Oncogene (2010) 29, 4874–4884

www.nature.com/onc

REVIEW

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-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*-nitrosonornicotine (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-HT_3 (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*.,

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Received 1 April 2010; revised 17 May 2010; accepted 27 May 2010; published online 28 June 2010

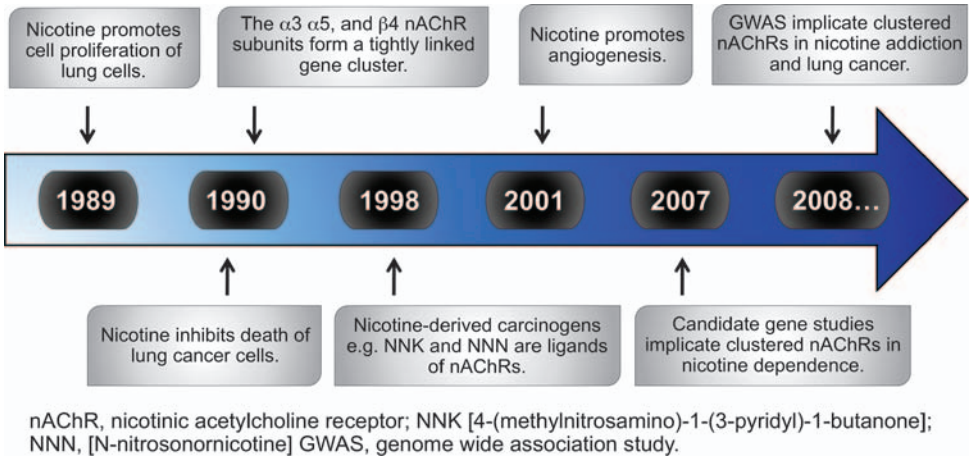

Figure 1 Timeline: key events implicating nAChRs in lung cancer etiology. GWAS, genome-wide association study; nAChR, nicotinic acetylcholine receptor; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine.

1993; Boyd, 1997; Gotti *et al*., 2006). 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, a cytoplasmic loop of variable length between the third and fourth 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; Saccone *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-

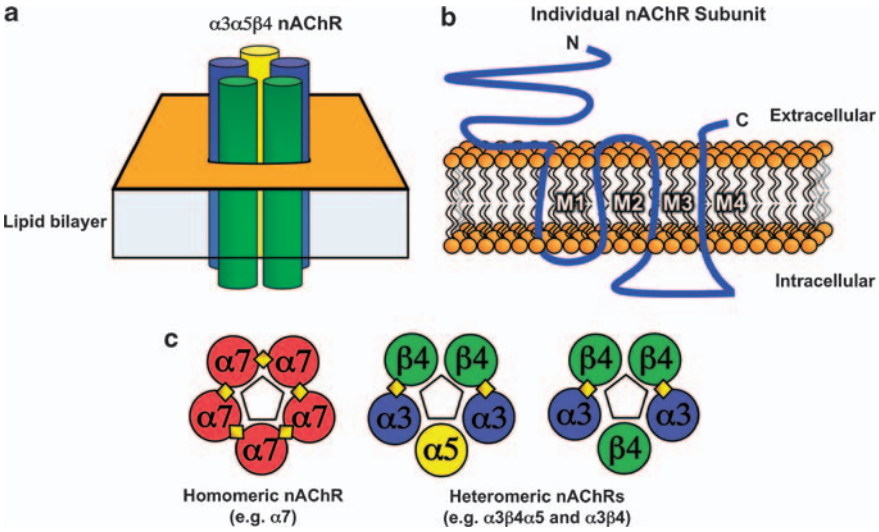

Figure 2 Structure of the nAChR. (**a**) Schematic representation illustrating the pentameric arrangement of subunits in an assembled nAChR. (**b**) Conserved domains of a nAChR subunit including the amino (N) and carboxy (C) terminals, transmembrane segments (M1–M4) and the intracellular loop. (**c**) 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.

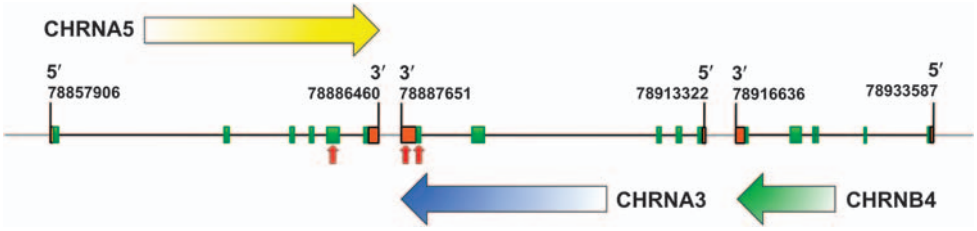

Figure 3 The human nAChR α3/α5/β4 gene cluster. Green boxes represent exons and orange boxes represent untranslated regions. Black lines located between green boxes represent introns whereas gray lines represent intragenic regions. The boundaries for each gene are labeled with corresponding Genbank annotations. Horizontal arrows indicate the direction of transcription. Vertical red arrows indicate SNPs associated with nicotine dependence and lung cancer.

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

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 achaete-scute complex homolog-1 (ASCL1), Brn-3a-c, c-Jun, hnRNPK, PHOX2A, Purα, Sox10, Sp3 and Tst-1/Oct6/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: β43′, found at the β4 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 α3 and α5 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 α4- 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). α4β2* 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 (affective) and physical (somatic) symptoms (De Biasi and Salas 2008). The α5- and β4-containing nAChRs as well as α7 nAChRs seem to be involved in the physical symptoms of withdrawal as somatic signs are diminished in α5, α7 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 α5 and α7 KO mice (Jackson *et al*., 2008; Portugal *et al*., 2008).

Results of the aforementioned genetic studies also support the role of the α3, α5 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 α5/α3/β4 gene cluster. Of particular interest is the non-synonymous SNP, rs16969968, found in the fifth exon of the α5 gene. This polymorphism changes an aspartic acid residue into asparagine at position 398 (D398N) in the second intracellular loop of α5. 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 α3 and rs578776 found in the α3 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 (Thorgeirsson *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 α5/α3/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 (Linnoila *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-lipooxygenase-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, Ca^{2+} 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 Cα 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 nicotine-mediated 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 α3-containing nAChRs. In the same study, increased Akt activation was observed in lung cancer tissue from smokers. Further evidence implicating α3 in Akt signal transduction is a recent report showing that small hairpin RNA-mediated depletion of the α3 subunit leads to a dramatic Ca^{2+} 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 α3 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 (Cooke and Ghebremariam, 2008). Nicotine also functions as a pro-angiogenic 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 Ghebremariam, 2008). Nicotine and its metabolite, cotinine, have also been shown to upregulate the expression of VEGF in endothelial cells (Conklin *et al*., 2002). In addition, second-hand smoke increases VEGF expression and elevates levels of circulating endothelial progenitor cells, promoting angiogenesis and tumor growth—an effect reduced by the non-selective 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 (Cooke and Ghebremariam, 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 α3 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 α3 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 α7 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 α-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 α5/α3/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 (Thorgeirsson *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 (Thorgeirsson *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.

References

ACS (2009). Cancer Facts And Figures 2009. American Cancer Society: Atlanta, Georgia, USA, pp 1–68.

Albuquerque EX, Pereira EF, Alkondon M, Rogers SW. (2009). Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol Rev* **89**: 73–120.

Amos CI, Wu X, Broderick P, Gorlov IP, Gu J, Eisen T *et al*. (2008). Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. *Nat Genet* **40**: 616–622.

Arneric SP, Holladay M, Williams M. (2007). Neuronal nicotinic receptors: a perspective on two decades of drug discovery research. *Biochem Pharmacol* **74**: 1092–1101.

Arredondo J, Chernyavsky AI, Grando SA. (2006a). The nicotinic receptor antagonists abolish pathobiologic effects of tobacco-derived nitrosamines on BEP2D cells. *J Cancer Res Clin Oncol* **132**: 653–663.

Arredondo J, Chernyavsky AI, Jolkovsky DL, Pinkerton KE, Grando SA. (2006b). Receptor-mediated tobacco toxicity: cooperation of the Ras/Raf-1/MEK1/ERK and JAK-2/STAT-3 pathways downstream of alpha7 nicotinic receptor in oral keratinocytes. *FASEB J* **20**: 2093–2101.

Arredondo J, Nguyen VT, Chernyavsky AI, Jolkovsky DL, Pinkerton KE, Grando SA. (2001). A receptor-mediated mechanism of nicotine toxicity in oral keratinocytes. *Lab Invest* **81**: 1653–1668.

Ball DW, Azzoli CG, Baylin SB, Chi D, Dou S, Donis-Keller H *et al*. (1993). Identification of a human achaete-scute homolog highly expressed in neuroendocrine tumors. *Proc Natl Acad Sci USA* **90**: 5648–5652.

Battaglioli E, Gotti C, Terzano S, Flora A, Clementi F, Fornasari D. (1998). Expression and transcriptional regulation of the human alpha3 neuronal nicotinic receptor subunit in T lymphocyte cell lines. *J Neurochem* **71**: 1261–1270.

Benfante R, Flora A, Di Lascio S, Cargnin F, Longhi R, Colombo S *et al*. (2007). Transcription factor PHOX2A regulates the human α3 nicotinic receptor subunit gene promoter. *J Biol Chem* **282**: 13290–13302.

Berrettini W, Yuan X, Tozzi F, Song K, Francks C, Chilcoat H *et al*. (2008). Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Mol Psychiatry* **13**: 368–373.

Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X *et al*. (2008). Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry* **165**: 1163–1171.

Bigger CB, Melnikova IN, Gardner PD. (1997). Sp1 and Sp3 regulate expression of the neuronal nicotinic acetylcholine receptor β4 subunit gene. *J Biol Chem* **272**: 25976–25982.

Blanchet MR, Israël-Assayag E, Cormier Y. (2004). Inhibitory effect of nicotine on experimental hypersensitivity pneumonitis *in vivo* and *in vitro*. *Am J Respir Crit Care Med* **169**: 903–909.

Boulter J, O'Shea-Greenfield A, Duvoisin RM, Connolly JG, Wada E, Jensen A *et al*. (1990). Alpha 3, alpha 5, and beta 4: three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. *J Biol Chem* **265**: 4472–4482.

Boyd RT. (1997). The molecular biology of neuronal nicotinic acetylcholine receptors. *Crit Rev Toxicol* **27**: 299–318.

Bruschweiler-Li L, Fuentes Medel YF, Scofield MD, Trang EBT, Binke SA, Gardner PD. (2010). Temporally- and spatially-regulated transcriptional activity of the nicotinic acetylcholine receptor β4 subunit gene promoter region. *Neurosci* **166**: 864–877.

Calabresi P, Lacey MG, North RA. (1989). Nicotinic excitation of rat ventral tegmental neurones *in vitro* studied by intracellular recording. *Br J Pharmacol* **98**: 135–140.

Campos-Caro A, Carrasco-Serrano C, Valor LM, Ballesta JJ, Criado M. (2001). Activity of the nicotinic acetylcholine receptor alpha5 and alpha7 subunit promoters in muscle cells. *DNA Cell Biol* **20**: 657–666.

Campos-Caro A, Carrasco-Serrano C, Valor LM, Viniegra S, Ballesta JJ, Criado M. (1999). Multiple functional Sp1 domains in the minimal promoter region of the neuronal nicotinic receptor alpha5 subunit gene. *J Biol Chem* **274**: 4693–4701.

Caporaso N, Gu F, Chatterjee N, Sheng-Chih J, Yu K, Yeager M *et al*. (2009). Genome-wide and candidate gene association study of cigarette smoking behaviors. *PLoS One* **4**: e4653.

CDC (2009). Cigarette Smoking among Adults and Trends in Smoking Cessation—United States, 2008. *Centers for Disease Control and Prevention* **58**: 1227–1232.

Conklin BS, Zhao W, Zhong DS, Chen C. (2002). Nicotine and cotinine up-regulate vascular endothelial growth factor expression in endothelial cells. *Am J Pathol* **160**: 413–418.

Conti-Fine BM, Navaneetham D, Lei S, Maus AD. (2000). Neuronal nicotinic receptors in non-neuronal cells: new mediators of tobacco toxicity? *Eur J Pharmacol* **393**: 279–294.

Cooke JP, Ghebremariam YT. (2008). Endothelial nicotinic acetylcholine receptors and angiogenesis. *Trends Cardiovasc Med* **18**: 247–253.

Corrigall WA, Coen KM. (1991). Selective dopamine antagonists reduce nicotine self-administration. *Psychopharmacology (Berl)* **104**: 171–176.

Corrigall WA, Franklin KB, Coen KM, Clarke PB. (1992). The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology (Berl)* **107**: 285–289.

Corrigall WA, Herling S, Coen KM. (1989). Evidence for a behavioral deficit during withdrawal from chronic nicotine treatment. *Pharmacol Biochem Behav* **33**: 559–562.

Corringer PJ, Le Novere N, Changeux J-P. (2000). Nicotinic receptors at the amino acid level. *Annu Rev Pharmacol Toxicol* **40**: 431–458.

Corriveau RA, Berg DK. (1993). Coexpression of multiple acetylcholine receptor genes in neurons: quantification of transcripts during development. *J Neurosci* **13**: 2662–2671.

Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N *et al*. (1990). A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. *Neuron* **5**: 847–856.

Dani JA, Bertrand D. (2007). Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* **47**: 699–729.

Dani JA, De Biasi M. (2001). Cellular mechanisms of nicotine addiction. *Pharmacol Biochem Behav* **70**: 439–446.

Dasgupta P, Chellappan SP. (2006). Nicotine-mediated cell proliferation and angiogenesis: new twists to an old story. *Cell Cycle* **5**: 2324–2328.

Davis R, Rizwani W, Banerjee S, Kovacs M, Haura E, Coppola D *et al*. (2009). Nicotine promotes tumor growth and metastasis in mouse models of lung cancer. *PLoS One* **4**: e7524.

De Biasi M, Salas R. (2008). Influence of neuronal nicotinic receptors over nicotine addiction and withdrawal. *Exp Biol Med* **233**: 917–929.

Di Chiara G. (2000). Role of dopamine in the behavioural actions of nicotine related to addiction. *Eur J Pharmacol* **393**: 295–314.

Di Chiara G, Imperato A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* **85**: 5274–5278.

Du Q, Melnikova IN, Gardner PD. (1998). Differential effects of heterogeneous nuclear ribonucleoprotein K on Sp1- and Sp3-mediated transcriptional activation of a neuronal nicotinic acetylcholine receptor promoter. *J Biol Chem* **273**: 19877–19883.

Du Q, Tomkinson AE, Gardner PD. (1997). Transcriptional regulation of neuronal nicotinic acetylcholine receptor genes. A possible role for the DNA-binding protein Puralpha. *J Biol Chem* **272**: 14990–14995.

Egleton RD, Brown KC, Dasgupta P. (2008). Nicotinic acetylcholine receptors in cancer: multiple roles in proliferation and inhibition of apoptosis. *Trends Pharmacol Sci* **29**: 151–158.

Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S. (1994). Alpha 9: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. *Cell* **79**: 705–715.

Elgoyhen AB, Vetter DE, Katz E, Rothlin CV, Heinemann SF, Boulter J. (2001). alpha10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. *Proc Natl Acad Sci USA* **98**: 3501–3506.

Falvella FS, Galvan A, Frullanti E, Spinola M, Calabro E, Carbone A *et al*. (2009). Transcription deregulation at the 15q25 locus in association with lung adenocarcinoma risk. *Clin Cancer Res* **15**: 1837–1842.

Flora A, Schulz R, Benfante R, Battaglioli E, Terzano S, Clementi F *et al*. (2000a). Transcriptional regulation of the human alpha5 nicotinic receptor subunit gene in neuronal and non-neuronal tissues. *Eur J Pharmacol* **393**: 85–95.

Flora A, Schulz R, Benfante R, Battaglioli E, Terzano S, Clementi F *et al*. (2000b). Neuronal and extraneuronal expression and regulation of the human alpha5 nicotinic receptor subunit gene. *J Neurochem* **75**: 18–27.

Freathy RM, Ring SM, Shields B, Galobardes B, Knight B, Weedon MN *et al*. (2009). A common genetic variant in the 15q24 nicotinic acetylcholine receptor gene cluster (CHRNA5-CHRNA3-CHRNB4) is associated with a reduced ability of women to quit smoking in pregnancy. *Hum Mol Genet* **18**: 2922–2927.

Fucile S. (2004). Ca2+ permeability of nicotinic acetylcholine receptors. *Cell Calcium* **35**: 1–8.

Gahring LC, Persiyanov K, Dunn D, Weiss R, Meyer EL, Rogers SW. (2004). Mouse strain-specific nicotinic acetylcholine receptor expression by inhibitory interneurons and astrocytes in the dorsal hippocampus. *J Comp Neurol* **468**: 334–346.

Gahring LC, Rogers SW. (2006). Neuronal nicotinic acetylcholine receptor expression and function on nonneuronal cells. *AAPS J* **7**: E885–E894.

Gerzanich V, Kuryatov A, Anand R, Lindstrom J. (1997). Orphan' alpha6 nicotinic AChR subunit can form a functional heteromeric acetylcholine receptor. *Mol Pharmacol* **51**: 320–327.

Gerzanich V, Wang F, Kuryatov A, Lindstrom J. (1998). alpha 5 Subunit alters desensitization, pharmacology, Ca++ permeability and Ca++ modulation of human neuronal alpha 3 nicotinic receptors. *J Pharmacol Exp Ther* **286**: 311–320.

Gotti C, Zoli M, Clementi F. (2006). Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol Sci* **27**: 482–491.

Grando SA. (2008). Basic and clinical aspects of non-neuronal acetylcholine: biological and clinical significance of non-canonical ligands of epithelial nicotinic acetylcholine receptors. *J Pharmacol Sci* **106**: 174–179.

Greenbaum L, Lerer B. (2009). Differential contribution of genetic variation in multiple brain nicotinic cholinergic receptors to nicotine dependence: recent progress and emerging open questions. *Mol Psychiatry* **14**: 912–945.

Grivennikov SI, Greten FR, Karin M. (2010). Immunity, inflammation, and cancer. *Cell* **140**: 883–899.

Hecht SS, Hoffmann D. (1988). Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis* **9**: 875–884.

Heeschen C, Jang JJ, Weis M, Pathak A, Kaji S, Hu RS *et al*. (2001). Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. *Nat Med* **7**: 833–839.

HHS (2004). 2004 Surgeon General's Report—The Health Consequences of Smoking. *US Department of Health and Human Services, Public Health Service, Office of the Surgeon General*.

Hung RJ, McKay JD, Gaborieau V, Boffetta P, Hashibe M, Zaridze D *et al*. (2008). A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. *Nature* **452**: 633–637.

Improgo MRD, Schlichting NA, Cortes RY, Zhao-Shea R, Tapper AR, Gardner PD. (2010). ASCL1 regulates the expression of the CHRNA5/A3/B4 lung cancer susceptibility locus. *Mol Cancer Res* **8**: 194–203.

Jackman DM, Johnson B. (2005). Small-cell lung cancer. *Lancet* **366**: 1385–1396.

Jackson KJ, Martin BR, Changeux JP, Damaj MI. (2008). Differential role of nicotinic acetylcholine receptor subunits in physical and affective nicotine withdrawal signs. *J Pharmacol Exp Ther* **325**: 302–312.

Jin Z, Gao F, Flagg T, Deng X. (2004). Nicotine induces multi-site phosphorylation of Bad in association with suppression of apoptosis. *J Biol Chem* **279**: 23837–23844.

Jull BA, Plummer III HK, Schuller HM. (2001). Nicotinic receptor-mediated activation by the tobacco-specific nitrosamine NNK of a Raf-1/MAP kinase pathway, resulting in phosphorylation of c-myc in human small cell lung carcinoma cells and pulmonary neuroendocrine cells. *J Cancer Res Clin Oncol* **127**: 707–717.

Karlin A. (2002). Emerging structure of the nicotinic acetylcholine receptors. *Nat Rev Neurosci* **3**: 102–114.

Kawashima K, Fujii T. (2003). The lymphocytic cholinergic system and its contribution to the regulation of immune activity. *Life Sci* **74**: 675–696.

Kenny PJ, Markou A. (2001). Neurobiology of the nicotine withdrawal syndrome. *Pharmacol Biochem Behav* **70**: 531–549.

Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP. (2001). Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J Neurosci* **21**: 1452–1463.

Lam DC, Girard L, Ramirez R, Chau W, Suen W, Sheriden S *et al*. (2007). Expression of nicotinic acetylcholine receptor subunit genes in non-small-cell lung cancer reveals differences between smokers and nonsmokers. *Cancer Res* **67**: 4638–4647.

Le Novere N, Changeux JP. (1995). Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells. *J Mol Evol* **40**: 155–172.

Leonard S, Bertrand D. (2001). Neuronal nicotinic receptors: from structure to function. *Nicotine Tob Res* **3**: 203–223.

Levey MS, Brumwell CL, Dryer SE, Jacob MH. (1995). Innervation and target tissue interactions differentially regulate acetylcholine receptor subunit mRNA levels in developing neurons *in situ*. *Neuron* **14**: 153–162.

Levey MS, Jacob MH. (1996). Changes in the regulatory effects of cell-cell interactions on neuronal AChR subunit transcript levels after synapse formation. *J Neurosci* **16**: 6878–6885.

Linnoila RI, Zhao B, DeMayo JL, Nelkin BD, Baylin SB, DeMayo FJ *et al*. (2000). Constitutive achaete-scute homologue-1 promotes airway dysplasia and lung neuroendocrine tumors in transgenic mice. *Cancer Res* **60**: 4005–4009.

Liu P, Vikis HG, Wang D, Lu Y, Wang Y, Schwartz AG *et al*. (2008). Familial aggregation of common sequence variants on 15q24-25.1 in lung cancer. *J Natl Cancer Inst* **100**: 1326–1330.

Liu Q, Huang Y, Xue F, Simard A, DeChon J, Li G *et al*. (2009). A novel nicotinic acetylcholine receptor subtype in basal forebrain cholinergic neurons with high sensitivity to amyloid peptides. *J Neurosci* **29**: 918–929.

Liu Q, Melnikova IN, Hu M, Gardner PD. (1999). Cell type-specific activation of neuronal nicotinic acetylcholine receptor subunit genes by Sox10. *J Neurosci* **19**: 9747–9755.

Lloyd GK, Williams M. (2000). Neuronal nicotinic acetylcholine receptors as novel drug targets. *J Pharmacol Exp Ther* **292**: 461–467.

Lustig LR, Peng H, Hiel H, Yamamoto T, Fuchs PA. (2001). Molecular cloning and mapping of the human nicotinic acetylcholine receptor alpha10 (CHRNA10). *Genomics* **73**: 272–283.

Macklin KD, Maus AD, Pereira EF, Albuquerque EX, Conti-Fine BM. (1998). Human vascular endothelial cells express functional nicotinic acetylcholine receptors. *J Pharmacol Exp Ther* **287**: 435–439.

Mai H, May WS, Gao F, Jin Z, Deng X. (2003). A functional role for nicotine in Bcl2 phosphorylation and suppression of apoptosis. *J Biol Chem* **278**: 1886–1891.

Maneckjee R, Minna JD. (1990). Opioid and nicotine receptors affect growth regulation of human lung cancer cell lines. *Proc Natl Acad Sci USA* **87**: 3294–3298.

Maneckjee R, Minna JD. (1994). Opioids induce while nicotine suppresses apoptosis in human lung cancer cells. *Cell Growth Differ* **5**: 1033–1040.

Marubio LM, Gardier AM, Durier S, David D, Klink R, Arroyo-Jimenez MM *et al*. (2003). Effects of nicotine in the dopaminergic system of mice lacking the alpha4 subunit of neuronal nicotinic acetylcholine receptors. *Eur J Neurosci* **17**: 1329–1337.

Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP *et al*. (2005). Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* **436**: 103–107.

Maus AD, Pereira EF, Karachunski PI, Horton RM, Navaneetham D, Macklin K *et al*. (1998). Human and rodent bronchial epithelial cells express functional nicotinic acetylcholine receptors. *Mol Pharmacol* **54**: 779–788.

McGehee DS, Role LW. (1995). Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annu Rev Physiol* **57**: 521–546.

McKeon A, Lennon VA, Lachance DH, Fealey RD, Pittock SJ. (2009). Ganglionic acetylcholine receptor autoantibody: oncological, neurological, and serological accompaniments. *Arch Neurol* **66**: 735–741.

Melnikova IN, Gardner PD. (2001). The signal transduction pathway underlying ion channel gene regulation by SP1-C-Jun interactions. *J Biol Chem* **276**: 19040–19045.

Melnikova IN, Lin HR, Blanchette AR, Gardner PD. (2000). Synergistic transcriptional activation by Sox10 and Sp1 family members. *Neuropharm* **39**: 2615–2623.

Milton NG, Bessis A, Changeux JP, Latchman DS. (1996). Differential regulation of neuronal nicotinic acetylcholine receptor subunit gene promoters by Brn-3 POU family transcription factors. *Biochem J* **317**(Pt 2): 419–423.

Nguyen VT, Hall LL, Gallacher G, Ndoye A, Jolkovsky DL, Webber RJ *et al*. (2000). Choline acetyltransferase, acetylcholinesterase, and nicotinic acetylcholine receptors of human gingival and esophageal epithelia. *J Dent Res* **79**: 939–949.

Nisell M, Nomikos GG, Svensson TH. (1994). Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. *Synapse* **16**: 36–44.

Orr-Urtreger A, Kedmi M, Rosner S, Karmeli F, Rachmilewitz D. (2005). Increased severity of experimental colitis in alpha 5 nicotinic acetylcholine receptor subunit-deficient mice. *NeuroReport* **16**: 1123–1127.

Paliwal A, Vaissière T, Krais A, Cuenin C, Cros MP, Zaridze D *et al*. (2010). Aberrant DNA methylation links cancer susceptibility locus 15q25.1 to apoptotic regulation and lung cancer. *Cancer Res* **70**: 2779–2788.

Patrick J, Sequela P, Vernino S, Amador M, Luetje C, Dani JA. (1993). Functional diversity of neuronal nicotinic acetylcholine receptors. *Prog Brain Res* **98**: 113–120.

Perry DC, Xiao Y, Nguyen HN, Musachio JL, Davila-Garcia MI, Kellar KJ. (2002). Measuring nicotinic receptors with characteristics of alpha4beta2, alpha3beta2 and alpha3beta4 subtypes in rat tissues by autoradiography. *J Neurochem* **82**: 468–481.

Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM *et al*. (1998). Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* **391**: 173–177.

Pidoplichko VI, DeBiasi M, Williams JT, Dani JA. (1997). Nicotine activates and desensitizes midbrain dopamine neurons. *Nature* **390**: 401–404.

Pillai SG, Ge D, Zhu G, Kong X, Shianna KV, Need AC *et al*. (2009). A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet* **5**: e1000421.

Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM *et al*. (2008). Crucial role of alpha4 and alpha6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J Neurosci* **28**: 12318–12327.

Pontieri FE, Tanda G, Orzi F, Di Chiara G. (1996). Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature* **382**: 255–257.

Portugal GS, Gould TJ. (2008). Genetic variability in nicotinic acetylcholine receptors and nicotine addiction: converging evidence from human and animal research. *Behav Brain Res* **193**: 1–16.

Portugal GS, Kenney JW, Gould TJ. (2008). Beta2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning. *Neurobiol Learn Mem* **89**: 106–113.

Pugh BF, Tjian R. (1991). Transcription from a TATA-less promoter requires a multisubunit TFIID complex. *Genes Dev* **5**: 1935–1945.

Punturieri A, Szabo E, Croxton TL, Shapiro SD, Dubinett SM. (2009). Lung cancer and chronic obstructive pulmonary disease: needs and opportunities for integrated research. *J Natl Cancer Inst* **101**: 554–559.

Quick MW, Ceballos RM, Kasten M, McIntosh JM, Lester RA. (1999). Alpha3beta4 subunit-containing nicotinic receptors dominate function in rat medial habenula neurons. *Neuropharmacology* **38**: 769–783.

Richardson CE, Morgan JM, Jasani B, Green JT, Rhodes J, Williams GT *et al*. (2001). Megacystis-microcolon-intestinal hypoperistalsis syndrome and the absence of the alpha3 nicotinic acetylcholine receptor subunit. *Gastroenterology* **121**: 350–357.

Role LW, Berg DK. (1996). Nicotinic receptors in the development and modulation of CNS synapses. *Neuron* **16**: 1077–1085.

Romanelli MN, Gratteri P, Guandalini L, Martini E, Bonaccini C, Gualtieri F. (2007). Central nicotinic receptors: structure, function, ligands, and therapeutic potential. *ChemMedChem* **2**: 746–767.

Rubin DT, Hanauer SB. (2000). Smoking and inflammatory bowel disease. *Eur J Gastroenterol Hepatol* **12**: 855–862.

Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML *et al*. (2009a). Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. *Am J Med Genet B Neuropsychiatr Genet* **150B**: 453–466.

Saccone NL, Wang JC, Breslau N, Johnson EO, Hatsukami D, Saccone SF *et al*. (2009b). The CHRNA5-CHRNA3-CHRNB4 nicotinic receptor subunit gene cluster affects risk for nicotine dependence in African-Americans and in European-Americans. *Cancer Res* **69**: 6848–6856.

Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA *et al*. (2007). Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet* **16**: 36–49.

Salas R, Cook KD, Bassetto L, De Biasi M. (2004a). The α3 and β4 nicotinic receptor subunits are necessary for nicotine-induced seizures and hypolocomotion in mice. *Neuropharmacology* **47**: 401–407.

Salas R, Main A, Gangitano D, De Biasi M. (2007). Decreased withdrawal symptoms but normal tolerance to nicotine in mice null for the alpha7 nicotinic acetylcholine receptor subunit. *Neuropharmacology* **53**: 863–869.

Salas R, Pieri F, De Biasi M. (2004b). Decreased signs of nicotine withdrawal in mice null for the beta4 nicotinic acetylcholine receptor subunit. *J Neurosci* **24**: 10035–10039.

Salas R, Pieri F, Fung B, Dani JA, De Biasi M. (2003). Altered anxiety-related responses in mutant mice lacking the beta4 subunit of the nicotinic receptor. *J Neurosci* **23**: 6255–6263.

Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC *et al*. (2004). Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol Pharmacol* **65**: 1526–1535.

Sartelet H, Maouche K, Totobenazara JL, Petit J, Burlet H, Monteau M *et al*. (2008). Expression of nicotinic receptors in normal and tumoral pulmonary neuroendocrine cells (PNEC). *Pathol Res Pract* **204**: 891–898.

Sasaki H, Hikosaka Y, Okuda K, Kawano O, Yukiue H, Yano M *et al*. (2010). CHRNA5 gene D398N polymorphism in Japanese lung adenocarcinoma. *J Surg Res* **162**: 75–78.

Scheid MP, Woodgett JR. (2001). PKB/AKT: functional insights from genetic models. *Nat Rev Mol Cell Biol* **2**: 760–768.

Schlaepfer IR, Hoft NR, Collins AC, Corley RP, Hewitt JK, Hopfer CJ *et al*. (2008). The CHRNA5/A3/B4 gene cluster variability as an important determinant of early alcohol and tobacco initiation in young adults. *Biol Psychiatry* **63**: 1039–1046.

Schoepfer R, Conroy WG, Whiting P, Gore M, Lindstrom J. (1990). Brain alpha-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. *Neuron* **5**: 35–48.

Schuller HM. (1989). Cell type specific, receptor-mediated modulation of growth kinetics in human lung cancer cell lines by nicotine and tobacco-related nitrosamines. *Biochem Pharmacol* **38**: 3439–3442.

Schuller HM. (1992). Nitrosamine-induced lung carcinogenesis and Ca2 + /calmodulin antagonists. *Cancer Res* **52**: 2723s–22726s.

Schuller HM. (2009). Is cancer triggered by altered signalling of nicotinic acetylcholine receptors? *Nat Rev Cancer* **9**: 195–205.

Schuller HM, Orloff M. (1998). Tobacco-specific carcinogenic nitrosamines. Ligands for nicotinic acetylcholine receptors in human lung cancer cells. *Biochem Pharmacol* **55**: 1377–1384.

Scofield MD, Bruschweiler-Li L, Mou Z, Gardner PD. (2008). Transcription factor assembly on the nicotinic receptor beta4 subunit gene promoter. *NeuroReport* **19**: 687–690.

Sher E, Codignola A, Passafaro M, Tarroni P, Magnelli V, Carbone E *et al*. (1998). Nicotinic receptors and calcium channels in small cell lung carcinoma. Functional role, modulation, and autoimmunity. *Ann NY Acad Sci* **841**: 606–624.

Sherva R, Wilhelmsen K, Pomerleau CS, Chasse SA, Rice JP, Snedecor SM *et al*. (2008). Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with 'pleasurable buzz' during early experimentation with smoking. *Addiction* **103**: 1544–1552.

Shields PG. (2002). Molecular epidemiology of smoking and lung cancer. *Oncogene* **21**: 6870–6876.

Shin VY, Wu WK, Ye YN, So WH, Koo MW, Liu ES *et al*. (2004). Nicotine promotes gastric tumor growth and neovascularization by activating extracellular signal-regulated kinase and cyclooxygenase-2. *Carcinogenesis* **25**: 2487–2495.

Shivji M, Burger S, Moncada CA, Clarkson ABJ, Merali S. (2005). Effect of nicotine on lung S-adenosylmethionine and development of Pneumocystis pneumonia. *J Biol Chem* **280**: 15219–15228.

Song P, Sekhon HS, Jia Y, Keller JA, Blusztajn JK, Mark GP *et al*. (2003). Acetycholine is synthesized by and acts as an autocrine growth factor for small cell lung carcinoma. *Cancer Res* **63**: 214–221.

Spindel ER. (2003). Neuronal nicotinic acetylcholine receptors: not just in brain. *Am J Physiol Lung Cell Mol Physiol* **285**: L1201–L1202.

Spitz MR, Amos CI, Dong Q, Lin J, Wu X. (2008). The CHRNA5-A3 region on chromosome 15q24-25.1 is a risk factor both for nicotine dependence and for lung cancer. *J Natl Cancer Inst* **100**: 1552–1556.

Stevens VL, Bierut LJ, Talbot JT, Wang JC, Sun J, Hinrichs AL *et al*. (2008). Nicotinic receptor gene variants influence susceptibility to heavy smoking. *Cancer Epidemiol Biomarkers Prev* **17**: 3517–3525.

Stolerman IP, Jarvis MJ. (1995). The scientific case that nicotine is addictive. *Psychopharmacology (Berl)* **117**: 2–10; discussion 14–20.

Sun X, Ritzenthaler JD, Zhong X, Zheng Y, Roman J, Han S. (2009). Nicotine stimulates PPARbeta/delta expression in human lung carcinoma cells through activation of PI3K/mTOR and suppression of AP-2alpha. *Cancer Res* **69**: 6445–6453.

Takahashi H, Ogata H, Nishigaki R, Broide DH, Karin M. (2010). Tobacco smoke promotes lung tumorigenesis by triggering IKKbeta- and JNK1-dependent inflammation. *Cancer Cell* **17**: 89–97.

Taly A, Corringer PJ, Guedin D, Lestage P, Changeux JP. (2009). Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. *Nat Rev Drug Discov* **8**: 733–750.

Tapper AR, McKinney SL, Marks MJ, Lester HA. (2007). Nicotine responses in hypersensitive and knockout alpha 4 mice account for tolerance to both hypothermia and locomotor suppression in wild-type mice. *Physiol Genomics* **31**: 422–428.

Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C *et al*. (2004). Nicotine activation of alpha4*receptors: sufficient for reward, tolerance, and sensitization. *Science* **306**: 1029–1032.

Terzano S, Flora A, Clementi F, Fornasari D. (2000). The minimal promoter of the human alpha 3 nicotinic receptor subunit gene. Molecular and functional characterization. *J Biol Chem* **275**: 41495–41503.

Thorgeirsson TE, Geller F, Sulem P, Rafnar T, Wiste A, Magnusson KP *et al*. (2008). A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature* **452**: 638–642.

Turner JR, Kellar KJ. (2005). Nicotinic cholinergic receptors in the rat cerebellum: multiple heteromeric subtypes. *J Neurosci* **25**: 9258–9265.

Unwin N. (2005). Refined structure of the nicotinic acetylcholine receptor at 4A resolution. *J Mol Biol* **346**: 967–989.

Valor LM, Campos-Caro A, Carrasco-Serrano C, Ortiz JA, Ballesta JJ, Criado M. (2002). Transcription factors NF-Y and Sp1 are important determinants of the promoter activity of the bovine and human neuronal nicotinic receptor beta 4 subunit genes. *J Biol Chem* **277**: 8866–8876.

Vernino S, Adamski J, Kryzer TJ, Fealey RD, Lennon VA. (1998). Neuronal nicotinic ACh receptor antibody in subacute autonomic neuropathy and cancer-related syndromes. *Neurology* **50**: 1806–1813.

Vernino S, Low PA, Fealey RD, Stewart JD, Farrugia G, Lennon VA. (2000). Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. *N Engl J Med* **343**: 847–855.

Wacholder S, Chatterjee N, Caporaso N. (2008). Intermediacy and gene-environment interaction: the example of CHRNA5-A3 region, smoking, nicotine dependence, and lung cancer. *J Natl Cancer Inst* **100**: 1488–1491.

Wang N, Orr-Urtreger A, Chapman J, Rabinowitz R, Nachman R, Korczyn AD. (2002). Autonomic function in mice lacking alpha5 neuronal nicotinic acetylcholine receptor subunit. *J Physiol* **542**: 347–354.

Wang N, Orr-Urtreger A, Chapman J, Rabinowitz R, Korczyn AD. (2003). Deficiency of nicotinic acetylcholine receptor beta 4 subunit causes autonomic cardiac and intestinal dysfunction. *Mol Pharmacol* **63**: 574–580.

Wang Y, Pereira EF, Maus AD, Ostlie NS, Navaneetham D, Lei S *et al*. (2001). Human bronchial epithelial and endothelial cells express alpha7 nicotinic acetylcholine receptors. *Mol Pharmacol* **60**: 1201–1209.

Weiss RB, Baker TB, Cannon DS, von Niederhausern A, Dunn DM, Matsunami N *et al*. (2008). A candidate gene approach identifies the CHRNA5-A3-B4 region as a risk factor for age-dependent nicotine addiction. *PLoS Genet* **4**: e1000125.

Wessler I, Kirkpatrick CJ. (2008). Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. *Br J Pharmacol* **154**: 1558–1571.

West KA, Brognard J, Clark AS, Linnoila IR, Yang X, Swain SM *et al*. (2003). Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. *J Clin Invest* **111**: 81–90.

WHO (2009). WHO Report on the Global Tobacco Epidemic, 2009: Implementing Smoke-Free Environments. *World Health Organization*.

Xin M, Deng X. (2005). Nicotine inactivation of the proapoptotic function of Bax through phosphorylation. *J Biol Chem* **280**: 10781–10789.

Xu W, Gelber S, Orr-Urtreger A, Armstrong D, Lewis RA, Ou C-N *et al*. (1999a). Megacystis, mydriasis, and ion channel defect in mice lacking the α3 neuronal nicotinic acetylcholine receptor. *Proc Natl Acad Sci USA* **96**: 5746–5751.

Xu W, Orr-Urtreger A, Nigro F, Gelber S, Sutcliffe CB, Armstrong D *et al*. (1999b). Multiorgan autonomic dysfunction in mice lacking the beta2 and the beta4 subunits of neuronal nicotinic acetylcholine receptors. *J Neurosci* **19**: 9298–9305.

Xu X, Scott MM, Deneris ES. (2006). Shared long-range regulatory elements coordinate expression of a gene cluster encoding nicotinic receptor heteromeric subtypes. *Mol Cell Biol* **26**: 5636–5649.

Yang X, Fyodorov D, Deneris ES. (1995). Transcriptional analysis of acetylcholine receptor alpha 3 gene promoter motifs that bind Sp1 and AP2. *J Biol Chem* **270**: 8514–8520.

Yang X, McDonough J, Fyodorov D, Morris M, Wang F, Deneris ES. (1994). Characterization of an acetylcholine receptor alpha 3 gene promoter and its activation by the POU domain factor SCIP/Tst-1. *J Biol Chem* **269**: 10252–10264.

Ye YN, Liu ES, Shin VY, Wu WK, Luo JC, Cho CH. (2004). Nicotine promoted colon cancer growth via epidermal growth factor receptor, c-Src, and 5-lipoxygenase-mediated signal pathway. *J Pharmacol Exp Ther* **308**: 66–72.

Zeidler R, Albermann K, Lang S. (2007). Nicotine and apoptosis. *Apoptosis* **12**: 1927–1943.

Zhang Q, Tang X, Zhang ZF, Velikina R, Shi S, Le AD. (2007). Nicotine induces hypoxia-inducible factor-1alpha expression in human lung cancer cells via nicotinic acetylcholine receptor-mediated signaling pathways. *Clin Cancer Res* **13**: 4686–4694.

Zheng Y, Ritzenthaler JD, Roman J, Han S. (2007). Nicotine stimulates human lung cancer cell growth by inducing fibronectin expression. *Am J Respir Cell Mol Biol* **37**: 681–690.

Zhou Y, Deneris E, Zigmond RE. (1998). Differential regulation of levels of nicotinic receptor subunit transcripts in adult sympathetic neurons after axotomy. *J Neurobiol* **34**: 164–178.

Zhu BQ, Heeschen C, Sievers RE, Karliner JS, Parmley WW, Glantz SA *et al*. (2003). Second hand smoke stimulates tumor angiogenesis and growth. *Cancer Cell* **4**: 191–196.

Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, Gotti C. (2002). Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. *J Neurosci* **22**: 8785–8789.