Acetylcholine Receptor Pathway and Lung Cancer

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Abstract: Genome-wide association studies revealed chromosome regions 15q24-25 were associated with a higher risk for development of lung cancer. The 15q24-25 region encompasses the nicotinic acetylcholine receptor subunit genes (nAchR α3, α5, and β4) that play a role in nicotine addiction. This review reports information of the acetylcholine receptor and lung cancer. In patients diagnosed with smoking-related lung cancer and who continue smoking, a negative correlation with lung cancer survival has been shown. The reduced treatment efficacy may well be explained by the pluriform effect of nicotine on tumor cell proliferation, apoptosis, epithelialmesenchymal transition, proinvasive, and angiogenic effects, which are reenforced by an autocrine/paracrine loop. Overall, there is no evidence that nicotine itself induces cancer, but nicotine promotes in vivo the growth of cancer cells and the proliferation of endothelial cells. This suggests that nicotine after initiation may contribute to the progression phase of cancer development. Continuation of smoking after lung cancer has been diagnosed should be discouraged because smoking may not only support tumor growth but also interferes with treatment.

Key Words: Acetylcholine receptor, Nicotine, Lung cancer, Review.

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This overview aims at providing insight on the acetylcholine receptor (AchR) pathway in lung cancer. Genome-wide association studies provide a powerful approach to identify common, low-penetrance disease loci without prior knowledge of their location or function.[1] Chromosome regions 15q24-25,[2,3] 15q15.33,[4] and 6p21.33[5] seem to have common sequence variants that may convey a higher risk for developing lung cancer. The odds ratios associated with these variants are generally between 1.2 and 1.5, denoting a definite but relatively weak association and support the supposition that susceptibility to lung cancer is polygenic. The 15q24-25 region encompasses the nicotinic acetylcholine receptor subunit genes (nAchR α3, α5, and β4), which play a role in nicotine addiction.[6,7]

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Two types of AchRs exist. The nAchR and muscarinic AchR (mAchR). The nAchR is a pentamer consisting of four related but genetically and immunologically distinct subunits. After assembly, the protein contains two α subunits, and one of each β, γ, and δ subunits.[8] Although in theory many different combinations of subunits exists, only a minority are detected in practice. Factors influencing the assembly are essentially unknown. The nAchR is a Ca^{++} or Na^{+} ion channel. The ratio of Ca^{++} to Na^{+} exchange depends on the AchR subtype, the α7 subtype has the largest Ca^{++} to Na^{+} ratio.

The mAchR receptor belongs to the family of G-protein coupled receptors with seven transmembrane spanning domains and has five subtypes (M1-M5). Similar to nAchR, mAchR is also a protein assembled with genetically distinct subunits. The M1, M3, and M5 subtypes have been linked to cell proliferation and are coupled with Gq, and on activation lead to increased levels of intracellularinositol triphosphate, diacylglycerol, and Ca^{++}. The M2 and M4 receptors are coupled to Gi and inhibit adenylyl cyclase formation.[9]

People who start smoking at an earlier age run a greater risk for long-term nicotine addiction. Recently, it was shown that in three different cohorts of long-term smokers with early nicotine exposure (before the age of 17 years), the ChRNα5-α3-β4 haplotypes were related to the severity of nicotine dependence. Other haplotypes were protective or neutral for dependence of nicotine.[7] The α5-α3 subtype nAchR association of smoking and lung cancer was specific for lung cancer because no evidence for elevated risk was found in never smoking patients with lung cancer or for other smoking-related cancer types (bladder and kidney).[6] The identification of an age- and smoking-dependent susceptibility haplotype reinforces the importance of preventing early exposure to tobacco through public health policies.

BRONCHIAL EPITHELIAL CELLS

Acetylcholine (Ach) is produced and degraded in airway epithelial cells.[10,11] Human bronchial epithelium (HBE) selectively expresses α3, α5, α7, β2, and β4 subunits of the nAchR genes.[12–14] Nicotine and the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(-3-pyridine)-1-butanone (NNK), are agonists for the nAchRs. Remarkably, the affinity of nicotine is greater than for the physiological agonist, Ach.[15]

In an epithelial cell culture model, stimulation with nicotinic derivates (NNK and *N*-nitrosonornicotine) resulted in temporary (1–2.5 hours) up-regulation of α7 subunits of the nAchR[14] and in increased proliferative activity.[16] The

increased proliferation was reduced by nicotinic antagonists (αBTX, see later).[16] Stimulation with NNK showed in one study the involvement of signal transduction effectors STAT1, GATA3, and NFκB, whereas *N*-nitrosonornicotine activates the first two.[16] Nicotine increases activation of AKT within minutes through.[17] Activated AKT increased phosphorylation of downstream substrates such as GSK-3, p70S6K, 4EBP-1, and FKHR.[17] This activation by nicotine was dependent on α3- or α4-containing nAchRs and for NNK on α7-containing nAchRs.[17] The nicotine or NNK concentration required for AKT activation is achievable in smokers.[17]

Incubation of bronchial epithelial cells with nicotine produces GM-CSF[18] that stimulates inflammatory cells.

Interestingly, apoptosis is induced in nonmalignant human bronchial epithelial cells treated with damaging agents such as etoposide, ultraviolet irradiation, or hydrogen peroxide. Nicotine or NNK attenuates this apoptotic effect and results partially in a transformed phenotype manifest as loss of contact inhibition and loss of dependence on exogenous growth factors or adherence to extracellular matrix.[17]

Thus, in epithelial cells, nicotine and NNK have a stimulatory effect on a growth, cell survival, and inflammation.

CANCER

In small cell lung cancer (SCLC) and non-SCLC (NSCLC), the same nAchRα3-α5-α7-β4 subunits as mentioned for the HBE cells are expressed.[17,19,20] In nonsmokers with NSCLC, a difference exists in nAchR expression, with higher expression of nAchR α6-β3 subunit genes present in nonsmokers than in NSCLC from smokers.[21] The mAchR is also expressed in most SCLC and many NSCLC.[22] In lung cancer cells, nicotine and NNK bind to nAchR,[23] whereas nicotine binds also to mAchR.[24] In NSCLC cell lines, nicotine leads to an increase in α7-nAchRs.[25]

Loop

Both NSCLC and SCLC cell lines synthesize and release Ach.[26] In squamous cell carcinoma (SqCC), the enzyme choline acetyltransferase is strongly up-regulated, whereas cholinesterases are down-regulated.[27,28] This combination causes prominent increase in Ach content in SqCC compared with normal lung.[28] The mechanism underlying the increased cholineacetyltransferase expression in SqCC is not clear, although nicotine itself stimulates Ach secretion.[28] In SCLC, mAchR and Ach are coexpressed.[22] Increased levels of Ach provide endogenous proliferative stimuli to both mAchR and nAchR. These findings suggest that an autocrine or a paracrine cholinergic pathway for mAchR and nAchR in SCLC and for nAchR in NSCLC exists see Figure 1.

Modulator of Receptor Activity

Lynx1 is a member of a newly described family of allosteric modulators of nicotinic receptor activity. It has been shown to attenuate responses to Ach and to increase the extent to which Ach and nicotine desensitize nAchR.[28] Lynx1, the nAchR accessory protein, is expressed in normal bronchial epithelium.[29] In SqCC, Lynx1 is expressed at significantly lower levels.[28] Therefore, decreases in Lynx1 may lead to potentiate responses to exogenous nicotine and endogenous Ach.

Proliferation

In lung cancer, nicotine induces proliferation of tumor cells by binding to the α7-nAchR. In this pathway, activation of SRC and Rb-Raf-1/pERK/p90RSK is involved.[30,24] Stimulation with nicotine induces a Ca^{++} influx within seconds, which may last for 15 min.[31,32] Nicotine stimulates tumor growth in a murine model where A549 cells orthotopically grafted.[24] Inhibitors of nicotine at the level of α7-nAchR (α-bungarotoxin and α-cobratoxin) suppress this proliferative effect, suggesting that nAchRs control the rate of proliferation.[24,33]

Stimulation of the mAchR in SCLC induced Ca^{++} release, MAPK, and AKT activation, which is associated with proliferation. Blocking experiments (antagonists and siRNA) showed that M3 subtype was responsible for Ca^{++} release and reduced MAPK, AKT, and inhibited proliferation.[22]

Nicotine induces an increase in fibronectin production.[25] Extracellular fibronectin binds to $\alpha5\beta1$ integrin, which subsequently leads to increased proliferation through ERK, PI3-K, and mTOR pathways. This indirect proliferative effect of nicotine is maximal at days 4 to 5[25] and could be blocked at the level of the $\alpha5\beta1$ integrin receptor. These data show that nicotine has a direct short term and an indirect longer term effect on proliferation.

Apoptosis

Similar to the effect in HBE, nicotine diminishes apoptosis in SCLC and NSCLC cell lines.[34–36] The antiapoptotic effects of nicotine were mediated by nAchR-α3 and required the AKT pathway, leading to an increased recruitment of E2F1 and concomitant dissociation of retinoblastoma tumor suppressor protein. The binding of E2F1 to the promoter of survivin and XIAP genes resulted in the up-regulation of these inhibitors of apoptosis.[35] α7-nAchR antagonists inhibited proliferation of NSCLC through mitochondria-associated apoptosis.[36] Thus, nicotine has a prolonged effect on tumor cell survival by preventing apoptosis.

Invasion

Pathways that contribute to the proinvasive effects of nicotine include α7-nAchR-mediated activation of Src, calcium channels, and up-regulation of EGFR. Nicotine induced down-regulation of epithelial markers, E-cadherin and β-catenin, whereas it caused concomitant increase of mesenchymal proteins fibronectin and vimentin in A549 human NSCLC.[37] These effects are part of the proinvasive epithelial-mesenchymal transition phenomenon.

Moreover, fibronectin may confer resistance to apoptosis induced by chemotherapy as has been shown in SCLC.[38] Beside the effect on fibronectin, nicotine also stimulated HIF-1α, with subsequent increase in VEGF, stimulating angiogenesis and invasion.[39] These data demonstrate proinvasive and angiogenic effects of nicotine.

Survival

In patients diagnosed with smoking-related lung cancer and who continue smoking, a negative correlation with lung

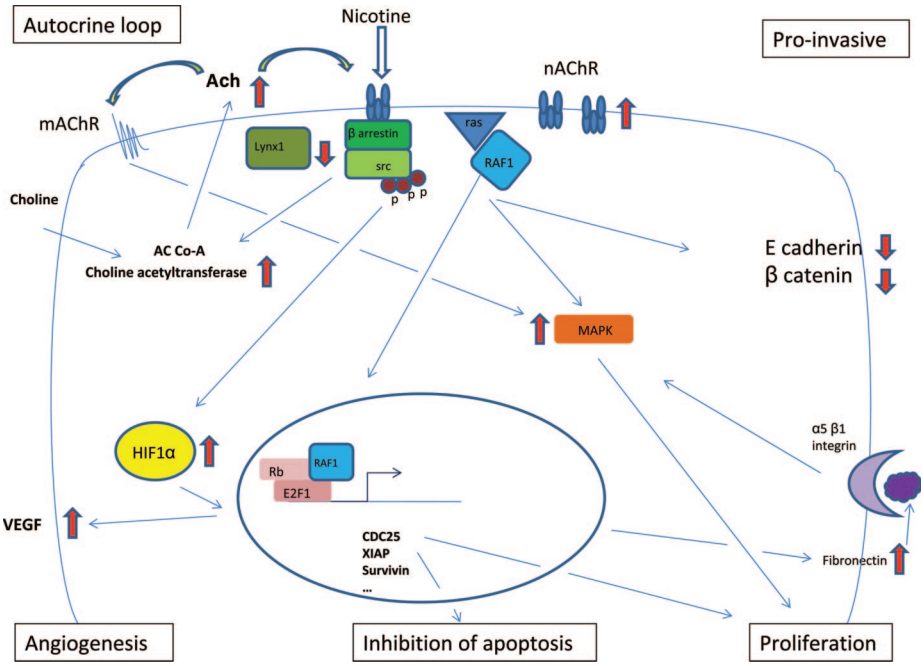

FIGURE 1. The acetyholine receptor (AchR) pathway plays a pivotal role in promotion of lung cancer. Nicotine binds with higher affinity than physiologic ligand acetylcholine (Ach) to nicotinic AchR (nAchR). Binding of ligand to nAchR results in Ca^{++} influx, binding of β-arrestin and SRC. One subsequent effect is increase of Ach synthesis. Ach binds to nAchR and to muscarinic AchR (mAchR). The coexpression of Ach and AchR has been shown in SCLC and NSCLC, pointing to an autocrine or paracrine loop. Lynx1 modulates the effect of activated nAchR. nAchRs may show less desensitization from endogenous Ach and exogenous nicotine in case of decreased levels of Lynx1. Ligand binding to nAchR and mAchR lead to activated MAPK and proliferation after a few hours. Nicotine leads to increase fibronectin synthesis, which binds extracellular to $\alpha 5\beta 1$ integrin. This in turn results in increase of MAPK, leading to proliferation with a peak after 5 days. Nicotine inhibits mitochondrial apoptosis induced by chemotherapy, UV radiation, and hydrogenperoxide, thereby, prolonging cell survival. Nicotine decreases E-cadherin and β-catenin and in combination with fibronectin it increases proinvasive effects possibly due to the loss of contact inhibition. Nicotine also leads to increase in HIF1α, with subsequent increase of VEGF stimulating neoangiogenesis. All these effects can be blocked at the level of the nAchR and the mAchR.

cancer survival has been shown.[40–42] The reduced treatment efficacy may be well explained by the pluriform effect of nicotine on tumor cell proliferation, apoptosis, epithelial-mesenchymal transition, proinvasive, and angiogenic effects, which are reenforced by the autocrine or paracrine loop.

Possible Treatment Options

These effects of nicotine in lung cancer raise the question whether inhibition of α7-nAchR activity by nontoxic agents or inhibition of its downstream mediators might open novel avenues for the therapy for cancers promoted by smoking.[37] Members of the type II α-neurotoxin subfamily have high affinity for α7-nAchR. Examples are α-CbT and α-bungarotoxin.[36,39] The latter acts as an antagonist to both α7-nAchR and mAchR.

Although treatment with nicotinic receptor antagonists can have significant effects on blood pressure, mAchR antagonists are better tolerated and are widely used for treatment of chronic obstructive pulmonary disease.[43] Therefore, it is not excluded that muscarinic antagonists might serve as a useful adjuvant to conventional chemotherapeutic regimens for SCLC and NSCLC, which express the autocrine cholinergic loop.

Overall, there is no evidence that nicotine itself induces cancer, but nicotine promotes in vivo the growth of cancer cells and the proliferation of endothelial cells. This suggests that nicotine after initiation may contribute to the progression phase of cancer development. Continuation of smoking after lung cancer has been diagnosed should be discouraged because smoking may not only support tumor growth but also interferes with treatment.

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