

# The Role of Nitrosamine (NNK) in Breast Cancer Carcinogenesis

Nomundelger Gankhuyag<sup>1</sup> · Kang-Hoon Lee<sup>1</sup> · Je-Yoel Cho<sup>1</sup> 

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**Abstract** Smoking cigarettes is one of the most concerning issues that leads to tobacco-related cancers and can even result in death. Therefore, these issues should be addressed with a great sense of urgency with low-cost and simple approaches. Over the past several years, the scientific community has attempted to find solutions to overcome this issue. Thus, a large number of excellent studies have been reported in this field, and summarizing these results and providing important roadmaps for future studies is currently of great importance. Finding an outstanding solution to address aforementioned issue would be of great value to the community and to the social. Tobacco contains thousands of chemicals, and sixty-nine compounds have been established as human carcinogens; specifically, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is the strongest carcinogen among the tobacco-specific nitrosamines. Tobacco carcinogens are also linked to mammary gland pathogenesis and increased risk of developing many cancers, including breast cancer, the most common cancer in women worldwide. This mini-review summarizes the role of NNK and the mechanisms of its receptor, nicotine acetylcholine receptor (nAChR), signaling in breast cancer based on publications identified using the keywords “secondhand smoke (SHS)”, “Nitrosamines” and “breast cancer”. Furthermore, this review considers the risk of NNK to the public in an effort to reduce exposure to SHS in women and their chances of developing breast cancer.

**Keywords** Second hand smoke · Nitrosamines · Breast cancer · Nicotinic acetylcholine receptor

## Abbreviations

Carex	carcinogen exposure
EMT	epithelial to mesenchymal transition
ETS	environmental tobacco smoke
FDA	food and drug administration
GWAS	genome-wide association studies
IARC	international agency for research on cancer
nAChR	nicotine acetylcholine receptor
NDEA	N-Nitrosodiethylamine
NDELA	N-nitrosodiethanolamine
NDMA	N-nitrosodimethylamine
NMEA	N-nitrosomethylethylamine
NMOR	N-nitrosomorpholine
NNAL	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N-nitrosornicotine
NPIP	N-nitrosopiperidine
NPYR	N-nitrosopyrrolidine
NSAR	N-nitrososarcosine
NSCLC	non-small cell lung carcinoma
PAHs	polycyclic aromatic hydrocarbons
PKA	protein kinase A
PKC	protein kinase C
SCLC	small-cell lung cancer
SHS	secondhand smoking

✉ Je-Yoel Cho  
jeycho@snu.ac.kr

<sup>1</sup> Department of Biochemistry, BK21 PLUS Program for Creative Veterinary Science Research and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, South Korea

## Introduction

The effect of smoking tobacco has been studied in patients with lung cancer or smoking-related cancer, including breast cancer,

due to heavy smoking [1, 2]. Breast cancer is the most common cancer in women; 1.7 million were diagnosed and 521,000 died worldwide in 2012 [3, 4]. Smoking tobacco has been suspected as one of the risk factors which can be regulated for breast [5, 6].

However, many recently published studies show that the risk of developing breast cancer is not directly associated with active smoking. Since 1986, an expert panel agency that includes the US Surgeon General and the International Agency for Research on Cancer (IARC) repeatedly concluded that the evidence does not support an association between smoking and the risk for breast cancer [7, 8]. Moreover, Hamajima et al. concluded that active smoking and breast cancer did not correlate based on their collated analysis of 53 epidemiological studies that included more than 150,000 cases [7].

On the other hand, secondhand smoke (SHS) (also known as passive smoking, involuntary smoking and environmental tobacco smoke (ETS)) has been identified as a toxic air contaminant since 2006 by Air Resources Board, California Environmental Protection Agency [9]. Non-smokers, who inhaled tobacco gases by being exposed to environmental tobacco smoke, are called passive and secondhand smokers. Throughout life, SHS occurs in the childhood household, in the adulthood household, and at work [10]. The CAREX (carcinogen exposure) database confirmed that in the European Union, 7.5 million workers are exposed to environmental tobacco smoke during ~75% of their workday, which is the second-most common environmental hazard after solar radiation (9.1 million workers) (<http://www.ttl.fi/Internet/English/Organization/Collaboration/Carex/default.htm>) [11]. Worse than active smoking, the association of SHS with the risk of breast cancer has been contested because the assessment of SHS is more difficult than that of active smoking due to lack of information about a major source of exposure [12, 13]. Notably, recent studies of large number of prospective cohorts provided evidence to suggest that breast cancer risk is elevated among women exposed to the highest levels of SHS [14]. In addition, a study by Johnson and Glantz comparing the strength of evidence from epidemiologic studies showed that SHS affects breast cancer more significantly than lung cancer [15].

Tobacco smoke is well known to contain thousands of chemicals, including more than 60 known carcinogens, which cause a variety of diseases and cancers [16]. Out of 60, at least 20 chemicals have been documented to induce mammary tumors in animal studies using rodent models [17] (Table 1). For the last few decades, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has been implicated in the risk of breast cancer development, it remains unclear in breast cancer development, specifically breast cancer induced by SHS [18, 19]. Moreover, NNK can reportedly bind to and activate nicotine acetylcholine receptors (nAChRs) [20, 21], and cancer cells express nAChRs, suggesting that these receptors play a crucial role in cancer development [22].

**Table 1** Summary of chemicals associated with increased mammary gland tumors in rodent models

Chemicals	Concentration in smoke of nonfilter cigarette	IARC group
benzo(a)pyrene	20–40 ng	2A
toluidine	30–337 ng	2B
4-aminobiphenyl N-heterocyclic amines	25–260	1
IQ	0.3	2A
hydrazine	24–43	2B
PhIP	11–23	2B
isoprene	450–1000 I g	2B
benzene	20–70 I g	1
styrene	10 I g	2B
nitromethane	0.5–0.6 I g	2B
nitrobenzen	25 I g	2B
acrylamide	Present	2B
acrylonitrile	3–15 I g	2A
vinyl chloride	11–15 I g	1
ethylene oxide	7 I g	1
propylene oxide	0–100 ng	2B
dibenz(a,h)anthracene	4 ng	2A
<i>N-nitrosamines</i>		
N-nitrosonomitocine	120–3700 ng	2B
N-nitrosodimethylamine	2–1000 ng	2A
N-nitrosodiethylamine	ND–2.8 ng	2A
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	80–770 ng	2B

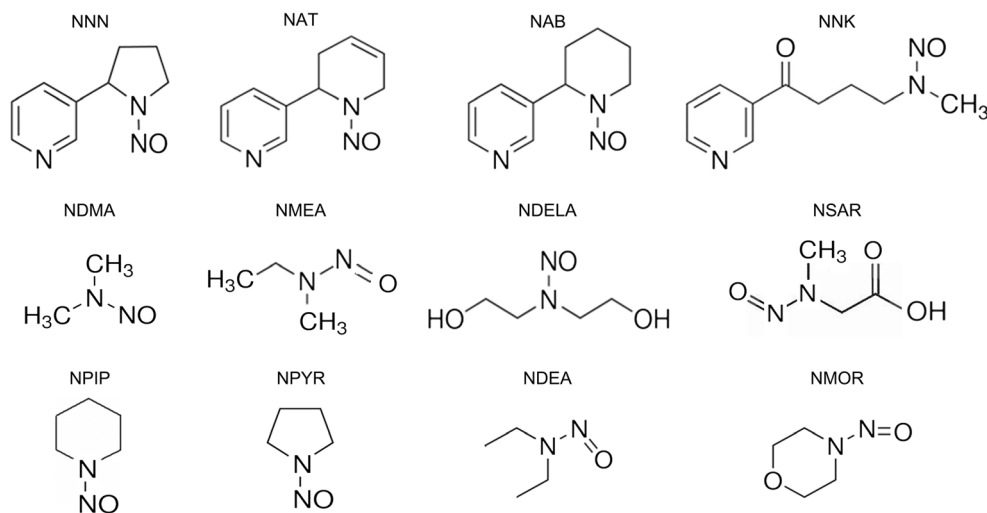
IQ: 3-methylimidazo[4,5-f]quinoline, PhIP: 2-Amino-1-Methyl-6-Phenylimidazo 4 5-b pyridine

Although active and passive smoke likely contain same chemicals but at different amounts, the fact that SHS is significantly more toxic than active smoke is surprising [23, 24]. This review focuses on the role of NNK in SHS-triggered breast cancer and the molecular mechanism underlying NNK-induced carcinogenesis.

### Tobacco Nitrosoamines

More than 70 tobacco species are known; tobacco is a green and leafy plant that originates in South America and contains nicotine, its main addictive compound, and thousands of other chemicals. Therefore, tobacco is often referred to as a chemical factory. The major chemical components of tobacco are converted to 5300 tobacco smoke compounds, such as nicotine derived N-nitrosamines, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, aldehydes and other inorganic and organic compounds [25]. Roberts classified unburned tobacco and cigarette smoke into a functional group of 25

**Fig. 1 The structures of the nitrosoamines found in tobacco smoke.** NNN (N-nitrosornicotine), NAT (N-nitrosoanatabine), NAB (N-nitrosoanabasine), NNK (4-methylnitrosamino-1-3-pyridyl-1-butanone), NDMA (N-nitrosodimethylamine), NMEA (N-nitrosomethylamin), NDELA (N-Nitrosodiethanolamine), NSAR (N-nitrososarcosine), NPIP (N-nitrosopiperidine), NPYR (N-nitrosopyrrolidine), NDEA (N-nitrosodiethylamine), NMOR (N-nitrosomorpholine)



classes of chemicals, and Hoffmann and coworkers subsequently added N-nitrosamines to this group. Gudzinowicz identified more chemical substances in tobacco leaf in 1980 [26]. The US Food and Drug Administration (FDA) and “Hoffman analytes” lists both have identified “harmful and potentially harmful” constituents in tobacco products, including two classes of compounds that have garnered significant attention: polycyclic aromatic hydrocarbons, mainly benzo[*a*]pyrene, and Nitrosamines (N-nitrosodiethylamine (NDEA), N-nitrosodiethanolamine (NDELA), N-nitroso dimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosomorpholine (NMOR), 4-(methyl nitrosamino)- 1-(3-pyridyl)-1-butanone (NNK), N-nitrosornicotine (NNN), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), and N-nitrososarcosine (NSAR)), especially NNK [27, 28]. (Fig. 1). Some of these chemical compounds, which are released when tobacco is burned, are carcinogenic and toxic [29]. Their carcinogenicity has been extensively examined both in vivo and in vitro systems [30–32]. Tobacco-specific *N*-nitrosamines, such as NNK, are derived from tobacco curing and smoking and their levels range from 50 to 200 ng per cigarette [33, 34].

### Tobacco-Specific Carcinogen Nitrosamine, NNK

As mentioned above, nicotine is an addictive but non-carcinogenic to healthy laboratory animals [33], and nicotine has consequently not been significantly researched in the context of cancer. However, the nitrosation of nicotine and alkaloids results in a “nicotine-derived nitrosamine ketone”, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [35], by replacing the N-H moiety with an N-N = O moiety, a change that was first reported in 1978 [30]. Among tobacco-specific nitrosamines, NNK is widely considered the most potent carcinogen to humans for tobacco-induced cancers and was evaluated by the International Agency for Research on Cancer [10, 36]. NNK has been detected in cigarettes, cigars, snuff tobacco and mainstream and sidestream tobacco smoke. Nitrosamines affect not only tobacco consumer but also non-smokers, who are exposed to environmental tobacco pollution [37]. Moreover, tobacco-specific nitrosamine, 63–74% of which consists of NNK, is formed from nicotine during smoking [38, 39](Table 2). Nicotine-derived nitrosamines can cause cancers or increase the risk of cancer in animals and humans [40–43], and humans may be exposed to nitrosamines by inhaling both

**Table 2** Tobacco-specific nitrosamines in both smokeless tobacco and cigarette smoke

Smoke type	NNN	NNK	NAB	Reference
Electronic cigarette (ng/15puffs)	7.7 0.00008–0.00043	ND 0.00011–0.00283	1.2–2.3 ng/g	Cressey [117] Grana [118]
Others (chewing, etc.) (ppm)	0.47–64	0.03–14	0.03–6.5	Hecht and Hoffmann [77]
Mainstream smoke (µg/cigarette)	0.33–12.454	0.004–4.2	0.33–4.6	Hecht and Hoffmann [77], Fisher et al. [119], Hoffmann et al. [120]
Sidestream smoke (µg /cigarette)	0.15–16.6	0.39–15.7	0.15–1.5	Hecht and Hoffmann [77] Hoffmann et al. [120]

NNN: N-nitrosornicotine, NNK: 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone, NNB: N-nitrosoanabasine

mainstream and sidestream smoke [44]. Accordingly, carcinogenic NNK and its metabolite are found more in the urine (0.06 to 1.4 pmol/mg creatinine) and blood ( $42 \pm 22$  fmol/mL) of smokers than urine (0.02 to 1.2 pmol/mg creatinine) and blood ( $<8$  fmol/mL) of nonsmoker [45, 46]. Current technical improvements have also directly detected nitrosamine-hemoglobin adducts in the blood of smokers [47]. Notably, raw sidestream smoke includes 20–30-fold more aromatic amines and 30–90% more particulate matter than mainstream smoke [48]. In addition, worldwide use of electronic cigarettes has increased rapidly. It is known that electronic cigarette liquids contain low levels of tobacco-specific nitrosamines but a few studies have tested that vapour from electronic cigarette is not free from NNK. Goniewicz et al. analyzed some carcinogens and toxicants in vapour from electronic cigarettes and found NNN (0.08–0.43 ng/15puffs) and NNK (0.11–2.83 ng/15puffs) smoke [49]. Farsalinos et al. also reported NAB at trace levels (1.2 ~ 2.3 ng/g) in vapour from electronic cigarettes [50].

### SHS and Breast Cancer

In fact, true non-smokers may not exist since all individuals are exposed to SHS at some point during their life, either in the childhood household, in the adulthood household, or at work [10]. In other words, most non-active smokers willingly or unwillingly inhale tobacco smoke from the environment. Moreover, previous meta-analysis studies have reported that breast cancer is associated with cigarette smoking because long periods of exposure to SHS increased breast cancer risk [2, 51, 52]. A. Wesley Horton reported that breast cancer and cigarette consumption are closely related in 20 countries, and the inhalation of tobacco smoke is considered a major environmental risk factor for breast cancer [14]. Furthermore, Chu et al. found that cigarette smoking significantly increases the risk of breast cancer in younger or premenopausal women, and this risk strongly correlates with smoking intensity and duration [53]. Similar studies have been reported in several countries, including Japan, Canada, and the USA [8, 54–56]. The main risk of cigarette smoking-associated breast cancer is presumably related to exposure to the potent carcinogens contained in tobacco smoke, including aromatic hydrocarbons, aromatic amines and nitrosamines, which may cause DNA mutations and DNA adducts [57]. Thus, many meta-analyses evaluated causal associations between environmental tobacco smoke and breast cancer and were reviewed by the California Environmental Protection Agency [58]. These studies demonstrated a causal relationship between exposure to SHS and breast cancer [59].

Reynolds et al. reported that high levels of SHS during adulthood may increase the risk of breast cancer in female lifetime non-smokers exhibiting the highest level of cumulative exposure (HR, 1.18; 95% CI, 1.00–1.40) [12]. Furthermore, breast cancer risk was strongly associated with

SHS, and SHS exposure is reportedly riskier than active smoking [60]. Schick and Glantz investigated unpublished *in vivo* research data on SHS obtained by the cigarette industry. Interestingly, animal experiments showed that inhaled sidestream smoke is approximately four times more toxic than mainstream cigarette smoke. Sidestream smoke can also damage the respiratory epithelium after 21 days of exposure, and the length of exposure directly correlates with the degree of damage. Overall, the study showed that whole sidestream smoke is more toxic than all of its major constituents combined [61]. Passive exposure is recognized as a high-risk factor because N-nitrosamines and carcinogens found in tobacco are more concentrated in sidestream than in mainstream smoke [10, 61]. Brunneemann et al. quantitatively analyzed N-nitrosamines in tobacco, fresh cigarette mainstream smoke and sidestream smoke using a thermal energy analyzer. Specifically, the representative nitrosamines in tobacco smoke, such as dimethylnitrosamine, methylethylnitrosamine, diethylnitrosamine, and nitrosopyrrolidine, were found at low levels (0.1 ng–97 ng) in mainstream smoke and higher concentrations (9 ~ 1770 ng) in sidestream smoke [62].

In cohort studies, Luo et al. observed that passive smokers who had never been active smokers had a 32% increased risk of breast cancer [63]. A relationship was also identified between breast cancer and the husband's smoking habit among Korean and Chinese women. Specifically, the risk directly correlated with the duration of the husband's smoking habit [64, 65]. In Thailand, sources of passive tobacco exposure include spouses (40.8%), the workplace (30.6%), and public areas (26.3%), and this exposure is an important risk factor for breast cancer among females in urban areas [66]. Moreover, an ecologic analysis indicated that women who live in smoke-free environments had a lower risk of breast cancer [67], and importantly, scientific data show that passive smoke is the most important risk factor for female breast cancer, despite the fact that passive smoke is more diluted than direct smoke [15]. These results suggest that effective preventative methods, such as smoke-free public environments, are needed to prevent breast cancer. Since 1985, many research groups have published relationships between breast cancer and SHS, as summarized in Table 3, which shows the results of 10 prospective cohort studies, 16 case-control studies and 7 meta-analyses.

### Nicotinic Acetylcholine Receptor (nAChRs) Signaling in Breast Cancer

#### nAChRs

The nicotine contained in tobacco products and its receptor, nAChR, have been well studied due to their numerous effects on the human body [68–70]. This receptor family plays a role in the synthesis and release of neurotransmitters

**Table 3** Studies of secondhand smoking and breast cancer

Study	Study Population (number of participants)	Follow-up (years)	SHS Exposure	Relative Risk of Breast Cancer in Women Exposed to SHS Compared to Women Not Exposed to SHS, RR (95% CI)
<b>Prospective cohort studies</b>				
Egan et al. [121]	78,206 (3140)	14	home or at work	0.90 (0.67–1.22)
Reynolds et al. [122]	116,544 (2005 cases)	5	home	0.94 (0.82–1.07)
Pirie et al. [123]	224,917 (2518 cases)	4	home	1.02 (0.86–1.16)
Lin et al. [124]	271,412 (208 cases)	11	home	1.24 (0.84–1.85)
Reynolds et al. [12]	57,523 (1754 cases)	10	Home, at work or social	1.13 (0.96–1.33)
Luo et al. [125]	41,022 (1660 cases)	10	home or at work	1.11 (0.92–1.34)
Xue et al. [126]	36,017 (2890 cases)	24	home or at work	0.97 (0.81–1.16)
Rosenberg et al. [127]	52,425 (1377 cases)	14	home	1.18 (0.98–1.42)
Dossus et al. [128]	183,608 (6264 cases)	11	home or at work	1.10 (1.01–1.20)
Wada et al. [129]	36,990 (15,719 cases)	16	home	1.98 (1.03–3.84)
<b>Case-control studies</b>				
	Cases	Controls		
Sandler et al. [2]	518	518	home	1.8 (1.0–3.7)
Johnson et al. [130]	2317	2438	home or at work	Premenopausal women: 2.6 (1.1–6.0) Postmenopausal women: 1.1 (0.6–1.8)
Kropp et al. [131]	197	459	home, at work	1.6 (1.1–2.4)
Shrubsole et al. [132]	1013	1117	home, at work or outside of home	1.1 (0.8–1.4)
Bonner et al. [133]	1166	2105	home, at work	Premenopausal women: 1.17 (0.54–2.56) Postmenopausal women: 1.29 (0.82–2.01)
Lissowska et al. [134]	2386	2502	home or at work	1.11 (0.85–1.46)
Roddam et al. [135]	639	640	home	Premenopausal women: 0.89 (0.64–1.25)
Slattery et al. [136]	1527	1601	home or at work	Premenopausal women: 1.2 (0.6–2.7) Postmenopausal women: 1.0 (0.6–1.7)
Young et al. [137]	6235	6533	home or at work	0.97 (0.88–1.08)
Anderson et al. [56]	3101	3471	home, at work or in social	Premenopausal women: 1.61 (0.74–3.52) Postmenopausal women: 1.03 (0.69–1.55)
Gao et al. [65]	669	682	home	1.47 (1.18–1.84)
Hu et al. [138]	196	211	Home, at work	1.54 (0.94–2.52)
Tong et al. [139]	312	312	home	1.46 (1.05–2.03)
Pimhanam et al. [66]	444	444	Home, at work or public	2.27(1.30–3.98)
Nishino et al. [140]	773	2057	home or at work	1.13 (0.90–1.42)
Li et al. [141]	877	890	home, at work	2.17 1.45–3.23
<b>Meta-analyses</b>				
Johnson et al. [13]	19 cohort and case-control studies		home or at work	1.27 (1.11–1.45)
Lee et al. [142]	22 cohort and case-control studies		home or at work	1.54 (1.17–2.04)
Miller et al. [58]	19 cohort and case-control studies		home or at work	1.25 (1.08–1.44)
Pirie et al. [123]	8 cohort studies		Home or at work	1.01 (0.96–1.06)
	17 case-control studies		Home or at work	1.21(1.11–1.32)
Yang et al. [143]	10 cohort studies		home, at work or public	1.01 (0.96–1.06)
Chen et al. [144]	8 case-control studies		home	1.67(1.27–2.21)
Macacu et al. [145]	11 cohort and 20 case-control studies		Home or at work	1.20 (1.07–1.33)

in the central nervous system and regulates all involuntary organ functions via the autonomic nervous system [71, 72]. The nAChR is a cation-selective channel receptor, and its activation is associated with cell proliferation, anti-

apoptosis, and angiogenesis processes [73]. Moreover, many biological reports have suggested that the effects of tobacco exposure are mediated by the actions of nitrosamine and nAChRs [20, 74, 75].

Cancer related-nAChR function was first described in 1989 using small-cell lung cancer cell lines [76]. Although follow-up studies have supported that nicotine inhibited apoptosis via nAChRs in lung cancer cell lines, it has not been highlighted until the binding of nicotine-derived nitrosamine to  $\alpha 7$ nAChR was found because nicotine itself does not cause cancer in laboratory animals [20, 30, 77]. In 1998, Schuller finally discovered that the nicotine-derived nitrosamine and powerful carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1 butanone (NNK) is an  $\alpha 7$ nAChR agonist with a more than 1300-fold higher affinity for the  $\alpha 7$ nAChR than nicotine. This finding provided a completely new direction in tobacco-associated cancer research [20, 76].

### nAChRs in Cancers

The neuronal system expresses homo-pentamers ( $\alpha 7$ - $\alpha 9$  subunits) or hetero-pentamers ( $\alpha 2$ - $\alpha 6$ ,  $\alpha 10$  subunits with  $\beta 2$ - $\beta 4$  subunits) of nAChRs, unlike the different combinations of nAChR subunits found in muscle ( $\alpha 1$  with  $\beta 1$ ,  $\gamma$ ,  $\delta$ , or  $\epsilon$  subunit) [78]. However, both arrangements are reportedly expressed and exhibit opposite functions in cancer cells.  $\alpha 7$ nAChR most effectively stimulates the growth of cancer cells, whereas  $\alpha 4\beta 2$ nAChR inhibits growth [79, 80].  $\alpha 7$ nAChR can bind to NNK with 1300 times higher affinity than to nicotine, whereas heteromeric  $\alpha\beta$  nAChRs exhibits a high affinity to NNN (~5000 times higher than to nicotine) [20, 21]. Notably, long-term exposure to nicotine and nicotine-derived nitrosamines, such as habitual tobacco smoking and exposure to SHS, upregulates all nAChRs but selectively leads the desensitization of  $\alpha 4\beta 2$ nAChR activity, whereas the activity of  $\alpha 7$ nAChR remains unchanged [7]. Nicotine-derived nitrosamines change the expression and function of the  $\alpha 7$ nAChR to hyperactivate all its downstream “accelerator functions” while simultaneously activating desensitized heteromeric nAChRs, which disables their “brake” functions. Therefore, smoking is a prominent risk factor for all human cancers [80], and  $\alpha 7$ nAChR may represent a major receptor subfamily that mediates cancer cell proliferation.

Many studies have demonstrated that nAChR is closely involved in the development of various cancers [81]. The most well-studied nAChR mechanism is that of  $\alpha 7$ nAChR in tobacco-related non-small cell lung carcinoma (NSCLC). Specifically, Paleari et al. demonstrated that nicotinic receptor antagonists inhibit the growth of NSCLC, accounting for 30% of all primary pulmonary malignancies both in vitro and in vivo. [82]. Moreover, Improgo et al. showed that nAChR, especially  $\alpha 3\beta 4\alpha 5$  nAChR, plays a crucial role in small-cell lung cancer (SCLC). Using publicly available data of multiple genome-wide association studies (GWAS) in lung cancer, they found that gene variants encoding  $\alpha 3\beta 4\alpha 5$  nAChR are closely associated with lung cancer and confirmed that  $\alpha 3\beta 4\alpha 5$  nAChR promotes SCLC growth [22]. Nicotine also

reportedly induces the secretion of different types of calpain, a proteolytic enzyme, from non-small cell lung cancer, which can promote the cleavage of various substrates in the extracellular matrix to result in metastasis and tumor progression [83]. In addition to lung cancer, the association of nicotine and nicotine derivatives with various types of nAChRs has been investigated in breast cancer [84, 85], colon cancer [86], leukemia [87, 88], cervical cancer [89], mesothelioma [90], medulloblastoma [91], and neuroblastoma [92].

### nAChR and Breast Cancer

Although  $\alpha 7$ nAChR is the oncogenic receptor responsible for most oncogenic responses in cancer,  $\alpha 9$ nAChR has recently become the focus of breast cancer research since  $\alpha 9$ nAChR is upregulated in estrogen receptor-positive breast cancer cells and stimulates the initiation and progression of breast cancer in coalition with the estrogen receptor [84]. Recent studies have documented that  $\alpha 9$ nAChR is highly associated with breast cancer [93–95]. Specifically, Chen et al. showed that the  $\alpha 9$ nAChR subunit played an important role in cell growth signaling in human breast cancer [95]. Lee et al. confirmed this finding both in vitro and in vivo to show that the down-regulation of  $\alpha 9$ nAChR results in decreased tumor volume for both the MDA-MB-231 human breast cancer cell line and xenografts in SCID mice [93]. Conversely, the stimulation of the  $\alpha 9$ nAChR led to breast cancer growth [96].

### nAChR Signaling in Cancers

The nAChR is the one of the best-characterized ligand-dependent cation channel receptors. nAChRs are expressed in autonomic ganglia and at the neuromuscular junction, and they play important roles in brain activity by being involved in synaptic and cellular function. Specifically, the opening of cation channel receptors results in the influx of sodium and calcium ions and exit of potassium ions, which causes membrane depolarization. [73].

Typically, nicotine- and NNK-mediated  $\alpha 7$ nAChR pathway activation has been observed to activate protein kinase A (PKA), the serine/threonine kinase Raf-1, the mitogen-activated kinases ERK1 and ERK2 and the transcription factors FOS, JUN and MYC in SCLC. [18, 22]. Moreover, PI3K-AKT and NF- $\kappa$ B activation by nicotine and NNK has been reported in NSCLC cell lines originating from large-cell carcinoma, squamous-cell carcinoma, and adenocarcinoma. Both pathways are known to be involved in cell proliferation [97]. In addition, Dasgupta et al. found that  $\alpha 3\beta 4$ nAChR-initiated and XIAP- and survivin-mediated signaling play an important role in nicotine-induced chemoresistance in NSCLC. Moreover, PI3K-AKT and downstream XIAP or NF- $\kappa$ B signaling promote the anti-apoptotic effects of nicotine in A549 human adenocarcinoma cells, resulting in cell increased survival [98]. In

other studies, both Mcl-1 and PPAR  $\beta\delta$  proteins were also reported to be involved in nicotine-stimulated survival [99].

Notably, nAChR signaling affects other major characteristics of cancer development, such as angiogenesis, epithelial to mesenchymal transition (EMT) and metastasis. Furthermore, ERK/MAP kinase, PI3K-AKT and NF- $\kappa$ B signaling have been implicated in nicotine-induced and  $\alpha$ 7nAChR-mediated pro-angiogenic effects in endothelial cells. Specifically, Zhang et al. showed that nicotine induced pro-angiogenic effects by upregulating HIF-1 $\alpha$ , and this effect was significantly attenuated by blocking the nAChR pathway, which consists of the Ca<sup>2+</sup>/calmodulin, Src, protein kinase C (PKC), PI3K-AKT, MAPK-ERK1/2 and mTOR pathways in NSCLC cell lines [100]. Additionally, the  $\alpha$ 7nAChR-mediated signaling pathway has recently been shown to be involved in the nicotine-induced invasion and migration of lung, breast and pancreatic cancer cells [98].

### nAChR Signaling in Breast Cancer

Although many studies have shown that nicotine and its derivatives from continuous tobacco smoking, including SHS, stimulate nAChRs to activate various intracellular signaling cascades that directly link to cancer characteristics (most were studied in lung cancer), little is known about the molecular mechanisms that connect nicotine to breast cancer.

In 2008, a signaling cascade involving PKC and cdc42 was identified in nicotine-induced mammary tumor migration. Guo et al. demonstrated that human breast cancer and normal cell lines constitutively express four types of nAChR subunits, and nicotine enhances the mobility of these cells. Thus, they concluded that nicotine initiates a signaling cascade that involves PKC and cdc42 and consequently promotes the migration of mammary epithelial or tumor cells by interacting with its receptor. However, they were not able to identify a receptor specific to breast cancer [83].

In 2010, Lee et al. identified  $\alpha$ 9nAChR as a nicotine-responsible receptor in breast cancer development [84, 93]. These studies found that  $\alpha$ 9nAChR plays a crucial role in human breast cancer cells that is mediated by nicotine and NNK. Moreover, they demonstrated that nicotine- and NNK-induced cancer cell proliferation was inhibited by knocking down  $\alpha$ 9nAChR using siRNA. By contrast, the over-expression of  $\alpha$ 9nAChR substantially increased tumor growth in a normal human breast cell line. Subsequently, a molecular pathway initiated by nicotine-mediated  $\alpha$ 9nAChR activation was studied in vivo and in vitro [101–103]. MAPK and PI3K-AKT can be activated by both nicotine and estrogen hormone stimulation. Importantly, the activation of  $\alpha$ 9nAChR features a strong positive feedback loop via the PI3K-AKT and MAPK pathways and is followed by the activation of AP1 and VDR transcription factors, which can bind to the promoter region of  $\alpha$ 9nAChR. These signal transduction cascades

eventually result in cancer development [104] (Fig. 2). Additionally, acetylcholine and NNN have been demonstrated to exhibit a higher binding affinity to heteropentameric nAChRs ( $\alpha$ 4 $\beta$ 2,  $\alpha$ 3 $\beta$ 2, or  $\alpha$ 3 $\beta$ 4) than to homopentameric nAChRs ( $\alpha$ 7,  $\alpha$ 9) [22, 105–107].

Indeed, the modulation of  $\alpha$ 9nAChR activity by nicotine and NNK in breast cells was associated with changes in proliferation, differentiation, migration, adhesion, cell contact, apoptosis, and angiogenesis [81].

### Conclusions

We herein showed that nicotine and its derivatives, i.e., NNK, in smoking tobacco products, and SHS are proven risk factors for cancer at all organ sites [108]. Mammary gland cancer is the most common cause of cancer death in females after lung cancer, and breast cancer caused by tobacco is referred to as tobacco-related primary and secondary cancer [109].

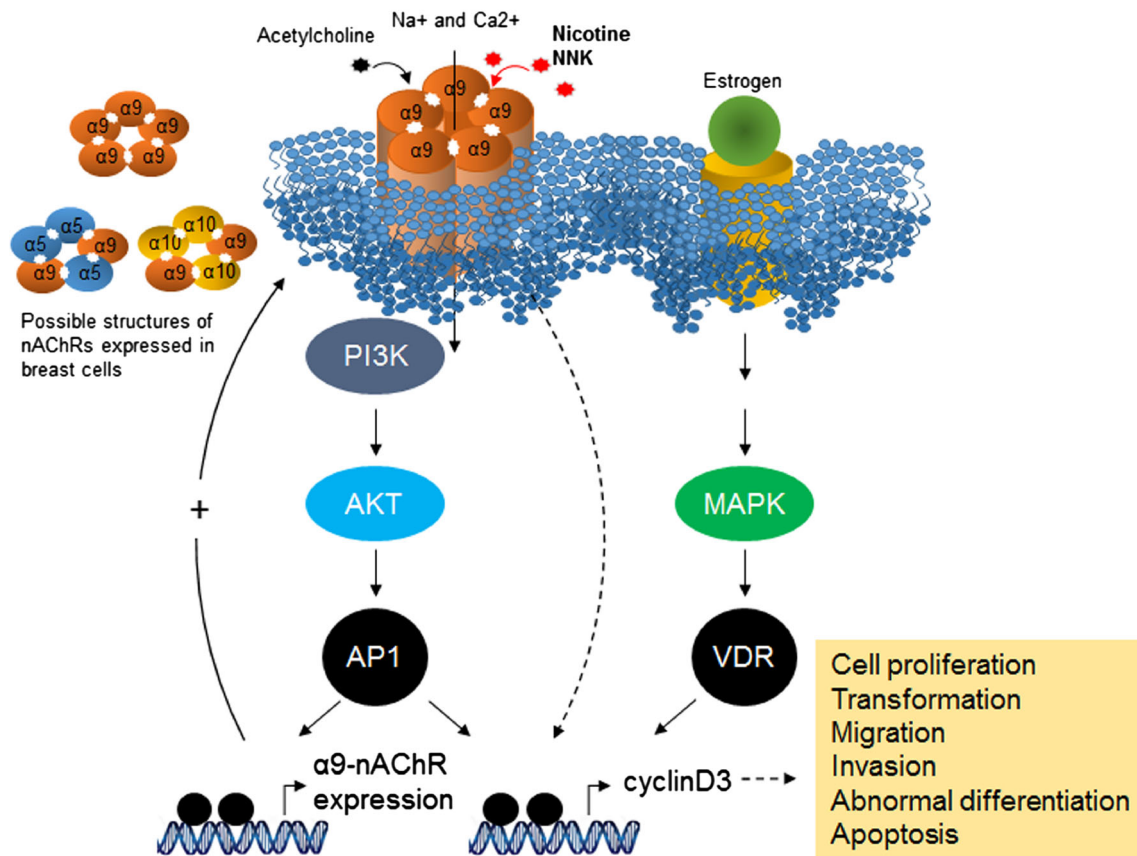
Long-term exposure to SHS among younger/primarily premenopausal women who had never smoked increased breast cancer risk by 60–70% because sidestream smoke contains more toxic chemicals than mainstream smoke [110].

The main component of tobacco smoke is nicotine, and it also contains more than 60 carcinogens. Nicotine is addictive but non-carcinogenic to healthy experimental animals, and the primary causes of cancer appear to be nicotine-derived nitrosamine and the powerful carcinogen NNK, which is an  $\alpha$ 7nAChR agonist with more than 1000-fold higher affinity to the receptor than nicotine. Additionally, it has been well documented that beta-adrenergic receptors and downstream cascades have a role in various cancer cell proliferation in response to NNK [111, 112]. Beta-adrenergic receptors, a family of G-protein coupled receptors, initiate multiple signaling cascades, including the adenylyl cyclase/cAMP/PKA/CREB pathway, which transactivates the epidermal growth factor receptor pathway [113–115].

Numerous studies have examined the role of nicotine and its receptors in the development of various cancers, especially lung cancer, but few articles have attempted to elucidate the molecular signaling cascades stimulated by NNK in breast cancer. Nevertheless,  $\alpha$ 9nAChR was identified as the receptor for nicotine and NNK in breast cells and is involved in nicotine-mediated cell proliferation in conjunction with estrogen hormone stimulation [93–95].

Although, all evidence provided in this review supports that SHS increases the risk of breast cancer development via NNK and its major target, the  $\alpha$ 9nAChR, extensive studies are necessary in the future to better understand the mechanism of tobacco-related breast cancer and assess the effect of low-dose exposures, such as that provided by SHS, on the metastatic process. Moreover, published data regarding the potential toxic effects of non-nicotine

## In Breast cancer



**Fig. 2**  $\alpha 9$ nAChR involved signal pathway in breast cancer. The three abundant  $\alpha$  subunits expressed in breast tissue are able to form one homopentameric nAChR ( $\alpha 9$ nAChR) and two possible heteropentameric structures ( $\alpha 9\alpha 10$ -,  $\alpha 9\alpha 5$ -) of nAChRs. NNK and nicotine with estrogen stimulation can activate downstream transcription factors, AP1 and VDR

chemicals incorporated in tobacco smoke, which can also include hazardous substances, are limited. In addition, the measurement of chronic tobacco smoke exposure has become essential to study the metabolic fate of tobacco products and their potential impact on public health, particularly in non-smokers. For example, Pérez-Ortuño developed a method to analyze nicotine, cotinine, NNN, NNK, and NNAL in human hair samples. Although this study was subject to several limitations, such as hair composition (e.g., the color of hair, cosmetic treatments, etc.), they concluded that the detection of NNK instead of nicotine in hair is a very good biomarker of long-term exposure to SHS. Therefore, monitoring the hair NNK levels among non-smokers irregularly exposed to SHS may serve as a good biomarker of individual carcinogenic risk [116].

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through the PI3K/Akt and MAPK signaling pathways. The activation of AP1 and VDR transcription factors has been known by association with various cancer phenotypes including cell proliferation and migration. Binding of these transcription factors on the promoter region of  $\alpha 9$ -nAChR leads to a strong positive feedback loop

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