From smoking to lung cancer: the CHRNA5/A3/B4 connection

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that modulate key physiological processes ranging from neurotransmission to cancer signaling. These receptors are activated by the neurotransmitter, acetylcholine, and the tobacco alkaloid, nicotine. Recently, the gene cluster encoding the α3, α5 and β4 nAChR subunits received heightened interest after a succession of linkage analyses and association studies identified multiple single-nucleotide polymorphisms in these genes that are associated with an increased risk for nicotine dependence and lung cancer. It is not clear whether the risk for lung cancer is direct or an effect of nicotine dependence, as evidence for both scenarios exist. In this study, we summarize the body of work implicating nAChRs in the pathogenesis of lung cancer, with special focus on the clustered nAChR subunits and their emerging role in this disease state.

Oncogene (2010) 29, 4874–4884; doi:10.1038/onc.2010.256; published online 28 June 2010

Keywords: nicotine addiction; lung cancer; nicotinic acetylcholine receptors; CHRNA5/A3/B4 gene cluster

Introduction

Tobacco use is the leading cause of preventable mortality around the world, resulting in more than 5 million deaths per year (WHO, 2009). Approximately 600,000 of these deaths are because of second-hand smoke, with one-third of the adult population exposed to second-hand smoke globally. In the United States, overall tobacco use has been declining but approximately 46 million adults still smoked in 2008 (CDC, 2009). If current trends persist, tobacco may kill a billion people by the end of this century.

The list of diseases caused by tobacco use is expanding, according to a recent Surgeon General’s report on the health effects of smoking (HHS, 2004). A causal relationship was reported between active smoking and cardiovascular diseases, respiratory diseases, reproductive disorders and several types of cancers, including cancers of the lung, bladder, cervix, esophagus, kidney, larynx, mouth, pancreas, stomach as well as leukemia.

Cigarette smoke contains 4000 chemicals, 250 of which are known to be harmful, and at least 50 of which are carcinogens (Shields, 2002). The most potent of these carcinogens are polycyclic aromatic hydrocarbons and nicotine metabolites such as 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosornornicotine (NNN). These nitrosamines form DNA adducts that cause mutations leading to cancer (Hecht and Hoffmann, 1988). In the following sections, we review evidence accumulated through the years (see Timeline in Figure 1) showing that nicotine, itself, promotes lung cancer through its interaction with nicotinic acetylcholine receptors (nAChRs).

Nicotinic acetylcholine receptors

nAChRs are a heterogeneous family of ligand-gated cation channels activated by the endogenous neurotransmitter acetylcholine (ACh) and exogenous chemicals such as nicotine and its metabolites. nAChRs were the first receptors to be characterized at the biochemical, biophysical, molecular and pharmacological levels and have served as prototypes for all other ligand-gated ion channels, including those activated by 5-HT3 (5-hydroxytryptamine), GABA_A and GABA_C (γ-aminobutyric acid) and glycine (Le Novere and Changeux, 1995, Taly et al., 2009). Ligand binding induces a conformational change causing the channel to open, thereby allowing the flow of Na^+, K^+ and Ca^{2+} ions down their electrochemical gradients. The propensity of nAChRs to flux intracellular calcium levels is important in the activation of downstream signaling cascades (Fucile, 2004).

nAChRs can be classified into two main categories: muscle or neuronal receptors. Muscle nAChRs are expressed primarily in skeletal neuromuscular junctions and are composed of the α1, β1, δ and ε or γ subunits (McGehee and Role, 1995). In contrast, neuronal nAChRs were originally cloned from neuronal-like cell lines and brain complementary DNA libraries, hence their name, and are expressed throughout the nervous system, in which they increase neuronal excitability and facilitate synaptic transmission (McGehee and Role, 1995; Dani and Bertrand, 2007; Albuquerque et al., 2009). In all, 12 neuronal nAChR subunits have been identified, namely α2–α10 and β2–β4 (Patrick et al.,...
Expression of these subunits has also been observed in many other cell types, including endothelial cells, gastrointestinal tissue, glia, immune cells, keratinocytes and lung tissue (Battaglioli et al., 1998; Macklin et al., 1998; Maus et al., 1998; Nguyen et al., 2000; Arredondo et al., 2001; Wang et al., 2001; Kawashima and Fujii, 2003; Spindel, 2003; Gahring et al., 2004; Gahring and Rogers, 2006; Wessler and Kirkpatrick, 2008).

nAChRs are integral membrane proteins composed of five subunits symmetrically arranged around a central pore (Figure 2a) (Corringer et al., 2000). Each nAChR subunit consists of a large extracellular amino-terminal domain, four transmembrane domains and a short extracellular carboxy-terminal domain (Figure 2b) (Unwin, 2005). The large extracellular domain of α-subunits contains adjacent cysteines important for ligand binding, whereas β-subunits lack these residues (Albuquerque et al., 2009). Unlike other α-subunits, however, α5 does not contribute to ligand binding as it is missing a key tyrosine residue (Karlin, 2002). Importantly though, incorporation of the α5 subunit into a mature receptor does alter receptor biophysical properties such as increasing the calcium conductance (Gerzanich et al., 1998).

The combination of different nAChR subunits can lead to the formation of a vast array of nAChR subtypes. The α2–α6 subunits can form heteromeric receptors with the β2–β4 subunits, whereas the α7–α9 subunits can form homomeric receptors that are blocked by α-bungarotoxin (Couturier et al., 1990; Schoepfer et al., 1990; Elgoyhen et al., 1994). In addition, α9 can form a heteromeric receptor with α10 (Elgoyhen et al., 2001; Lustig et al., 2001) and α7 can form a heteromeric receptor with β2 (Liu et al., 2009). Each of these receptor subtypes has distinct electrophysiological and pharmacological properties (Role and Berg, 1996; Boyd, 1997; Gerzanich et al., 1997; Albuquerque et al., 2009).

The functional diversity by the nAChR family offers abundant prospects for the design of novel therapeutics. Hence, nAChRs are being actively investigated as drug targets for nervous system disorders, including Alzheimer’s disease, anxiety, attention deficit hyperactivity disorder, depression, epilepsy, pain, Parkinson’s disease, schizophrenia, Tourette’s syndrome and nicotine addiction (Lloyd and Williams, 2000; Arneric et al., 2007; Romanelli et al., 2007).

The α3/α5/β4 nAChR subunit gene cluster

In recent years, a series of linkage analyses, candidate-gene association studies and genome-wide association studies have pointed to a possible role for the α3, α5 and β4 nAChR subunits in both nicotine addiction and lung cancer (Schlaepfer et al., 2008; Amos et al., 2008; Berrettini et al., 2008; Bierut et al., 2008; Hung et al., 2008; Portugal and Gould, 2008; Spitz et al., 2008; Stevens et al., 2008; Thorgeirsson et al., 2008; Wacholder et al., 2008; Weiss et al., 2008; Caporaso et al., 2009; Freathy et al., 2009; Pillai et al., 2009; Sacci et al., 2009a; Sasaki et al., 2010). The genes that encode the α3, α5 and β4 nAChR subunits lie in a genomic cluster in strong linkage disequilibrium with each other (Figure 3) (Boulter et al., 1990). These three genes encode a predominant nAChR subtype expressed in the peripheral nervous system (Leonard and Bertrand, 2001).

The function of the clustered subunits can be gleaned from knockout (KO) animal studies. These studies have shown that the α3 subunit is necessary for survival, with homozygous KO mice dying perinatally because of multiorgan dysfunction (Xu et al., 1999a). α3 KO mice have enlarged bladders, causing bladder infection, dribbling urination and urinary stones—a phenotype resembling that of a rare human condition called megacystis-microcolon-intestinal hypoperistalsis syn-
drome. Patients with this disease also do not appear to express α3 mRNA (Richardson et al., 2001). α3 KO mice also show extreme mydriasis and lack of pupil contraction in response to light, with loss of bladder contraction in response to nicotine (Xu et al., 1999b). Furthermore, α3 heterozygous mice are partially resistant to nicotine-induced seizures compared with wild-type littermates (Salas et al., 2004a). In contrast, α5 and β4 KO mice are both viable and lack any gross abnormalities (Xu et al., 1999b; Wang et al., 2002, 2003). However, α5 and β4 KO mice do show autonomic dysfunction and are less sensitive to nicotine. Mice lacking α5 are also more susceptible to experimentally induced inflammatory bowel disease (Orr-Urtreger et al., 2005) whereas β4 KO mice show less anxiety in behavioral tests (Salas et al., 2003).

The observation that the α3, α5 and β4 genes are co-expressed and co-regulated in many cell types in the nervous system is consistent with the hypothesis that their expression is because of coordinated transcriptional regulation. The three subunits are highly expressed in the peripheral nervous system as well as in several regions of the brain, including the brain stem, cerebellum, hippocampus, interpeduncular nucleus, medial habenula, pineal gland and the ventral tegmental area (Quick et al., 1999; Xu et al., 1999a; Flora et al., 2000b; Klink et al., 2001; Perry et al., 2002; Zoli et al., 2002; Salas et al., 2003; Gahring et al., 2004; Salminen et al., 2004; Turner and Kellar, 2005). Furthermore, mRNA levels of the three genes are coordinately upregulated during neural development and differentiation (Corriveau and Berg, 1993; Levey et al., 1995; Levey and Jacob, 1996; Zhou et al., 1998).

Efforts have been made to understand the regulatory mechanisms governing the expression of the clustered nAChR subunit genes. Sequencing of the individual gene promoters has revealed that each promoter lacks classical CAAT and TATA boxes (Boulter et al., 1990). Instead, the promoters are GC rich and contain several binding sites for the transcription factor, Sp1. Sp1 regulates transcription of each of the clustered subunit genes through multiple binding sites in each individual promoter (Yang et al., 1995; Bigger et al., 1997; Campos-Caro et al., 1999, 2001; Melnikova et al., 2000; Terzano et al., 2000; Flora et al., 2000a; Melnikova and Gardner, 2001; Valor et al., 2002). Chromatin immunoprecipitation experiments have confirmed Sp1 binding in the context of native chromatin.

Figure 2 Structure of the nAChR. (a) Schematic representation illustrating the pentameric arrangement of subunits in an assembled nAChR. (b) Conserved domains of a nAChR subunit including the amino (N) and carboxy (C) terminals, transmembrane segments (M1–M4) and the intracellular loop. (c) Assembly of heteromeric and homomeric nAChR subtypes. Individual nAChR subunits are represented as colored circles, with diamonds representing ligand-binding sites. Pentagons in the center of each pentamer represent the pore region.

Figure 3 The human nAChR α3/α5/β4 gene cluster. Green boxes represent exons and orange boxes represent untranslated regions. Black lines located between green boxes represent introns whereas gray lines represent intragenic regions. The boundaries for each gene are labeled with corresponding Genbank annotations. Horizontal arrows indicate the direction of transcription. Vertical red arrows indicate SNPs associated with nicotine dependence and lung cancer.
for all three promoters (Benfante et al., 2007; Scofield et al., 2008). It is likely that Sp1 is involved in tethering the basal transcription machinery to the TATA-less nAChR subunit gene promoters (Pugh and Tjian, 1991). In addition to the Sp1 regulation common to all three promoters, other transcription factors have been found to govern expression of the clustered genes either independently or coordinately, including achaeolecute complex homolog-1 (ASCL1), Brn-3a-c, c-Jun, hnRNPK, PHOX2A, Pur and SCIP (Yang et al., 1994; Milton et al., 1996; Bigger et al., 1997; Du et al., 1997, 1998; Liu et al., 1999; Melnikova et al., 2000; Benfante et al., 2007; Improgo et al., 2010). Two regulatory elements have also been found that direct the expression of the clustered nAChR genes in a tissue-specific manner: the 3′-untranslated region, and CNR4, a conserved non-coding region located 20 kb upstream of β4 (Xu et al., 2006). Recently, we showed that a 2.3-kb fragment of the β4 gene promoter directs spatially and developmentally regulated expression of a reporter gene in vivo (Bruschweiler-Li et al., 2010). Whether this region also regulates expression of the z3 and z5 genes remains to be determined.

Role of nAChRs in nicotine addiction

Nicotine is one of the most widely consumed psychoactive drugs in the world and is the primary reinforcing chemical in tobacco (Stolerman and Jarvis, 1995). Nicotine addiction is initiated upon nicotine-mediated activation of nAChRs in the mesolimbic dopaminergic pathway, known as the reward circuitry of the brain (Corrigall et al., 1992; Di Chiara, 2000; Dani and De Biasi, 2001). Dopaminergic neurons in this pathway originate in the ventral tegmental area and project to the nucleus accumbens (NAc) and the prefrontal cortex. Activation of nAChRs expressed in the ventral tegmental area ultimately causes an increase in the firing of dopaminergic neurons, resulting in an increase of dopamine release in the NAc (Calabresi et al., 1989; Nisell et al., 1994; Pontieri et al., 1996; Pidoplichko et al., 1997). Expression of z4- and β2-containing receptors in the ventral tegmental area is necessary and sufficient for nicotine-mediated dopamine elevation in the NAc (Picciotto et al., 1998; Marubio et al., 2003; Maskos et al., 2005; Pons et al., 2008). z4β2 nAChRs are also critical for nicotine reward/reinforcement, sensitization and tolerance (Picciotto et al., 1998; Tapper et al., 2004, 2007; Pons et al., 2008). Elevation of dopamine levels in the NAc reinforces drug use and is critical for the onset and maintenance of nicotine dependence (Di Chiara and Imperato, 1988). Conversely, inhibiting dopamine elevation through lesions or pharmacological blockade attenuates the rewarding effects of nicotine (Corrigall and Coen, 1991).

Nicotine dependence is a consequence of both positive reinforcement and avoidance of the aversive effects of cessation (Kenny and Markou, 2001). Smoking cessation produces withdrawal symptoms, which account for the high incidence of relapse in people attempting to quit smoking (Corrigall et al., 1989; Kenny and Markou, 2001). The withdrawal syndrome involves both mood-oriented (afffective) and physical (somatic) symptoms (De Biasi and Salas, 2008). The z5- and β4-containing nAChRs as well as z7 nAChRs seem to be involved in the physical symptoms of withdrawal as somatic signs are diminished in z5, z7 and β4 KO mice (Salas et al., 2004b, 2007; Jackson et al., 2008). Conversely, affective symptoms are absent in β2 KO mice but are readily observable in z5 and z7 KO mice (Jackson et al., 2008; Portugal et al., 2008).

Results of the aforementioned genetic studies also support the role of the z3, z5 and β4 subunits in nicotine dependence. In a candidate-gene study targeting 348 genes, smokers of European descent who developed nicotine dependence were compared with smokers who were not dependent (Saccone et al., 2007). In this study, several single-nucleotide polymorphisms (SNPs) associated with nicotine dependence were found within the z5/z3/β4 gene cluster. Of particular interest is the non-synonymous SNP, rs16969968, found in the fifth exon of the z5 gene. This polymorphism changes an aspartic acid residue into asparagine at position 398 (D398N) in the second intracellular loop of z5. Receptors expressing the aspartic acid variant show greater maximal response to nicotine, causing higher intracellular calcium levels (Bierut et al., 2008). Individuals with one copy of the minor allele were found to have a 1.3-fold increased risk for nicotine dependence, whereas individuals with two copies of this risk variant have almost a twofold increase in risk (Saccone et al., 2007). In addition, rs16969968 was found to be associated with pleasurable responses during smoking initiation among Caucasians (Sherva et al., 2008).

Other SNPs highly correlated with rs16969968 also influence the risk for nicotine dependence, such as rs1051730 found in exon 5 of z3 and rs578776 found in the z3 3′-untranslated region (Saccone et al., 2007). The latter had an even stronger association with nicotine dependence. These same SNPs were associated with increased smoking intake in an independent study analyzing 219 European American families (Bierut et al., 2008). Furthermore, these SNPs were associated with early-onset smoking, a phenotype associated with more severe nicotine dependence in adults (Weiss et al., 2008). In addition, rs1051730 was found to be strongly associated with smoking quantity in an Icelandic population (Thorgerisson et al., 2008) and was associated with decreased likelihood of quitting during pregnancy in women of European descent (Freathy et al., 2009). These studies provide compelling evidence for the role of the z5/z3/β4 gene cluster in nicotine dependence.

Role of nAChRs in lung cancer

Smoking is the major risk factor associated with lung cancer, the leading cause of cancer-related deaths for...
both men and women (ACS, 2009). Lung cancer is also the second most common form of cancer in both sexes, with an overall 5-year survival rate of 15%. The two major histopathological types of lung cancer are small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). NSCLC can be subdivided into adenocarcinoma, squamous cell, bronchioalveolar and large cell lung carcinoma. In SCLC, >95% of patients have a history of cigarette smoking and the 5-year survival rates for these patients can reach as low as 2% (Jackman and Johnson, 2005).

Several lines of evidence indicate that nAChRs have a role in lung carcinogenesis as discussed in the following sections. nAChRs are expressed in both normal and lung cancer cells (Schuller, 1989; Maneckjee and Minna, 1990; Maus et al., 1998; Wang et al., 2001; Song et al., 2003; Lam et al., 2007; Sartelet et al., 2008; Improgo et al., 2010). The clustered nAChR subunits, in particular, are overexpressed in SCLC (Improgo et al., 2010). This overexpression seems to be regulated by ASCL1 (Improgo et al., 2010), a basic helix-loop-helix transcription factor that is also overexpressed in SCLC (Ball et al., 1993). Transgenic mice that constitutively express ASCL1 and the SV40 large T antigen develop aggressive lung tumors with SCLC features (Linnola et al., 2000). Upregulation of the clustered nAChRs by ASCL1 provides a mechanism by which the effects of nicotine and other nAChR ligands are potentiated in SCLC, contributing to the aggressiveness of this type of lung cancer (Improgo et al., 2010). Additional evidence for a role of the clustered nAChR genes in lung cancer comes from the recent demonstration that the α3 subunit gene is a frequent target of aberrant DNA hypermethylation and silencing in lung cancer (Paliwal et al., 2010).

nAChRs and cell proliferation

The various ligands that activate nAChRs promote the development and progression of lung cancer through different mechanisms. First, ACh is synthesized by and acts as an autocrine growth factor for SCLC (Song et al., 2003). ACh has also been shown to activate signaling pathways vital for growth and differentiation of human epithelial cells (Grando, 2008). Similarly, nicotine can induce cell proliferation in a manner reminiscent of classical growth factors activating cancer signaling pathways. Specifically, nicotine treatment has been shown to cause physical interactions between the retinoblastoma protein and the signaling kinase Raf-1, leading to downstream events such as inactivation of cyclins and cyclin-dependent kinases, dissociation of the transcription factor E2F1 from retinoblastoma protein, binding of E2F1 to proliferative promoters causing their transcription and entry into S phase (Dasgupta and Chellappan, 2006; Egleton et al., 2008). In addition, nicotine treatment can increase the levels of growth factors such as brain-derived neurotrophic factor, hepatocyte growth factor, platelet-derived growth factor, transforming growth factor-α and -β, vascular endothelial growth factor (VEGF) and VEGF-C as well as their corresponding receptors (Conti-Fine et al., 2000). Moreover, nicotine activation of epidermal growth factor receptor seems to involve increases in intracellular calcium levels (Sher et al., 1998). Nicotine also stimulates NSCLC cell proliferation by upregulating fibronectin expression while downregulating epithelial markers such as E-cadherin and β-catenin (Zheng et al., 2007; Davis et al., 2009). Nicotine-induced fibronectin expression is associated with activation of the extracellular signal-regulated kinase and the phosphoinositide 3-kinase/mammalian target of rapamycin signaling pathways and is abrogated by treatment with the α7 nAChR antagonist, α-bungarotoxin (Zheng et al., 2007). This group also showed that nicotine induces NSCLC cell proliferation by stimulating the expression of the nuclear hormone receptor, peroxisome proliferator-activated receptor-β/δ, an effect that can be blocked by α-bungarotoxin, α7 nAChR short interfering RNA and phosphoinositide 3-kinase inhibitors (Sun et al., 2009). Taken together, these results suggest that nicotine increases peroxisome proliferator-activated receptor-β/gene expression through α7 nAChR-mediated activation of phosphoinositide 3-kinase/mammalian target of rapamycin signals leading to cell proliferation (Zheng et al., 2007; Sun et al., 2009). Nicotine also promotes cell proliferation in other types of cancers: it promotes growth of gastric tumors by activating extracellular signal-regulated kinase and cyclooxygenase-2 and promotes growth of colon cancer through epidermal growth factor receptor, c-Src and 5-lipoxygenase-mediated signaling pathways (Shin et al., 2004; Ye et al., 2004).

nAChRs and apoptosis

Maneckjee and Minna (1994) first showed that low concentrations of nicotine confer resistance to apoptosis in lung cancer cells. Since then, nicotine has been shown to inhibit apoptosis induced by various stress stimuli, including ultraviolet radiation, oxidative stress and exposure to opioids, Ca2+ ionophores, neurotoxins and anticancer drugs (Zeidler et al., 2007; Egleton et al., 2008). This apoptotic inhibition seems to involve several signaling pathways. One mechanism involves phosphorylation and consequent activation of the anti-apoptotic protein, B cell lymphoma gene 2 by protein kinase Cz and phospholipase C (Mai et al., 2003). Consistently, nicotine inactivates the pro-apoptotic functions of Bax and Bad (Jin et al., 2004; Xin and Deng, 2005). Another mechanism involves nicotinemediated activation of Akt (also called protein kinase B), a serine-threonine kinase whose activation leads to apoptotic inhibition and tumorigenesis (Scheid and Woodgett, 2001). Nicotine exposure causes site-specific phosphorylation of Akt at Thr308 and Ser473 as well as phosphorylation of downstream Akt substrates such as mammalian target of rapamycin, FKHR, elf-4, GSK3B, tuberin and S6K (West et al., 2003). The use of
pharmacological agents suggests that this process involves z3-containing nAChRs. In the same study, increased Akt activation was observed in lung cancer tissue from smokers. Further evidence implicating z3 in Akt signal transduction is a recent report showing that small hairpin RNA-mediated depletion of the z3 subunit leads to a dramatic Ca2+ influx in a NSCLC cell line that was followed by activation of the Akt pathway (Paliwal et al., 2010). In this study, NSCLC cells, in which the z3 subunit was depleted, were resistant to apoptosis-inducing drugs.

nAChRs and angiogenesis

Endothelial cells express nAChRs as well as key molecules for cholinergic signaling such as choline acetyltransferase and acetylcholinesterase (Macklin et al., 1998; Wang et al., 2001). In these cells, ACh is thought to act in an autocrine or paracrine manner to stimulate angiogenesis (Cook and Ghrebremariam, 2008). Nicotine also functions as a proangiogenic agent, activating both physiological and pathological angiogenesis through the phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways (Heeschen et al., 2001). Analogous to angiogenic cytokines, nicotine promotes endothelial cell migration, proliferation, survival, tube formation and nitric oxide production and can be as potent as fibroblast growth factor (Cooke and Orloff, 1998). NNK preferentially activates a3 nAChR antagonist mecamylamine (Zhu et al., 2003). Even in the absence of exogenous nicotine, angiogenic processes stimulated by VEGF or fibroblast growth factor can be blocked by nAChR antagonists such as mecamylamine and hexamethonium and the α7-selective antagonist α-bungarotoxin (Cook and Ghrebremariam, 2008). In lung cancer cells, nicotine also induces the expression of hypoxia-inducible factor-1α, a transcription factor that promotes hypoxia-induced angiogenesis (Zhang et al., 2007).

nAChRs and the immune system

The function of nAChRs in immunity and cancer has two aspects. The first involves the complex interplay between the inflammatory effects of irritants in cigarette smoke and the anti-inflammatory effects of nicotine (Gahring and Rogers, 2006). Chronic inflammation triggered by tobacco smoke has been shown to promote lung carcinogenesis (Takahashi et al., 2010). Inflammation induced by cigarette smoke also promotes chronic obstructive pulmonary disease, a disease associated with increased lung cancer risk (Punturieri et al., 2009; Grivennikov et al., 2010). Chronic inflammation increases cancer risk by influencing every stage of cancer from initiation, promotion, invasion and metastasis through induction of oncogenic mutations and genomic instability, local immunosuppression and angiogenesis (reviewed in Grivennikov et al., 2010). In contrast, nicotine itself seems to suppress immune function and has been shown to be protective against inflammatory diseases such as pneumonia and ulcerative colitis (Rubin and Hanauer, 2000; Blanchet et al., 2004; Shivji et al., 2005). Suppression of the immune response by nicotine may affect immune surveillance, preventing the clearance of nascent tumor cells (Gahring and Rogers, 2006; Grivennikov et al., 2010).

The second aspect of nAChR function in immunity and cancer involves the production of autoantibodies against nAChRs in cancer patients with paraneoplastic syndromes (Gahring and Rogers, 2006). In particular, antibodies against z3 nAChRs have been detected in the serum of SCLC patients who show autonomic neuropathy (Vernino et al., 1998, 2000). Dysautonomia caused by these autoantibodies is characterized by symptoms such as impaired papillary light reflex, gastrointestinal dysmotility and bladder dysfunction that are reminiscent of those observed in z3 KO mice (Xu et al., 1999a; McKeon et al., 2009).

Carcinogenic nitrosamines as nAChR ligands

Nicotine-derived nitrosamines such as NNK and NNN activate nAChRs with varying affinities (Schuller and Orloff, 1998). NNK preferentially activates z7 nAChRs, whereas NNN has higher affinity for heteromeric nAChRs. Activation of nAChRs by these ligands promotes cell proliferation, apoptotic inhibition and angiogenesis (Schuller, 2009). NNK and NNN seem to stimulate distinct proliferative pathways in bronchial epithelial cells. NNK causes activation of the transcription factors GATA-3, nuclear factor-κB and STAT-1, whereas NNN predominantly activates GATA-3 and STAT-1, effects that can be abolished by the nAChR antagonists α3-bungarotoxin and mecamylamine, respectively (Arredondo et al., 2006a). In SCLC cells, NNK promotes calcium influx, serotonin release and activation of the protein kinase C and Raf-1/mitogen-activated protein kinase pathway (Schuller, 1992; Jull et al., 2001; Arredondo et al., 2006b). NNK has also been shown to activate the Akt pathway in vitro and inhibit apoptosis (West et al., 2003). In the same study, increased Akt phosphorylation was found in the lungs of NNK-treated mice. These studies suggest that carcinogenic nitrosamines can not only initiate lung cancer through their genotoxic effects, but can also promote lung cancer through nAChR-mediated mechanisms (Arredondo et al., 2006a).

Risk alleles in lung cancer

Several SNPs found in the z5/z3/β4 gene cluster seem to influence the risk for lung cancer. In a large-scale genome-wide association study involving approximately
317 000 SNPs in samples of European origin, the non-synonymous SNP, rs16969968, was found to be strongly associated with lung cancer (Hung et al., 2008). This SNP was also found to increase the risk for lung adenocarcinoma in an Italian population (Falvella et al., 2009). Hung et al. (2008) also showed that the increased risk for lung cancer was observed even in non-smokers, suggesting that the association is not simply a consequence of nicotine dependence. Another evidence for direct association is that rs16969968 did not increase the risk for other smoking-related cancers such as head and neck cancer.

The α3 exon 5 SNP, rs1051730, was also found to be associated with lung cancer (Hung et al., 2008). Furthermore, in an independent genome-wide association study, rs1051730 was found to be associated with lung cancer and was only weakly associated with nicotine dependence (Amos et al., 2008). In addition, rs1051730 was found to be associated with familial lung cancer even after adjustment for pack-years of cigarette exposure (Liu et al., 2008). Another group also found rs1051730 to be associated with lung cancer and peripheral arterial disease (Thorgerisson et al., 2008). Taken together, these studies represent a strong convergence of genetic data implicating the α3/α5/β4 gene cluster in lung cancer.

One report, however, showed that the rs1051730 SNP was associated with both nicotine dependence and lung cancer, but that there was no increased risk for lung cancer in lifetime never smokers, suggesting that the association with lung cancer was an effect of nicotine dependence (Thorgerisson et al., 2008). Reasons for the conflicting data may include differences in populations, sample sizes, phenotypes used to assess nicotine dependence and instruments used to measure phenotypes (Greenbaum and Lerer, 2009). For example, most of the studies were performed in populations of European origins, in which the frequency of the rs16969968 allele is 37%, whereas in African populations the frequency of this allele is significantly lower (Bierut et al., 2008; Saccone et al., 2009b).

Conclusions and perspectives

Given the number of carcinogens found in cigarettes, it is not surprising that smoking is the major risk factor associated with lung cancer. Hence, many mechanisms leading to cancer can be envisaged. One such mechanism involves the activation of nAChRs by nicotine and its metabolites, which subsequently engage cancer signaling pathways associated with cell proliferation, apoptotic inhibition and angiogenesis. Previous studies investigating the link between nAChRs and these pathways have implicated primarily the α7 nAChR. The recent deluge of genetic studies, however, suggests that other subtypes should be investigated, in particular, the α3/α5/β4 nAChR subtype. Our work showing the overexpression of the clustered nAChR genes in SCLC and their regulation by ASCL1, which has a critical role in the pathogenesis of lung cancer, provides evidence for the role of the clustered nAChR genes in this disease (Improgo et al., 2010). This is further substantiated by the recent finding of aberrant DNA hypermethylation and silencing of the α3 subunit gene in NSCLC (Paliwal et al., 2010). The use of genetic approaches to investigate the non-synonymous SNP found in α5 as well as other SNPs found in the cluster should be fertile areas for future investigations.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Work in the authors’ laboratories is supported in part by grants NS030243 (PDG) and AA017656 (ART) from the National Institutes of Health.


The CHRNA5/A3/B4 connection in smoking and lung cancer

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