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Review

Lung carcinoma signaling pathways activated by smoking

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Abstract

Lung cancer is the leading cause of cancer death in men and women worldwide, with over a million deaths annually. Tobacco smoke is the major etiologic risk factor for lung cancer in current or previous smokers and has been strongly related to certain types of lung cancer, such as small cell lung carcinoma and squamous cell lung carcinoma. In recent years, there has been an increased incidence of lung adenocarcinoma. This change is strongly associated with changes in smoking behavior and cigarette design. Carcinogens present in tobacco products and their intermediate metabolites can activate multiple signaling pathways that contribute to lung cancer carcinogenesis. In this review, we summarize the smoking-activated signaling pathways involved in lung cancer.

Key words Lung cancer, signaling pathways, carcinogenesis, cigarette smoking

Lung cancer is the leading cause of cancer death in men and women worldwide, with over a million deaths annually[1]. Tobacco smoke is the predominant etiologic risk factor for lung cancer. Approximately 80% to 90% of lung cancer patients in the United States involve smoking[2].

Histopathologically, lung cancer is classified as squamous cell carcinoma, small cell carcinoma, adenocarcinoma, or large cell carcinoma with other types accounting for a small percentage of cases[3]. Small cell carcinoma and squamous cell carcinoma are strongly related to cigarette smoking. Recent increases in the incidence of lung adenocarcinoma also appear to be associated with cigarette smoking and are probably due to changes in smoking behavior and lower tar content in cigarettes[4,5].

Cigarette smoke contains over 60 chemicals that have been identified as carcinogens by the International Agency for Research in Cancer. The most potent carcinogens are polycyclic aromatic hydrocarbons, such as benzo[a]pyrene and the tobacco-specific nitrosamine known as nicotine-derived nitrosoaminoketone (NNK)[6]. Carcinogens present during smoking or produced in their intermediate metabolites can activate cell proliferation and survival signals, resulting in preneoplastic changes in bronchial epithelial cells and inducing lung cancer in laboratory animals[6].

β-Adrenergic Receptor–Mediated Signaling

β-Adrenergic receptors (β-ARs) are members of the G-protein–coupled receptor family. The tobacco-specific nitrosamine, nicotine-derived NNK, is structurally similar to the classical β-AR agonist[7,8] and can bind β-AR on pulmonary epithelial cells. This binding stimulates cell proliferation signaling pathways by triggering β-AR activation[9], suggesting that β-ARs play an important role in NNK-induced lung cancer (Figure 1). Once G-protein–coupled receptor family signaling is activated in NNK-treated lung adenocarcinoma cells, it can trigger the activation of adenylyl cyclase and cyclic AMP (cAMP) and the subsequent activation of protein kinase A (PKA). PKA further activates phospholipase-A2 and causes an NNK concentration-dependent release of arachidonic acid (AA) from cell-membrane phospholipids, increasing DNA synthesis and cell proliferation in adenocarcinoma. This signaling pathway can be completely blocked by β-AR antagonists[7]. In addition, both aspirin, a cyclooxygenase (COX) inhibitor, and ML-886, a lipoxygenase inhibitor, can partially inhibit DNA synthesis in cells after being exposed to NNK, suggesting that the

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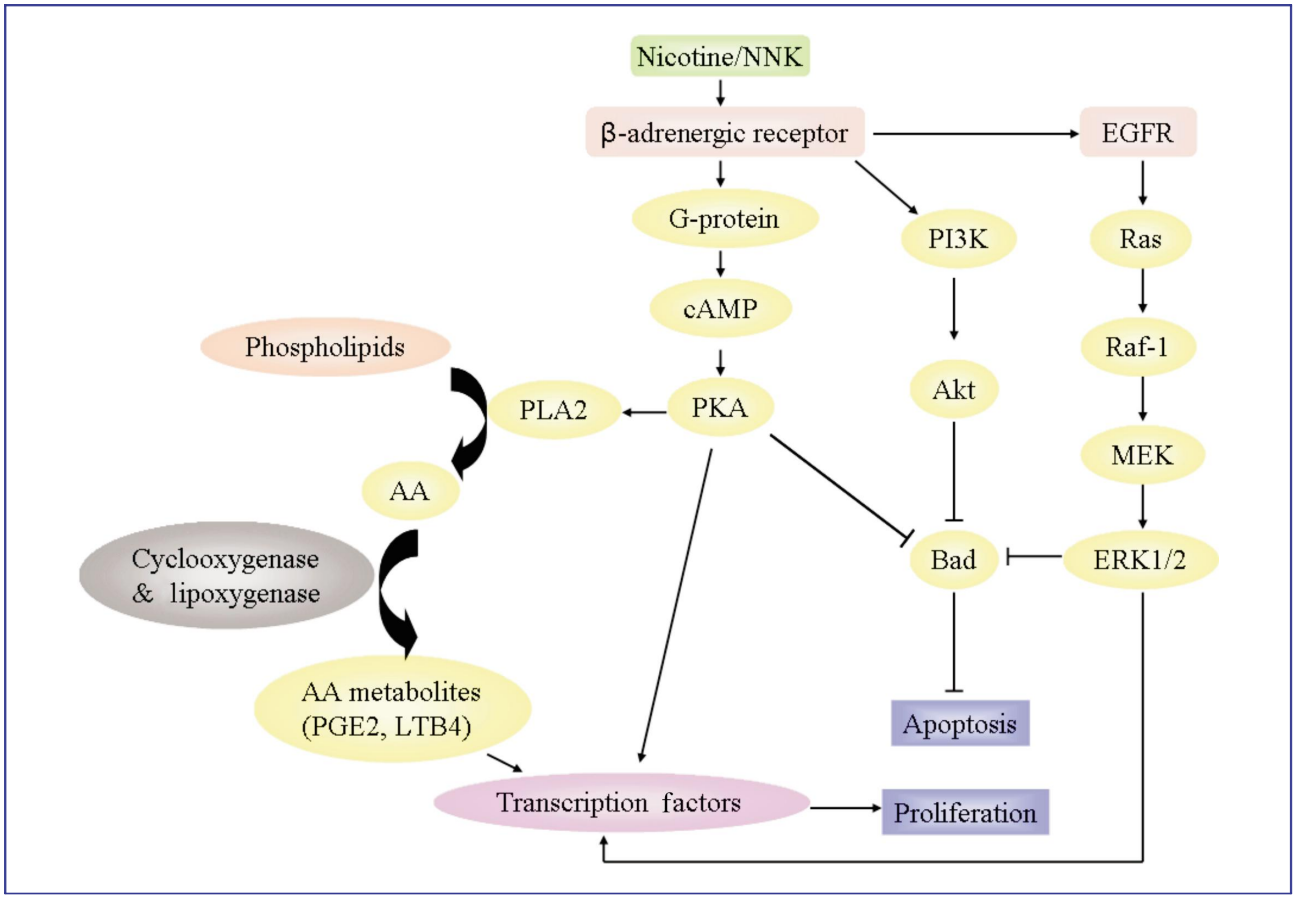

Figure 1. **β-AR–mediated signaling pathways in lung cancers.** Binding of nicotine-derived nitrosoaminoketone (NNK) to β-adrenergic receptors (β-AR) on pulmonary epithelial cells results in cAMP/PKA activation. Protein kinase A (PKA) causes arachidonic acid (AA) release by activating phospholipase-A2, leading to increased DNA synthesis. Epidermal growth factor receptor (EGFR) and PI3K/Akt pathways may be activated as downstream of β-AR to promote cell proliferation and inhibit cell apoptosis.

AA-dependent mitogenic signaling transduction cascade's role in smoking-related lung carcinogenesis involves both COX- and lipoxygenase- dependent messengers[7]. High systemic levels of AA-metabolizing enzymes COX-2[10], AA metabolites prostaglandin E2 and leukotriene B4[11] have been identified in lung cancers in NNK-treated mice. Consistently, COX-2–positive tumors are more common in smokers (32%, 29 of 90) than in non-smokers (10%, 1 of 10, $P = 0.15$)[10].

In addition to AA metabolites, several transcription factors can be stimulated as downstream targets of β-AR/PKA signaling, such as the PKA-dependent overexpression and phosphorylation of cAMP response element binding protein (CREB). Phosphorylation of CREB induced by NNK in human small airway epithelial cells and adenocarcinoma cells[12] and overexpression of p-CREB in NNK-induced adenocarcinomas[13,14] have been reported. CREB regulates the expression of certain genes, including cyclins, Bcl-2 family members and *Egr-1*, whose aberrant expression promotes oncogenesis[15]. An inhibitor of the CREB signaling pathway can block CREB activation in lung cancer cells by arresting the cell cycle at the G_2/M phase and by inducing apoptosis through suppression of Bcl-2 and Bcl-X_L expression[16]. However, CREB activation may not be specific in smoking-related lung cancers. An immunohistochemical analysis of a tissue microarray containing adenocarcinoma, bronchioloalveolar carcinoma, and squamous cell carcinoma specimens collected from 310 patients revealed a significant association between decreased survival and CREB or p-CREB overexpression in non-smokers but not in current or former smokers with lung cancer[17].

β-AR–initiated cAMP signaling may transactivate the epidermal growth factor receptor (EGFR) pathway, including overexpression of EGFR-specific phosphorylated tyrosine kinase, Raf-1, and extracellular signal-regulated kinase 1/2 (ERK1/2) and ERK1/2 phosphorylation (Figure 1). cAMP signaling and the EGFR/Raf-1/ERK1/2 pathway can synergistically regulate

the growth and development of NNK-induced lung adenocarcinomas [12,13]. NNK phosphorylates EGFR at tyrosine residues 991, 1068 and 1173 via β-adrenergic stimulation[12]. However, in one study, no mutations were detected in *EGFR* exons 18–21, which typically occur in non-smokers with lung adenocarcinomas [12]. The phenomenon suggests that the EGFR pathway plays an important role in carcinogenesis through distinct mechanisms in smoking- and non-smoking–related lung adenocarcinomas.

Another signal transduction pathway, phosphatidylinositol 3-kinase (PI3K)/Akt, is reported to induce Bad phosphorylation downstream of β-AR/PKA signaling (Figure 1). In human lung adenocarcinoma A549 cells, nicotine induces ERK1/2, Akt, and PKA activation through an upstream β-AR to trigger multi-site Bad phosphorylation. Bad phosphorylation blocks apoptosis and subsequently promotes cell survival[18].

Nicotin ic Acetylcholine Receptor–Mediated Signaling

Nicotinic acetylcholine receptors (nAChRs) are ion channels located in the plasma membrane of mammalian cells. Initially identified at the neuromuscular junction, nAChRs are classified as neuronal or muscular. Neuronal nAChRs are composed of five identical α7, α8, or α9 subunits or a combination of α2–α6 or α10 subunits and β2–β4 subunits, whereas muscle nAChRs are composed of combinations of α1 subunits with β1, γ, δ, or ε subunits[19]. Binding of agonist to nAChRs results in conformational changes to the receptors, with consequent ion influxes. These can result in the release of neurotransmitters or the stimulation of various intracellular signaling cascades[20]. Nicotine can mimic the actions of acetylcholine as an agonist by binding to the α subunits of nAChRs[20]. Up-regulated expression and activation of nAChRs has been reported in both lung cancer and normal bronchial epithelial cells by exposure to nicotine or NNK[21-24].

Raf/mitogen-activated protein kinase signaling

Recent studies have found that the proliferation of pulmonary neuroendocrine cells and small cell carcinoma can be regulated by neuronal nAChR, which contains the α7 subunit[25-27]. In small cell carcinoma cells, binding of nicotine or NNK to α7 nAChR on the cell membrane results in cation (primarily Ca^{2+}) influx and activation of the mitogenic signal transduction pathway[28,29] (Figure 2). Specifically, increased intracellular Ca^{2+} can trigger the release of serotonin, an autocrine

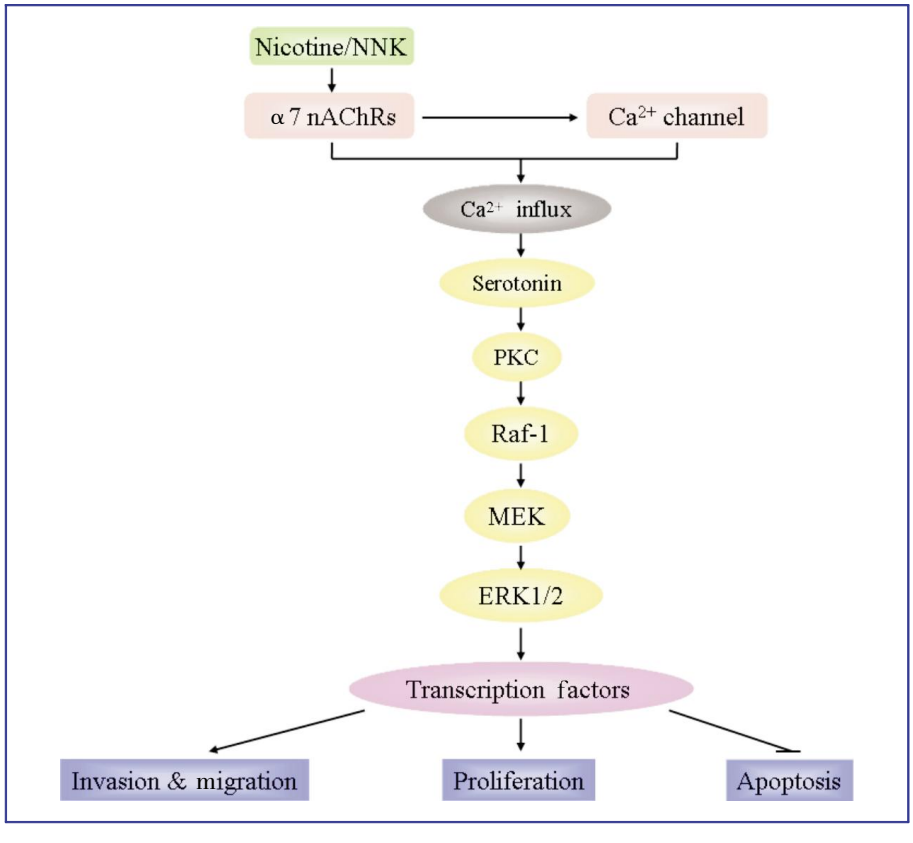

Figure 2. **Subunit α7 of nicotinic acetylcholine receptors (α7 nAChR)-mediated signaling pathways in small cell lung cancers.** In fetal pulmonary neuroendocrine cells and small cell carcinoma cells, binding of NNK to α7 nAChR on the cell membrane results in Ca^{2+} influx and serotonin release, which activates protein kinase C (PKC) and the downstream Raf-1 mitogen–activated protein kinase/MAPK kinase cascade, leading to DNA synthesis and cell proliferation.

growth factor. Serotonin activates protein kinase C (PKC) and the downstream kinase cascade that involves overexpression and activation of the serine-threonine protein kinase (Raf-1), and mitogen-activated protein kinase (MAPK), leading to DNA synthesis and cell proliferation in fetal pulmonary neuroendocrine cells and small cell carcinoma cells [29,30]. The serotonin re-uptake inhibitor α-BTX (imipramine), the PKC inhibitor (sphingosine), and the MAPK kinase inhibitor (PD98059) block NNK-induced DNA synthesis and mitogenesis[30,31].

Unlike small cell carcinoma, which primarily expresses α7 nAChR, squamous cell carcinoma, adenocarcinoma, and benign bronchial and small airway epithelial cells express multiple nAChR subtypes[21,27,32,33]. These subtypes (α3β2 and α4β2), along with α7-containing nAChR, participate in smoking-induced squamous and adenocarcinoma cell proliferation and invasion[32,33] (Figure 3). Unlike activation via Ca^{2+} influx stimulated PKC in small cell carcinoma, nAChR-mediated MAPK activation in squamous cell carcinoma and adenocarcinoma is mediated by the scaffcolding protein, β-arrestin, which is independent of PKC [34,35]. β-arrestin binds and recruits Src to the receptors; activated Src further stimulates the Raf/MAPK pathway[35].

MAPK activation can induce phosphorylation and activate transcription factors, such as c-Myc [30] and activator protein-1 [36], further regulate downstream gene transcription and protein expression, including bcl-2 [34,37], cyclin D1[38], proliferating cell nuclear antigen[36], interleukin (IL)-8[39], hypoxia-inducible factor-1α[40], fibronectin[23], and contactin-1 [41]. MAPK-associated extracellular signal-regulated kinases (ERK1/2) can also activate bcl-2 [42],

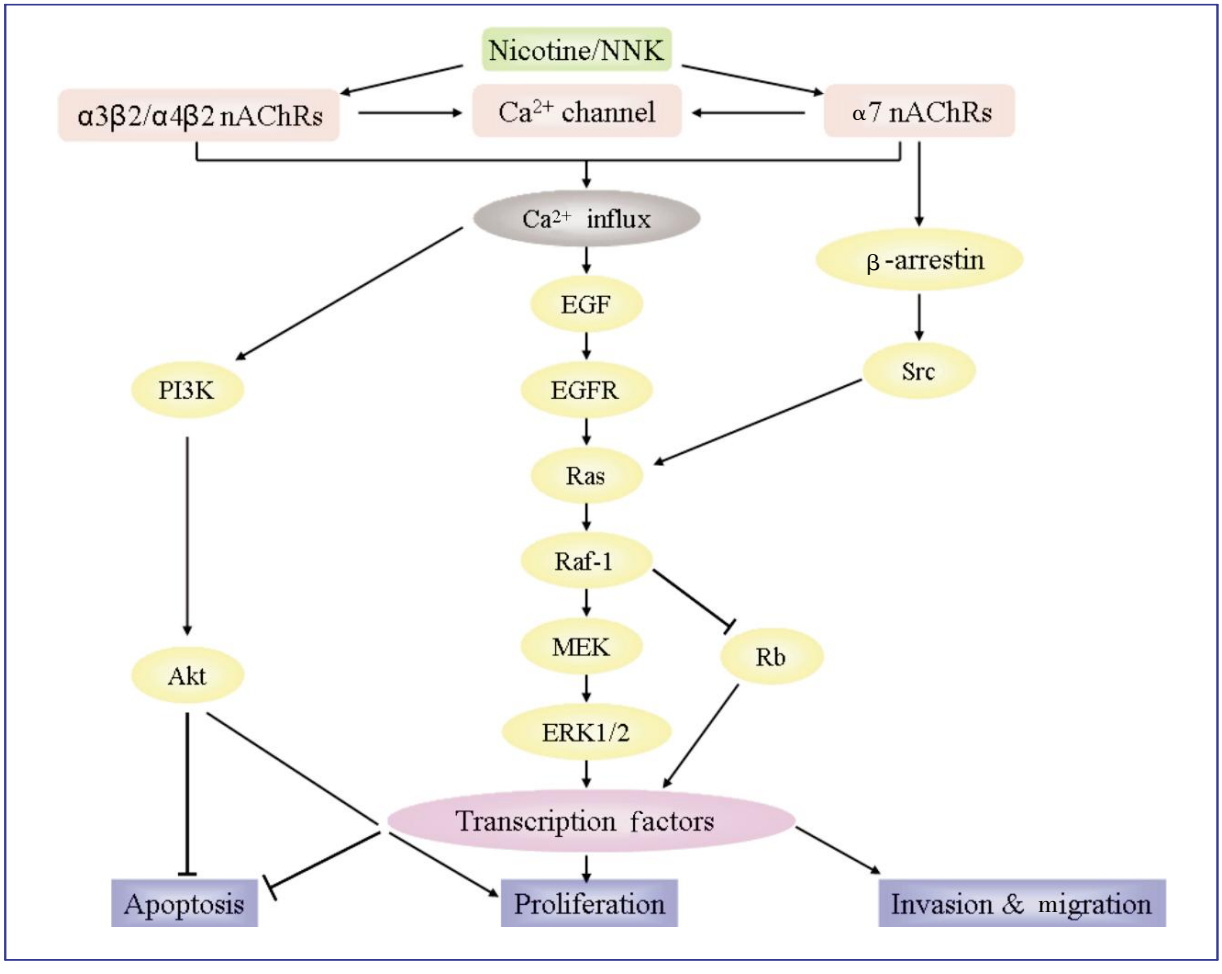

Figure 3. **nAChR-mediated signaling in lung squamous cell carcinomas and adenocarcinomas.** nAChR-mediated mitogen-activated protein kinase (MAPK) activation in squamous cell carcinoma and adenocarcinoma is mediated by the scaffolding protein β-arrestin, which binds to and recruits *Src* to the receptors and stimulates the Raf/MAPK pathway. MAPK activation functions with the PI3K/Akt and the EGFR/Raf/MAPK signaling pathways, both activated by Ca^{2+} influx through nAChRs, to induce phosphorylation and activation of transcription factors, further regulate downstream gene transcription and protein expression, provoke cell proliferation, anti-apoptosis, tumor invasion, and therapy resistance.

p90RSK[43], m-calpains and μ-calpains[44,45], cyclin D/cdk4 and cyclin E/cdk2 [35,38] cascades. Raf-1, which is activated by Src[35], inactivates retinoblastoma (Rb) gene products and enhances Rb's dissociation with and E2F1's recruitment to proliferation promoters, such as cdc6 and cdc25A [35]. Therefore, nicotine stimulation affects various components to provoke cell proliferation, anti-apoptosis, tumor invasion, and therapy resistance.

Some MAPK downstream proteins have been found to be related to lung squamous cell cancer and adenocarcinoma in smokers. Cyclin D1 is expressed more often in lung carcinomas in smokers (77%) than in those from non-smokers (57%)[46]. In another study, cyclin D1 expression in lung squamous cell cancer and adenocarcinoma was associated with heavy smoking (>40 pack-years; P = 0.02) and a shorter overall survival duration (P = 0.01)[47].

nAChR-mediated, MAPK signaling–induced mitogenic effects varied by CO_2 level in normal pulmonary neuroendocrine cells derived from fetal hamster lungs and cell lines derived from human neuroendocrine lung carcinoma[26]. *In vivo*, exposure to NNK under hyperoxic conditions induced small cell carcinoma–like neuroendocrine lung tumors in hamsters, whereas under ambient air conditions, β-AR–regulated lung adenocarcinoma developed[48,49]. The finding of suboptimal O_2 level and nAChR activation levels has important clinical implications. Because small cell carcinoma is more strongly associated with smoking than are other lung cancer types, smokers with chronic obstructive pulmonary disease usually have high nAChR expression and are at high risk of developing small cell carcinoma[50].

Researchers have also studied the roles of another two major mammalian MAPKs, c-Jun N-terminal kinase (JNK) and p38 MAPK, in nicotine-mediated carcinogenesis. ERK, JNK, and p38 MAPK were all activated by nicotine in rat lungs[36,51], although in one report[34] nicotine activated the ERK1/2 MAPK signaling pathway had no effect on JNK and p38 MAPK activity. A gene expression profile analysis of human bronchial epithelial cells revealed that ERK1/2 and JNK, but not p38 MAPK, are activated in response to nicotine[39]. This variation in MAPK activation may be a result of different extracellular stimuli with distinct downstream targets and cellular responses. For instance, MAPK activation was reported to provoke smoking-induced apoptosis in rat lung tissues by up-regulating FasL, Bax, t-Bid, cytochrome C and caspase 3, down-regulating bcl-2, and increasing Fas and p53 phosphorylation [51]. However, JNK and p38 MAPK activation is more common in non-smokers than in smokers[52]. The selective activation of p38 MAPK contributes to cell growth in adenocarcinoma cell lines from non-smokers[53].

PI3K/Akt signaling

The PI3K/Akt pathway may be activated by nicotine or NNK binding to α7 nAChR or α3β2/α4β2 nAChRs[54] (Figure 3). Akt activity maintenance is necessary for the survival of preneoplastic and transformed lung epithelial cells[55]. The identified downstream proteins of this nicotine- or NNK-activated pathway include glycogen synthase kinase-3, ribosomal protein S6 kinase, eukaryotic translation initiation factor 4E binding protein 1, forkhead transcription factor[32], Bax[56], Bad[57], XIAP[33,37], survivin[33], hypoxia-inducible factor-1α, vascular endothelial growth factor[40] and fibronectin[23], contributing to cell proliferation, anti-apoptosis, differentiation, cell migration, and tumor invasion.

Activated Akt was detected in lung cancers from NNK-treated A/J mice and human lung cancer cells derived from smokers[32]. However, one study revealed no association between phosphorylated Akt expression and adenocarcinoma in smokers or smoking status[58]. In another report, higher phosphorylated Akt levels were observed in non-smokers with lung adenocarcinoma than in smokers[59]. Further research is needed to clarify the role of Akt in smoking-related lung cancers.

Nuclear Factor–κB Signaling

Nuclear factor–κB (NF-κB) is a ubiquitous nuclear transcription factor. The activation of NF-κB by smoke may be mediated by ERK1/2 signaling[60,61] or through a pathway similar to that of tumor necrosis factor[62]. NF-κB is in the inactive state in the cytoplasm as a heterotrimer consisting of p50, p65, and IκBα subunits. Upon activation, IκBα undergoes sequential phosphorylation by IκBα kinase and ubiquitination and degradation; the p50/p60 heterodimer is released and translocated to the nucleus, where it binds to specific sequences in the promoter regions of target genes[63].

In squamous cell lung cancer and lung adenocarcinoma, smoke has been reported to induce NF-κB activation, which regulates the expression of downstream molecules such as COX-2[62], cyclin D1, matrix metalloproteinase-9[64,65], p21, c-IAP2, Bcl-2, and Bad[61,62,66], consequently promotes cancer cell proliferation and survival.

Furthermore, NF-κB directly exerts carcinogenic effects to enhance cancer cell growth. Cigarette smoking induces the production of pro-inflammatory factors, such as COX-2, prostaglandin E2[67], IL-8[68], IL-6, and macrophage inflammatory protein 2[69], via NF-κB activation in normal human lung fibroblasts and alveolar macrophages, creating a pro-inflammatory environment. Long-term smoking induces chronic inflammatory lung diseases. Emerging evidence suggests that chronic inflammation plays a significant role in lung cancer

pathogenesis by promoting tumor formation[70] and NF-κB activation may be a link between chronic inflammation and lung cancer.

EGFR Signaling

EGFR may be activated by its autocrine ligands, which are produced by oxygen radicals during cigarette smoking. In lung cancer cells, tobacco smoke-generated oxygen radicals stimulate the tumor necrosis factor-α-converting enzyme, resulting in an increased shedding of EGFR pro-ligands, such as transforming growth factor-α , heparin-binding EGF-like growth factor, and amphiregulin. By binding to EGFR, these ligands activate EGFR phosphorylation and induce the expression of downstream targets such as IL-8 and mucin, resulting in lung cancer cell proliferation[71-73].

Gamma-Aminobutyric Acid Signaling

Gamma-aminobutyric acid (GABA) is the most common inhibitory neurotransmitter in the central nervous system. In a recent study, significant GABA underexpression was reported in NNK-induced hamster lung adenocarcinoma [74]. An *in vitro* study using the immortalized human small airway epithelial cell line HPL1D and human lung adenocarcinoma cell line NCI-H322 revealed that signaling via the GABA receptor strongly inhibited base-level and isoproterenol-induced cAMP, p-CREB, element-luciferase and pERK1/2, effectively blocking DNA synthesis and cell migration [74]. Therefore, GABA may have a tumor-suppressor function in small airway epithelial cells and lung adenocarcinomas. GABA down-regulation by NNK may contribute to cancer development in smokers.

Conclusions

The number of smokers in the United States is decreasing. However, tobacco smoke is still the major etiologic risk factor for lung cancer, which is more common in previous smokers than in current smokers. A comprehensive understanding of the signals activated by smoking-related carcinogens may help us develop targeted therapy for lung cancer patients with a history of smoking.

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