell transformation upon long-term (>1 mo) exposure to a low concentration of NNK (100 p.m.) (37). In addition, we recently showed that nicotine (10 μM) can induce transformation in normal breast epithelial cells (MCF-10A) upon forced expression of α9-nAChR (28). Another important concern is that nAChRs can be induced in response to nicotine, NNN, and NNK exposure, which produces a detrimental feedback effect on receptor-ligand binding signaling. Such results indicate that receptor-mediated effects may play an important role in
nicotine-induced carcinogenesis. Therefore, understanding nAChR-mediated mechanisms could aid in the design of novel cancer therapies.

**nAChR activation-mediated tumorigenesis.** Cell-type-specific oncogenesis occurs in response to different nAChR combinations. For example, high levels of α7-nAChR expression promote cancer cell proliferation and metastasis in lung, gastrointestinal, and bladder tissues through ERK and Akt signaling transduction (43). Apart from α7-nAChRs, α4β2-nAChRs are evolutionarily the oldest heteromeric nAChR receptor and are predominantly expressed in mammalian brain and lung tissues (43). It has also been shown that the α4β2-nAChR subtype is crucial for mediating the effects of nicotine-induced dopamine release (44). These effects promote cigarette-smoking behavior through uncontrolled nicotine addiction and thus play a major role in disease progression (45). The α9-nAChR homopentamer was originally identified in hair cells of the inner ear, and is involved in synaptic transmission between efferent nerves and hair cells (46). α9-nAChR was found to have diverse functions in keratinocyte adhesion (47), immune response (48), neuropathic pain (49), and even breast cancer formation (28). The correlation between α9-nAChR mRNA expression level and disease outcome in breast tumor patients was evaluated in a recent study (28). In that study, 186 (67.3%) of 276 paired samples expressed α9-nAChR mRNA at higher levels (mean: 7.84-fold) in breast cancer than in surrounding normal tissue. The highest α9-nAChR mRNA expression levels were detected in smoking-related, advanced-stage breast cancer tissues. These observations led to the conclusion that nicotine binding to nAChRs may play a crucial role in human breast cancer formation.

**nAChR activation-mediated angiogenesis.** Angiogenesis is a complex combinatorial process that is regulated by a balance between pro- and antiangiogenic molecules (16–18). An imbalance between pro- and antiangiogenic factors can result in pathological situations involving either deficit conditions (e.g., inefficient healing and tissue ischemia) or excess angiogenesis (e.g., atherosclerotic plaque development, diabetic retinopathy, and cancer). Emerging evidence shows that nicotine potently induces angiogenesis by mediating its effect on the PI3K/Akt, MAPK, and NF-κB pathways, as shown by abrogation of network formation by the PI3K inhibitor LY294002 (53). In a further investigation, α7-nAChR knockout mice exhibited an attenuated angiogenic response to ischemia and inflammation, including a 27% abrogation of the angiogenic response. Of note, antagonists of nAChRs (e.g., mecamylamine and α-Bgtx) markedly attenuate VEGF-induced angiogenesis (17). Together, these findings indicate that an intricate interplay occurs between the proangiogenic factors VEGF and βFGF and the nAChR cholinergic pathway. Modulation of the activity of this pathway may represent a new therapeutic avenue for disorders characterized by inadequate or pathological angiogenesis.

**nAChR activation-mediated metastasis.** Cell invasion and metastasis are crucial processes in tumor development. Numerous factors, including growth factors, kinases, phosphatases, and extracellular matrix components, play a role in regulating this process. Many growth factors and their downstream effectors are aberrantly activated or overexpressed, contributing to growth deregulation in cancer. It has been reported that the addition of nicotine increases EGF receptor (EGFR) expression in lung, breast, gastric, and colon cells (54–57). After recruiting downstream effectors, growth-related receptors often exert their functions by organizing their downstream effectors to initiate signaling cascades in such pathways as PKC, PI3K/Akt, and MAPK. These signaling cascades affect various biological processes, including cell migration and cancer cell invasion (58, 59). Nicotine has been shown to induce the phosphorylation of multiple subtypes of calpains, resulting in enhanced cell migration and specifically lung cancer metastasis (60, 61). Emerging evidence shows that nicotine potently induces the secretion of multiple types of calpain from lung cancer cells, which promotes the cleavage of various substrates in the extracellular matrix and facilitates metastasis and tumor progression (62). Accordingly, nicotine-treated mice have shown markedly higher tumor recurrence (59.7%) than vehicle-treated mice (19.5%). Nicotine was also found to increase the metastasis of dorsally implanted Line-1 tumors to the lungs 9-fold. Treatment with α-Bgtx significantly inhibited nicotine-induced angiogenesis of Line-1 cells, suggesting that an nAChR subunit is mainly responsible for mediating the proliferative effect of nicotine. Another study also confirmed that NNK enhances the migration of HT29 and DLD-1 colon cancer cells through α7-nAChR-mediated down-regulation of the adhesion molecule E-cadherin and up-regulation of its transcriptional repressors Snail and ZEB1 (7). Interference with α7-nAChR protein expression or treatment with an α7-nAChR–specific inhibitor (MLA) can significantly attenuate α7-nAChR mediated NNK-enhanced colon cancer cell migration.

**Clinical-Translational Advances**

**nAChRs are molecular targets for drug development**

It is well documented that brain nAChRs participate in physiological functions such as attention, memory, and cognition. Clinical data also suggest their involvement in...
neuronal nAChR subunits, and their gene products (9). The difficulty in drug design is that at least 12 genes encode the nAChR structure and function (66). However, the main ligand-bound forms has greatly increased our knowledge of meric nAChRs (65).

However, the majority of known nAChR ligands and types requires the development of subtype-specific drugs. Accordingly, successful treatment of a broad diversity of pentamers with distinct pharmacological properties. Hence, the development of subtype-specific drugs is a major challenge in drug design.

Table 1. nAChR inhibitors with potential clinical applications for cancer therapy

<table>
<thead>
<tr>
<th>Compound</th>
<th>Primary indication</th>
<th>Receptor</th>
<th>Trial stage</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydro-β-erythroidine (DHβE)</td>
<td>Laboratory inhibitor</td>
<td>α4β2</td>
<td>—</td>
<td>(71)</td>
</tr>
<tr>
<td>Eryosidine</td>
<td>Laboratory inhibitor</td>
<td>α4β2</td>
<td>—</td>
<td>(72)</td>
</tr>
<tr>
<td>α-Conotoxin-MII</td>
<td>Laboratory inhibitor</td>
<td>α3β2</td>
<td>—</td>
<td>(75, 76)</td>
</tr>
<tr>
<td>Mecamylamine (TRIDMAC, Inversine)</td>
<td>Depression hypertension</td>
<td>α3β4</td>
<td>Launched</td>
<td>(85, 86)</td>
</tr>
<tr>
<td>MLA</td>
<td>Anxiety</td>
<td>α7</td>
<td>Phase III</td>
<td>(78, 79)</td>
</tr>
<tr>
<td>n-Bgtx</td>
<td>Laboratory inhibitor</td>
<td>α3β2</td>
<td>—</td>
<td>(69, 70)</td>
</tr>
<tr>
<td>α-Bgtx</td>
<td>Laboratory inhibitor</td>
<td>α7α9</td>
<td>—</td>
<td>(80)</td>
</tr>
<tr>
<td>It14a</td>
<td>Pain</td>
<td>α9</td>
<td>—</td>
<td>(87, 88)</td>
</tr>
<tr>
<td>α-ImI, α-ImII</td>
<td>Pain</td>
<td>α9</td>
<td>—</td>
<td>(90, 91)</td>
</tr>
<tr>
<td>α-RGlA</td>
<td>Pain</td>
<td>α9</td>
<td>—</td>
<td>(50, 89)</td>
</tr>
<tr>
<td>Vc1.1 (ACV1)</td>
<td>Pain</td>
<td>α9</td>
<td>Phase II</td>
<td>(92)</td>
</tr>
<tr>
<td>Garcinol</td>
<td>Laboratory inhibitor</td>
<td>α9</td>
<td>—</td>
<td>(8)</td>
</tr>
<tr>
<td>Luteolin</td>
<td>Laboratory inhibitor</td>
<td>α9</td>
<td>—</td>
<td>(10)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Laboratory inhibitor</td>
<td>α9</td>
<td>—</td>
<td>(10)</td>
</tr>
<tr>
<td>EGCG</td>
<td>Laboratory inhibitor</td>
<td>α9</td>
<td>—</td>
<td>(93)</td>
</tr>
</tbody>
</table>

The pathogenesis of several disorders, including Alzheimer’s disease, Parkinson’s disease, schizophrenia, and depression. On the basis of a variety of preclinical and (to a lesser extent) clinical models, it has been hypothesized that nAChRs may be a therapeutic target for studying the effects of L-form nicotine (63). In 2001, the crystal structure of the nAChR protein allowed its characterization as an ACh-binding protein (64). Currently, there are two rapidly developing fields of research involving nicotinic receptor target drugs. One field is focused on aiding drug discovery by bridging the gap between the assembly, activity, and conformational transitions of nicotinic receptors. The other field relies on the development of therapeutically applicable nicotinic receptor ligands that are either competitive (e.g., agonist and antagonist binding) or noncompetitive (e.g., allosteric ligand binding). The positions of noncompetitive (allosteric) ligand-binding sites on heteropentameric nAChRs differ in that the non-α neuronal subunit takes the place of the γ or δ subunit. Competitive ligand-binding sites are formed at the interfaces between homopentameric neuronal α subunits (α7, α8, and α9 nAChRs) and between α (α2–4 or α6) and β subunits (β2 or β4) in heteropentameric nAChRs (65).

Over the last few years, the availability of high-resolution X-ray crystal structures of nAChRs in their ligand-free and ligand-bound forms has greatly increased our knowledge of nAChR structure and function (66). However, the main difficulty in drug design is that at least 12 genes encode the neuronal nAChR subunits, and their gene products (9α and 3β subunits) assemble in various combinations, resulting in a broad diversity of pentamers with distinct pharmacological properties. Accordingly, successful treatment of pathological conditions that involve various neuronal subtypes requires the development of subtype-specific drugs. However, the majority of known nAChR ligands and designed drugs are not subtype-specific (11). Studies in preclinical models showed that nicotine can enhance wound healing via nAChR stimulation (18). Similar results have been obtained in several neurological diseases associated with aging and reduced angiogenesis. For example, decreased levels of nAChRs were detected in the cerebrovascular cells of patients with Alzheimer’s disease (67), suggesting that nicotine-based therapy may be appropriate for neurological disorders. However, another study showed that α7-nAChR agonists used for therapeutic revascularization after myocardial infarction may have proatherogenic activity (31). Additional studies should be done to assess the optimum balance of nAChR activity regulation in clinical patients.

nAChR antagonists as potential agents for molecular cancer therapy

Neurotoxins are commonly used to distinguish between nAChR subunit combinations (68). The neurotoxins lophotoxin, neosurugatoxin, and Bgtx (69, 70), and the alkaloids DHβE (71) and eryosidine (72) are competitive nAChR antagonists that display selectivity for β2-containing nAChRs, particularly the α4β2 subtype (73). Among these nAChRs, the α7-nAChRs are known to be overexpressed in small-cell lung carcinomas associated with smoking (74). Results from in vitro experiments suggest that malignant growth can be halted with the use of snake neurotoxins (α-neurotoxins) or snail conotoxins (α-conotoxins) (75, 76) because they are competitive antagonists of α7-nAChR (77). In vivo animal studies have further shown that α7-nAChR inhibitors, such as MLA (78, 79) and α-Bgtx (80), can reverse the proangiogenic effects of nicotine and inhibit cancer cell growth (16, 17, 19, 81, 82). In a lung airway epithelial cell model (83), normal human bronchial epithelial (NHBE) cells were forced to transform by nicotinic activation of Akt, altering their growth characteristics.
Dysregulated NHBE growth after nicotine administration is consistent with in vivo observations that active smokers have increased proliferative indices compared with former smokers. Protection from prolonged serum deprivation-induced apoptosis conferred by nicotine was found to be attenuatable by LY294002 or DHβE, and protection conferred by NNK was attenuatable by LY294002 or α-BTX. These studies show that in addition to promoting cellular survival and/or transformation, nicotine-induced nAChR activation or NNK-induced Akt signaling is required to diminish contact inhibition and reduce cellular dependence on exogenous growth factors and the extracellular matrix. The results also indicate that specific antagonists can decrease the high levels of α7-nAChR expression in human cancer cells. In addition to α7-nAChR–specific antagonists, α9-nAChR–specific antagonists reduce ascending afferent excitatory pain pathway signaling and have been shown to have analgesic effects in in vivo studies (50, 84). As shown in Table 1, several nicotinic antagonists have either launched or entered phase II clinical trials (85, 86). Unfortunately, most of these antagonists can also cause a variety of side effects resulting from the lack of nAChR subtype specificity. Among these α9-nAChR–specific antagonists (50, 87–91), Vc1.1 has been shown to have profound analgesic effects in a variety of animal pain models and is currently in phase II clinical trials (92). Vc1.1 may have off-label use against α9-nAChR–mediated cancer formation in the future.

Interference with α9-nAChR expression as a novel strategy for drug development

In a recent study, we showed that α9-nAChR was preferentially overexpressed in human breast tumor tissue in comparison with normal tissue (28). We transformed normal breast epithelial cells (MCF-10A) by long-term nicotine treatment (10 μM, >2 mo) together with forced expression of α9-nAChR using the Tet-inducible adenovirus system. The established α9-nAChR overexpressing cells (Tet-Off group) were transplanted into nude mice, resulting in increased tumor growth volume (2.33-fold) when compared with the control (Tet-On) group. Moreover, specific inhibition of α9-nAChR expression using RNA-interference (siRNA) or compounds derived from plants was previously found to concomitantly inhibit cancer cell growth, soft-agar colony formation, and tumor growth in SCID mice (8, 10, 93). Nicotine-induced breast cancer cell proliferation can be inhibited by garcinol (1 μM) derived from the edible fruit *Garcinia indica* through down-regulation of α9-nAChR and cyclin D3 expression (94). A combination of luteolin and quercetin (0.5 μM each) synergistically down-regulates α9-nAChR expression in human breast cancer cells (10). Another study revealed that the tea polyphenol (−)-epigallocatechin-3-gallate (EGCG, 10 μM) attenuates estradiol (10 nM)– and nicotine (10 μM)–induced α9-nAChR protein expression in human breast cancer cells (93). This study further showed that combined treatment with EGCG profoundly inhibits [3H]−Nic/α9-nAChR binding activity, resulting in reduced soft-agar colony formation (>50%) in MCF-7 breast cancer cells (93). Several other studies also showed that in vivo growth of lung and breast cancer cell lines with functional ERs is initiated by estradiol (1–10 nM) stimulation (9, 94–97). These results suggest that EGCG (or other natural compounds such as garcinol, luteolin, and quercetin) may be able to block smoking (nicotine)- or hormone (estradiol)-induced cancer cell proliferation by inhibiting the nAChR signaling pathway.

Conclusion

Over the past few years, the technique of high-resolution X-ray crystallography has revealed the structures and functions of nAChRs in both their ligand-free and ligand-bound forms. It has been shown that nAChRs are selectively overexpressed in a variety of cancers, such as lung cancer (α7-nAChR) and breast cancer (α9-nAChR). Studies have shown that inhibition of nAChR protein levels can significantly attenuate nicotine and NNK-induced cell proliferation in tumor models. Several nAChR-specific agonists and antagonists have been assessed in clinical trials for the treatment of various diseases (excluding cancer); however, a selective antagonist that could specifically target the overexpressed nAChR subtypes that appear in human cancer cells would provide a novel therapeutic strategy. The development of nAChR-specific antagonists for clinical translation is both timely and relevant, and it will enhance our understanding of the carcinogenic role of nAChRs.


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