Tobacco carcinogen NNK-induced lung cancer animal models and associated carcinogenic mechanisms

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Abstract

Tobacco usage is a major risk factor in the development, progression, and outcomes for lung cancer. Of the carcinogens associated with lung cancer, tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is among the most potent ones. The oncogenic mechanisms of NNK are not entirely understood, hindering the development of effective strategies for preventing and treating smoking-associated lung cancers. Here, we introduce the NNK-induced lung cancer animal models in different species and its potential mechanisms. Finally, we summarize several chemopreventive agents developed from these animal models.

Key words: NNK, lung cancer, oncogenic mechanisms, animal model

Introduction

Lung cancer is the leading cause of cancer deaths worldwide, especially in areas where cigarette smoking is prevalent. The earliest studies on the connection between tobacco smoking and lung cancer were described over 100 years ago [1] with the first quantitative analysis in 1929 [2]. Since these initial studies, mounting evidence has led to the generally accepted conclusion that cigarette smoking is the major risk factor in lung cancer [3], with confirmation studies done in epidemiology, animal experiments, cellular pathology, and experiments on cancer-associated chemicals present in cigarette smoke [4].

While the general connection between cigarette smoking and lung cancer is widely accepted, the mechanism by which tobacco affects lung cancer development has not been entirely elucidated. Tobacco contains a variety of carcinogens, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), polycyclicaromatic hydrocarbons, nitrosamines, and aromatic amines. Among these carcinogens, NNK has recently been demonstrated to have a significant association with lung cancer [5]. Numerous studies have found that NNK could be activated by several cytochrome P450s through three distinct reactions such as α-hydroxylation, pyridine oxidation, and carbonyl reduction [6–11], with the α-hydroxylation products including α-hydroxy NNKs and α-hydroxy 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL) capable of forming DNA adducts. When DNA adducts are not repaired quickly, these changes persist and can cause permanent gene mutations that may lead to uncontrolled cell proliferation and transformation. Similarly, NNK is known to activate phosphatidylinositol-3-kinase (PI3K)/protein kinase B (PKB/AKT) [12,13], protein kinase C (PKC) [14], nuclear factor kappa B (NF-κB) [15], and other signaling pathways [16], which all promote cell proliferation, survival, and angiogenesis, contributing to the development of smoking-associated lung cancers.

To date, oncogenic mechanisms of NNK have been extensively studied. Here, we briefly summarize the recent findings in NNK-induced lung cancer animal models, the functional mechanisms of NNK, and some potential chemopreventive agents.

NNK-induced Lung Cancer in Animal Models

NNK is a potent carcinogen that has been found to effectively induce various types of lung cancer in several species including A/J mice, rat,
hamster, and ferret. Studies on these animal models showed that different methodologies and different dosages of NNK were capable of inducing lung cancer malignancies among the different species (Table 1). In 6-week-old A/J mice, a single dose of NNK (100 mg/kg) intraperitoneal injection (i.p.) led to the development of hyperplasia along the lung alveolar septa after 14 weeks. Later, between 34 and 42 weeks, the A/J mice exhibited increased frequency of adenomas, and by 54 weeks, carcinomas increased and composed >50% of the observed pulmonary lesions [18]. In an independent study, female A/J mice (7 weeks old) were treated with eight consecutive doses of NNK (3 μmol each week) by either intragastric gavage (i.g.) or i.p.. Twenty-six weeks later, all the mice developed lung adenoma [19]. To test whether exposure to tobacco smoke could affect progeny, the transplacental tumorigenicity of NNK was assessed in A/J mice administered i.p. on days 14, 16, and 18 of gestation at a dose of 100 mg/kg each time [17]. After 24 weeks, 12 of 66 progeny was found to develop large tumors, and 13 of the 14 mothers also developed lung tumors, indicating a much higher tumor incidence and multiplicity with an average of >20 lung tumors per mouse in mothers. These results suggest that NNK induces lung cancer in progeny. The effects of NNK on infant mice were also investigated in Swiss mice. At intervals of 1, 4, 7, 10, and 14 days after birth, infant Swiss mice were given 50 mg/kg NNK i.p.; 13–15 months later, 57% of the males and 37% of the females developed lung cancer. Some mice also developed hepatocellular tumors [20].

In addition to mice, studies on golden hamsters showed that this species was also susceptible to NNK in a dose-dependent manner. A single subcutaneous injection (s.c.) of either 1, 3.3, or 10 mg of NNK appeared to respectively induce respiratory tract tumors including tumors of either the lung, nasal mucosa, and/or trachea at 15%, 35%, and 42.1% of the animal subjects after 72 weeks [22]. In another study, Schuller et al. [28] demonstrated that hamsters exposed simultaneously to hyperoxia and NNK developed neuroendocrine lung tumors with an origin of type II cells. However, under ambient air conditions, NNK-induced adenomas and adenocarcinomas of Clara cell origin in hamsters. These results suggested that the altered pulmonary oxygen levels caused a change of lung tumor types in Syrian golden hamsters when treated with NNK [28]. Moreover, Hoffmann et al. [21] investigated the carcinogenicity of NNK and N’-nitrosonornicotine with different treatment schemes, and found that in golden hamsters NNK was a more powerful tobacco carcinogen leading to the tumor types of lung adenomas or adenocarcinomas.

In ferrets, a combination of monthly injection of 50 mg/kg NNK administered for four consecutive months and daily exposure to cigarette smoke for 6 months resulted in the development of grossly identifiable neoplastic lesions in the lung in 50% of the tested ferrets, with histopathological types of squamous cell carcinoma, adenosquamous carcinoma and adenocarcinoma similar to those in humans, while no ferrets in the control group developed any lesions during this time [23]. Aizawa et al. [24] also used this scheme in male ferrets (3–5-month old) without smoke exposure. They found that 16.7% of ferrets developed tumors 24 weeks after NNK injection (50 mg/kg), and after 26 and 32 weeks, the proportion increased to 40% and 66.7%, respectively. These results indicated that the injection of NNK alone could induce both preneoplastic lesions (squamous metaplasia, dysplasia, and atypical adenomatous hyperplasia) and tumors (squamous cell carcinoma, adenocarcinoma, and adenosquamous carcinoma) in lung, which were commonly seen in humans [24].

A key finding of NNK’s correlation with lung cancer is that NNK was shown to be capable of inducing lung cancer via numerous disparate treatments. Both F344 rats and Wistar rats treated with NNK, either by s.c., feeding via water, or intratracheal instillation developed lung cancer [25–27,29]. These results highlight the potential of NNK as a compound capable of inducing lung cancer in different animal species by different exposure methods.

Carcinogenesis Mechanism of NNK

NNK induces gene mutation and chromosomal instability

Several studies have been carried out to explore the carcinogenic mechanisms of NNK. NNK is metabolized into NNAL (the major carcinogenic form of NNK) [30] or NNAL-Gluc (the detoxication product) [31,32] by metabolic enzymes, such as cytochrome P450s. These metabolic intermediates will react with DNA to form DNA adducts, which causes DNA mutations if not repaired [33–35]. In the event that key tumor suppressor genes mutate, normal cells can be transformed into cancerous cells. Moreover, NNK is capable of inducing chromosomal instability.

Gross chromosomal alterations were observed in NNK-induced mouse lung adenocarcinomas [36,37]. More detailed studies should be performed to determine whether chromosomal changes in NNK-induced animal models are consistent with the changes in lung cancer patients upon tobacco exposure, which may help to find novel biomarkers that are critical for the early detection and risk management of lung cancer. Recent studies revealed that urinary levels of tobacco-specific nitrosoamine metabolites could be potentially used to predict risk and development of lung cancer [38–43].

NNK promotes lung cancer development in part through activating the α7 nicotinic acetylcholine receptor/extracellular-signal-regulated kinase/contactin 1 pathway

Numerous lines of evidence have established that NNK induces several kinds of cancer in different animal models, and that the underlying mechanisms are carcinogen-mediated DNA mutations and abnormal signaling pathways activated by associated receptors, such as α7 nicotinic acetylcholine receptor (α7-nAChR) and β-adrenergic receptor (β-AR). Contactin 1 is a neural cell adhesion molecule belonging to the immunoglobulin superfamily located in the plasma membrane through glycosyl phosphatidylinositol anchor. This molecule is capable of interacting with several other membrane proteins or extracellular matrix to activate the downstream signaling pathways [44,45].

Contactin 1 was previously demonstrated to regulate the development of several types of cancers including lung adenocarcinoma [46,47], and reported to promote lung cancer cell invasion and metastasis [48–50]. Hung YH and Hung WC [51] found that NNK up-regulated the expression of contactin 1 in low invasive CL1.0 lung adenocarcinoma cell line in a dose-dependent manner (Fig. 1). The induction of contactin 1 was blocked by α-bungarotoxin, an inhibitor of α7 nAChR [52,53]. NNK also induced the contactin 1 expression through extracellular-signal-regulated kinase (ERK) activated by α7 nAChR [54,55]. Additionally, NNK-promoted α7-nAChR/ERK/contactin 1-dependent adhesion and invasion in CL1.0 cells. Alongside the α7-nAChR/ERK pathway, contactin 1 was also capable of enhancing human lung adenocarcinoma cell line A549 cell invasion and metastasis via the VEGF-C/Flt-4-mediated Src–p38-C/EBP pathway [48–50]. Knockdown of contactin 1 was found to inhibit the invasion and metastasis of lung adenocarcinoma and increase the survival in animal models via a RhoA-mediated mechanism [47]. Furthermore, contactin 1 was also found to activate AKT, in part by preventing...
Table 1. NNK-induced lung cancer in different animal models

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Injection</th>
<th>Dose</th>
<th>Lung cancer incidence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/J mice</td>
<td>Days 14, 16, and 18 of gestation</td>
<td>i.p.</td>
<td>100 mg/kg, three times</td>
<td>24 weeks mothers: 13/14 (92.9%)</td>
<td>[17]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Progeny: 12/66 (18.2%)</td>
<td></td>
</tr>
<tr>
<td>A/J mice</td>
<td>6 weeks</td>
<td>i.p.</td>
<td>100 mg/kg</td>
<td>34–42 weeks, adenomas (50%)</td>
<td>[18]</td>
</tr>
<tr>
<td>A/J mice</td>
<td>Female, 7 weeks</td>
<td>i.p. or (i.g.)</td>
<td>Total 24 μmol, eight consecutive weekly treatments of NNK, each weekly dose was one-eighth of the total indicated</td>
<td>26 weeks, 100%</td>
<td>[19]</td>
</tr>
<tr>
<td>Swiss mice</td>
<td>Infant mice at postnatal days 1, 4, 7, 10, and 14</td>
<td>i.p.</td>
<td>50 mg/kg, five times</td>
<td>13–15 months, male: 57%, female: 37%</td>
<td>[20]</td>
</tr>
<tr>
<td>Golden hamster</td>
<td>8–10 weeks</td>
<td>s.c.</td>
<td>A: 0.048 mmol, three times weekly for 6.3 weeks, total dose: 0.91 mmol; B: 0.012 mmol, three times weekly for 25 weeks, total dose = 0.91 mmol</td>
<td>A: 16 months, 19/30 (63.3%); B: 17 months, 16/20 (80%)</td>
<td>[21]</td>
</tr>
<tr>
<td>Golden hamster</td>
<td>8 weeks</td>
<td>s.c.</td>
<td>Single 1.0 mg, 3.3 mg, or 10.0 mg, and then exposed to cigarette smoke for the next 72 weeks</td>
<td>8/19 (42.1%), 7/20 (35%), and 3/20 (15%)</td>
<td>[22]</td>
</tr>
<tr>
<td>Ferret</td>
<td>Adult male ferrets</td>
<td>Smoke exposure</td>
<td>50 mg/kg once a month, four times and daily exposure of cigarette smoke for 6 months</td>
<td>6/12 (50%) developed neoplastic lesions in the lung</td>
<td>[23]</td>
</tr>
<tr>
<td>Ferret</td>
<td>Male ferrets 3–5-monthsold</td>
<td>i.p.</td>
<td>50 mg/kg once a month, four times</td>
<td>16.7, 40.0, and 66.7% for 24, 26, and 32 weeks</td>
<td>[24]</td>
</tr>
<tr>
<td>F344 rats</td>
<td>7 weeks</td>
<td>s.c.</td>
<td>Three times weekly for 20 weeks, total dose 702 mg</td>
<td>12 months later, 67% lung tumors</td>
<td>[25]</td>
</tr>
<tr>
<td>F344 rats</td>
<td>Male, 8 weeks</td>
<td>Drinking water</td>
<td>0.5, 1.0, or 5.0 ppm in drinking water for 128, 120, and 108 weeks</td>
<td>9/80 (11.25%), 20/80 (25%), and 27/30 (90%)</td>
<td>[26]</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>Female, 6–7 weeks</td>
<td>Intratracheal instillation</td>
<td>Single dose of 25 mg/kg</td>
<td>92 days, 10/18 (55.6%) alveolar atypical dysplasia; 6/18 (33.3%) alveolar hyperplasia</td>
<td>[27]</td>
</tr>
</tbody>
</table>
nnK increases α7-nAChR-mediated expression of contactin 1, followed by activating RhoA and down-regulating E-cadherin, thereby promoting tumor invasion and metastasis. In addition, contactin 1 is induced by VEGF/C/Flt-4/Src/p38 MAPK/CREB/α pathway; (ii) NNK promotes arachidonic acid metabolism pathway to synthesize more TxA2, which then binds to its corresponding receptor TP, and thus activates the downstream PI3K/AKT/CREB pathway, resulting in angiogenesis and cell survival; and (iii) NNK induces HO-1 expression through NF-κB and ERK1/2 pathways, which correlates with tumor invasiveness, advanced stages, and poor prognosis.

NNK regulates gene expression through DNA methyltransferase 1-mediated epigenetic modifications

In lung cancer cells, DNA methyltransferase 1 (DNMT1) is over-expressed, and the over-expression correlates with tumor progression and poor clinical outcomes [56,57]. Apparently, DNMT1 methylates numerous tumor suppressor genes at the CpG islands in their promoters, and then down-regulates their expression levels [58]. Given the importance of DNMT1 in lung cancer and the finding that NNK-induced DNA methylation in several genes [59–62], it seems that increased DNA methylation level may be mediated in a DNMT1-dependent manner in NNK-induced lung cancers.

Lin et al. found that NNK increased the expression of DNMT1 in A549 cells by blocking its proteasomal degradation. Upon NNK treatment, AKT is activated and then promotes glycogen synthase kinase-3β (GSK3β) phosphorylation at Ser9 to form inactive GSK3β, which subsequently attenuates the ability of beta-transducin repeats-containing proteins (βTrCPs) to degrade DNMT1 protein, because inactive GSK3β fails to phosphorylate DNMT1 and only the phosphorylated DNMT1 protein could be recognized by βTrCPs [63,64]. AKT also enhances the ability of heterogeneous nuclear ribonucleoprotein U (hnRNPU) to transport nuclear βTrCPs into cytoplasm (Fig. 2). Potentially, nuclear accumulation of DNMT1 epigenetically silences several important tumor suppressor genes. Similar results were observed in esophageal squamous cell carcinoma [65].

Considering the important function of DNMT1 in NNK-related cancer development, inhibition of DNMT1 may be a promising approach for lung cancer patients with long-time and chronic smoking habits. Indeed, evidence from several studies indicates that inhibiting DNMT1 via siRNA or 5-aza-2′-deoxycytidine leads to promoter demethylation and gene re-expression in several types of cancer cells such as hepatocellular carcinoma cell, esophageal squamous cell carcinoma cell, and human non-small-cell lung cancer (NSCLC) cell [66–72]. The development of demethylation strategies targeting DNMT1 may be a promising and better approach for preventing or treating smoking-associated lung cancer [70,73,74].

NNK activates the TxA2/TxA2/TP/PI3K/AKT/CREB and β-AR/arachidonic acid signaling pathways

Huang et al. [75] demonstrated that NNK increased the expressions of thromboxane synthase TxA2 and thromboxane receptor (TP) in lung cancer cells. TxA2 is the enzyme responsible for the synthesis of TxA2 (thromboxane A2) from PGH2 (prostaglandin H2) which is synthesized from arachidonic acid by COX1/2. Aside from TxA2, PGH2 can also be metabolized into PGE2, PGD2, PGF2α, and PGI2 by prostaglandin synthases [76]. Recent studies demonstrated that TxA2 activated the TP signaling pathway, and promoted cell proliferation, survival, and invasion [77–80]. TP also promoted tumor growth and angiogenesis via induction of VEGF in A549 cells and NSCLC patients [81,82].
The critical role played by TxA2 in lung cancer development suggests that TxA2 may be involved in the NNK-mediated lung cancer development. This assumption is supported by the finding that NNK-stimulated TxA2 synthesis and that the TxAS inhibitor or TxA2 receptor antagonist inhibited NNK-promoted cell proliferation in NCI-H23 and CRL-2066 cells [75]. More explicitly, NNK activates PI3K/AKT and ERK pathways through TP, phosphates CREB, induces the expressions of PCNA and Bcl-2, and thereby promotes lung cancer cell survival [75]. The mechanism by which NNK induces the expression of TxA2 has previously been explored [83], whereby NNK increases the TPα protein level and increases the TxAS transcription via the COX-2/ERK/NF-κB pathway. The increased TxAS synthesized new TxA2, and further activated TPα, which formed an autoregulatory feedback loop for TPα activation. NNK also enhanced TxAS expression by inhibiting the miR-34b/c, which negatively regulated TxA2 ([Fig. 1](#fig1)) [93–95]. Furthermore, NNK increased the expressions of HO-1 downstream pro-survival proteins, such as c-IAP2 and Bcl-2, conferring resistance to apoptosis. Interestingly, HO-1 also activated the NF-κB pathway, and in A549 cells HO-1 promoted metastasis via the NF-κB pathway-mediated up-regulation of MMPs [96]. In endothelial cells, CO generated by HO-1 activated the NF-κB pathway to exert an anti-apoptotic effect [97].

Existing evidence shows that the TxA2/TP signaling pathway plays a critical role in NNK-induced lung cancer development, and accordingly targeting this pathway may be a promising approach to develop novel therapies. As expected, several studies showed that TxA2 antagonist, TxAS inhibitor, or TP antagonist could inhibit cancer cell growth and induce apoptosis by increasing the ROS level, inhibiting the NF-κB pathway, increasing the nuclear p27 level, or blocking ERK/CREB signaling in lung cancer, bladder cancer, and glioblastoma [83,85–91].

NNK induces the expression of heme oxygenase-1
Heme oxygenase-1 (HO-1) is a stress induced rate-limiting enzyme in heme catabolism which can degrade heme to carbon monoxide (CO), biliverdin, and ferrous iron [92]. It is reported that NNK significantly induced the expression of HO-1 in human NSCLC cells NCI-H23 via NF-κB and ERK1/2 pathways, because blocking either pathway inhibited the stimulatory effect of NNK on HO-1 ([Fig. 1](#fig1)) [93–95]. Furthermore, NNK increased the expressions of HO-1 downstream pro-survival proteins, such as c-IAP2 and Bcl-2, conferring resistance to apoptosis. Interestingly, HO-1 also activated the NF-κB pathway, and in A549 cells HO-1 promoted metastasis via the NF-κB pathway-mediated up-regulation of MMPs [96]. In endothelial cells, CO generated by HO-1 activated the NF-κB pathway to exert an anti-apoptotic effect [97].

The expression of HO-1 is increased in lung cancer patients, which is correlated with high tumor invasiveness, high grades, and poor prognosis [98,99]. Additionally, HO-1 promotes cancer cell proliferation, differentiation, metastasis, angiogenesis, and anti-tumor immune response, which has been excellently reviewed by Was et al. [100]. These results indicated that HO-1 may be a potential target for future cancer treatments [95,96,101–104].
NNK induces immune suppression

Due to the important role of immune system in tumor initiation and development, the effect of tobacco carcinogens on the immune system has drawn increasing attention. In A/J mice, the NNK-induced lung adenomas/adenocarcinomas showed an increase in the number of tumor cells following the depletion of NK cells, suggesting that NNK might inhibit NK cells in lung cancer [105,106]. Recovering NK cells from NNK-induced immunosuppression status may provide a promising method to treat lung cancer [107]. Further studies should focus on elucidating the immunosuppression mechanisms of NNK, which will help to develop compounds capable of activating the immune system and inhibiting tumor growth more effectively.

NNK was also shown to affect cytotoxic T lymphocyte (CTL) activation and reduce memory programming. An in vitro study showed that NNK could enhance the expression of adhesion molecule CD62L in CTLs, but had no effects on the expansion and production of effector molecules [108]. NNK pretreatment causes an early loss of CTL expansion after transplanted into recipient mice. The exposure of CTLs to NNK resulted in reduced formation of memory CTL. Therefore, NNK could induce immune suppression in lung cancer.

NNK activates other signaling pathways

Shen et al. [109] demonstrated that NNK-promoted cell migration and invasion by activating Src, focal adhesion kinase (FAK), and PKC. Knockdown of Src, FAK, and PKC by siRNA inhibited NNK-induced H1299 cell migration and invasion. In A549 cells, NNK binds to β-AR and activates c-Src/PKC (Fig. 2), and subsequently PKC triggers Bad phosphorylation at multiple sites, leading to the dissociation of Bcl-XL, which abolishes the pro-apoptotic activity of Bad, thereby resulting in cell survival and chemoresistance [110]. Pham et al. [111] also reported that NNK could bind to β-AR and activate FAK/Src/ERK in pancreatic cancer cells. These findings imply that NNK could potentially induce cell migration and invasion by activating Src/PKC/FAK in α7-nAChR-dependent manner [109,110,112,113]. Guo et al. [115] further demonstrated that NNK-induced IKKβ expression via STAT3 in human NSCLC cells, and knockdown of IKKβ abolished nicotine-induced cell survival and induced apoptosis in NSCLC cells [114]. Moreover, NNK increased the PKA-dependent CREB phosphorylation via β-AR in lung adenocarcinoma cells [115] (Fig. 2).

Chemopreventive Agents Against NNK-induced Lung Cancer

In mice, hamsters, rats, and ferrets, NNK was found to be a potent carcinogen that effectively induces lung cancer. The successes of these models, together with the intrinsic advantages of such models, suggested that animal models could be effectively used to develop and evaluate anti-tumor or chemopreventive agents in a way that clinical trials could not. Here, we summarized several anti-tumor or chemopreventive agents and their functional mechanisms (Table 2). Retinoic acid and the retinoic acid receptor RARβ signaling pathway usually inhibit tumor development [143], but in several cancers (including lung cancer) this pathway is frequently inactivated due to the loss of RARβ expression [144–146]. Chemical agents that can restore the abnormal inactivated RARβ signaling may be developed into promising drugs for lung cancer treatments. Some antioxidants (β-carotene, α-tocopherol, and ascorbic acid) have been demonstrated

Table 2. Chemopreventive agents against NNK-induced lung cancer

<table>
<thead>
<tr>
<th>Agent</th>
<th>Species</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9cRA</td>
<td>A/J mice</td>
<td>Increasing expression of RARβ</td>
<td>[116]</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>A/J mice</td>
<td>Restoring expression of lung SIRT1, p53, and RARβ to that of the control group and decreasing the level of lung IL-6 mRNA and phosphorylation of AKT</td>
<td>[117]</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>A/J mice</td>
<td>Inhibiting AKT/mTOR pathways</td>
<td>[118]</td>
</tr>
<tr>
<td>5F</td>
<td>A/J mice</td>
<td>Inducing apoptosis and suppressing cell proliferation</td>
<td>[119]</td>
</tr>
<tr>
<td>β-Escin</td>
<td>A/J mice</td>
<td>Inhibiting ALDH activity and RhoA/Rock signaling</td>
<td>[120]</td>
</tr>
<tr>
<td>Black tea or green tea</td>
<td>A/J mice</td>
<td>Inhibition of cell proliferation; inhibition DNA lesion partly by antioxidant properties</td>
<td>[121–124]</td>
</tr>
<tr>
<td>PEITC-NAC plus MI or I3C</td>
<td>A/J mice</td>
<td>Changing expression pattern of carcinogenesis-related proteins</td>
<td>[125]</td>
</tr>
<tr>
<td>Kava (beverage, dihydromethysticin)</td>
<td>A/J mice</td>
<td>Inhibiting proliferation and promoting apoptosis; reducing DNA adducts</td>
<td>[126,127]</td>
</tr>
<tr>
<td>Changkii saponins</td>
<td>A/J mice</td>
<td>Inhibiting proliferation (PCNA)</td>
<td>[128]</td>
</tr>
<tr>
<td>CP-31398 and Prima-1</td>
<td>A/J mice</td>
<td>Reducing proliferation by activating function of nut-p53</td>
<td>[129]</td>
</tr>
<tr>
<td>Suberyolamidine hydroxamic acid</td>
<td>A/J mice</td>
<td>Inhibiting α-hydroxylation pathway of NNK</td>
<td>[130]</td>
</tr>
<tr>
<td>PEITC-NAC plus MI</td>
<td>A/J mice</td>
<td>Inhibition of cell proliferation and induction of apoptosis</td>
<td>[131]</td>
</tr>
<tr>
<td>β-Carotene, α-tocopherol, ascorbic acid</td>
<td>Ferrets</td>
<td>Maintaining normal tissue level of RA, inhibiting phosphorylation of JNK and ERK</td>
<td>[132]</td>
</tr>
<tr>
<td>Indole-3-carbinol</td>
<td>A/J mice</td>
<td>Inhibition of cell proliferation and induction of apoptosis</td>
<td>[133,134]</td>
</tr>
<tr>
<td>p-XSC</td>
<td>F344 rats</td>
<td>Inhibiting the formation of DNA adducts</td>
<td>[135]</td>
</tr>
<tr>
<td>Aspirin and phenethylisothiocyanate</td>
<td>Wistar rats</td>
<td>Inhibiting cell proliferation</td>
<td>[27]</td>
</tr>
<tr>
<td>C93</td>
<td>A/J mice</td>
<td>Inhibiting fatty acid synthase</td>
<td>[136]</td>
</tr>
<tr>
<td>Tea polyphenols and caffeine</td>
<td>A/J mice</td>
<td>Decreased phosphorylation level of c-Jun and ERK1/2</td>
<td>[137]</td>
</tr>
<tr>
<td>Metformin</td>
<td>A/J mice</td>
<td>Inhibiting IGF-I/insulin receptor and mTOR signaling</td>
<td>[138,139]</td>
</tr>
<tr>
<td>Acetylsalicylic acid and NS-398</td>
<td>A/J mice</td>
<td>Inhibiting COX2-mediated bioactivation of NNK</td>
<td>[140]</td>
</tr>
<tr>
<td>Green tea polyphenols and atorvastatin</td>
<td>A/J mice</td>
<td>Promoting cell apoptosis</td>
<td>[141]</td>
</tr>
<tr>
<td>4-Ipomeanol analogs</td>
<td>A/J mice</td>
<td>Inhibiting NNK metabolism</td>
<td>[142]</td>
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</table>
to effectively prevent NNK-induced lung carcinogenesis in smoke-exposed ferrets through restoring retinoic acid to the normal level and inhibiting the JNK and MAPK pathways [132]. Moreover, β-cryptoxanthin [117] and 9-cis-retinoic acid [116] were also found to exhibit anti-tumor activities via restoration of the nicotine-inhibited expression of lung SIRT1, p53, and RAR. These results imply that RARβ signaling pathway may be a potential target for lung cancer treatments.

Since NNK activates several aberrant signaling pathways in lung carcinogenesis, compounds that can attenuate these signaling pathways, such as AKT/mTOR inhibitor rapamycin [118], RhôA/Rock inhibitor β-escin [120], NF-κB pathway inhibitor kava [126], fatty acid synthase inhibitor C93 [136], MAPK inhibitor tea polyphenols [137], and IGF-1R/IR inhibitor metformin [138], will reduce carcinogenesis in NNK-induced lung cancer animal models.

Furthermore, the NNK metabolism pathway may provide a good target for chemopreventive agents. Compounds such as 4-ipomeanol (a competitive inhibitor of NNK metabolism), NS-398 (a COX-2-specific inhibitor), and SAHA (an inhibitor of a-hydroxylation pathway) exert effective anti-tumor activities by targeting at a-hydroxylation pathway, an NNK bioactivation pathway [130,140,142]. The NNK metabolite-mediated DNA lesions were also reduced by p-XSC [135], dihydromethystine [127], and EGCG [121]. These results indicate that NNK-induced lung cancer models are useful for developing new chemopreventive agents.

Conclusions and Perspectives on Future Research

While the general mechanism of NNK and the association of NNK with lung cancer have both been well studied, the precise mechanism of NNK-mediated carcinogenesis remains to be further investigated. For example, NNK-induced miRNA changes should be studied in lung cancer models [147–149]. A whole genome and transcriptome analysis should be performed in NNK-induced lung cancers, which will provide information of a new paradigm of mechanism of NNK-induced carcinogenesis, and help to explain the differences of lung cancer risk among different individuals and populations [150–152]. In addition, these NNK-induced lung cancer animal models should be further improved because of their relatively long latency. NNK may be used together with other potent carcinogens and by improved schemes. Studies with novel animal models will provide more insights into NNK’s carcinogenesis mechanisms, and develop novel drugs for clinical treatments.

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NNK induces lung cancer


