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Review

The Molecular Mechanisms of Tobacco-Related Oral Carcinogenesis

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Abstract: Oral squamous cell carcinoma (OSCC) causes a serious loss of facial function or death, and its morbidity is highly related to the usage of tobacco products. Uncovering the mechanisms of tobacco-related OSCC plays a vital role in the prevention and treatment of OSCC. The present review systematically and comprehensively discusses the known mechanisms of tobacco-related OSCC and offer a foundation for the prevention, diagnosis, and treatment of tobacco-mediated OSCC. Scientific literature related to the incidence of tobacco-related OSCC and studies on mechanisms related to tobacco components are included, both in humans and animals. Among the 129 articles cited, three perspectives of the incidence of tobacco-related OSCC were evaluated: DNA adducts, receptor binding, and cocarcinogenic pathways. Tobacco-associated carcinogens cause OSCC by covalently binding to DNA to form DNA adducts or by binding to the receptors, and through the combined action of cocarcinogenic pathways. Three tobacco carcinogens that bind to DNA to form DNA adducts, two receptors that bind to carcinogens, five downstream pathways, and three cocarcinogen-related pathways were listed. This work evaluated the present research status of tobacco-related OSCC to enhance the pathogenesis knowledge of OSCC and offer a foundation for further research endeavours on the prevention, diagnosis, and treatment of tobacco mediated OSCC.

Keywords: Cigarette Smoking; Tobacco; Carcinogen; Signalling Pathways; Oral Squamous Cell Carcinoma

1. Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent type of HNSCC, ranked as the sixth most common cancer in the world [1]. According to Sung et al. [1], lip and oral cavity cancer are highly frequent in South Central Asia (e.g., India, Sri Lanka, and Pakistan) as well as Melanesia (Papua New Guinea). The primary risk factor for OSCC is tobacco smoking, which is three times more likely in smokers than nonsmokers. Other risk factors include betel quid (BQ) chewing, alcohol consumption and poor dietary habits lacking fruits and vegetables. Additionally, it is estimated that only 3% of OSCC prevalence is related to high-risk human papillomavirus (HR-HPV) infection, compared to more than 90% of oropharyngeal squamous cell carcinoma (OPSCC) cases [2–5]. Despite breakthroughs in medical technology and treatment protocols, the 5-year overall OSCC survival rate remains at 65% [6]. Therefore, it is crucial to understand the evidence-based mechanisms underlying the development of tobacco related OSCC to fully implement effective preventive healthcare strategies.

According to accumulating evidence, cigarette smoke (CS) produces over 5000 distinct chemical constituents,

of which over 60 are carcinogenic [7]. In this regard, the International Agency for Research on Cancer (IARC) has reviewed several carcinogens, including nitrosamines, polyaromatic hydrocarbons (PAH), aromatic amines, aldehydes, phenols, volatile hydrocarbons, nitro compounds, and other organic and inorganic substances. Among these, nitrosamines mainly refer to N'-nitrosonornicotine (NNN) and 4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), while PAH mainly contains Benzo[a]pyrene (B[a]p) and DB[a,l]P: Dibenzo [def,p]chrysene (Dibenzo[a,l]pyrene, DB[a,l]P). Animal or human research has provided sufficient evidence for each of the discovered carcinogens. For example, B[a]p, NNK, and NNN are group 1 carcinogens, while DB[a,l]P and acrolein are group 2A carcinogens [8]. It is established that these tobacco derived carcinogens give rise to the development of lung, oral, nose, larynx, oropharynx, hypopharynx, esophagus, stomach, liver, pancreas, bladder, kidney, and cervical cancers, as well as myeloid leukemia [9–17].

Nicotine, the primary component of CS, is also extremely addictive. The ability of nicotine to cause cancer per se has been a subject of debate for several decades [18]. Despite not being recognised as a carcinogen, previous studies have shown it is genotoxic and tumor promoting. However, some studies have demonstrated that nicotine causes cancer in A/J mice and epithelial cells in culture, suggesting that it should be designated as a carcinogen [19–23]. By turning nicotine into nitrosamines (NNK and NNN), nicotine's ability to cause cancer increases.

Tumorigenesis is a complex process involving multiple factors and multiple signalling pathways. Fourteen hallmarks of cancer have been identified, delineating the complex multistep progression of the malignant changes, involving mechanisms mainly related to genomic instability and mutation, resisting cell death, sustaining proliferative signalling and activating invasion and metastasis [24]. The primary focus of this review is to summarize the literature on how tobacco-related components, such as nicotine, nitrosamines (NNN and NNK), PAH, and acrolein, play an important role in oral carcinogenesis.

2. DNA Adducts in CS Induced Oral Carcinogenesis

According to Hecht [25], DNA adducts play a crucial role in the carcinogenic process brought on by the burning of tobacco in CS (Table 1) [26–31]. Nitrosamines (NNN and NNK) and PAH (B[a]P and DB[a,l]P) require metabolic activation to exert their carcinogenicity. Therefore, in humans, using oral mucosa cells to assess DNA adducts as biomarkers of tobacco smoke exposure and molecular changes that may be linked to cancer is a great way to collect data due to its high metabolic activity [32]. As the exposed organism strives to convert them to more easily eliminated forms, most carcinogens in CS are enzymatically converted into a series of metabolites, mainly by cytochrome P450 enzymes [33]. The final product becomes electrophilic and reacts with DNA, generating DNA adducts, which play a central role in CS-related cancer [34].

Table 1. DNA adducts related to OSCC formed by carcinogens in tobacco products.

Carcinogens	DNA Adducts
NNK, NNN	4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) DNA adducts [26]
	pyridyloxobutyl (POB) DNA adducts [16]
PAH	
B[a]P	BPDE-N2-dG adducts [27]
DB[a,l]P	DB[a,l]PDE-N6-dA adducts [28, 29]
	DB[a,l]PDE-N2-dG adducts [30]
Acrolein	Acr-dG DNA adducts [31]

2.1. DNA Adducts Formed by NNN and NNK

Several DNA adducts, i.e., hydroxy-1-(3-pyridyl)-1-butanone (HPB) DNA adducts, methyl DNA adducts, pyridyloxobutyl (POB) DNA adducts, and the minor adducts formed from the NNN metabolite formaldehyde have all been thoroughly described. According to a recent study by Li and Hecht [35], POB DNA base and phosphate adducts are formed at the 2' locations of NNN, while DNA base adducts and DNA phosphate adducts are formed at the 5' locations of NNN. Additionally, the oral cavity is more susceptible to the carcinogenic effect of NNN, as it is demonstrated to be more effective with lower doses inducing oral cancer [36]. The prospective Shanghai Cohort Study shows a strong correlation between cancer risk in smokers and NNN and NNK rat target tissues [37]. The most substantial

evidence for the relevance of F344 rat target tissues of tobacco-specific nitrosamines to those in humans was found in smokers who had the highest levels of urinary NNN, followed by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a metabolite of NNK [37, 38].

The pyridyloxobutylation of DNA by NNK, NNN, and perhaps other tobacco-specific chemicals is measured by HPB releasing DNA adducts [39]. When HPB DNA is treated with acid, many pyridyloxobutyl adducts are hydrolysed, releasing HPB (Figure 1). It has been shown that smokers with HNSCC had a median level of HPB releasing DNA adducts that were 6.6 times higher than smokers without HNSCC [40].

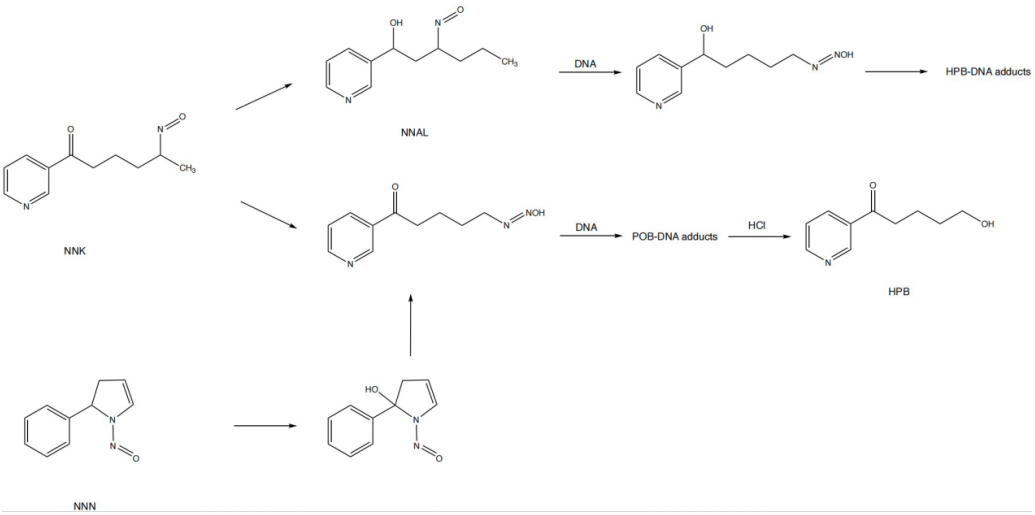

Figure 1. The pyridyloxobutylation of DNA by nitrosamines (NNK and NNN) forming 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) and pyridyloxobutyl (POB) DNA adducts followed by acid hydrolysis releasing HPB [26].

2.2. DNA Adducts Formed by PAH

B[a]P, a prototype environmental PAH carcinogen, caused tumours at distal locales and tongue papillomas and carcinomas in mice when it was provided to them as part of their food for two years [41]. B[a]P can be metabolically activated by cytochrome P4501A1/1B1 and epoxide hydrolase via intermediate B[a]P dihydrodiol (B[a]pDHD), ending up with the ultimate carcinogen anti-BPDE (7,8-dihydroxyanti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene) (Figure 2), which can react with deoxyguanosine (dG) to form BPDE-N2-dG adducts [42].

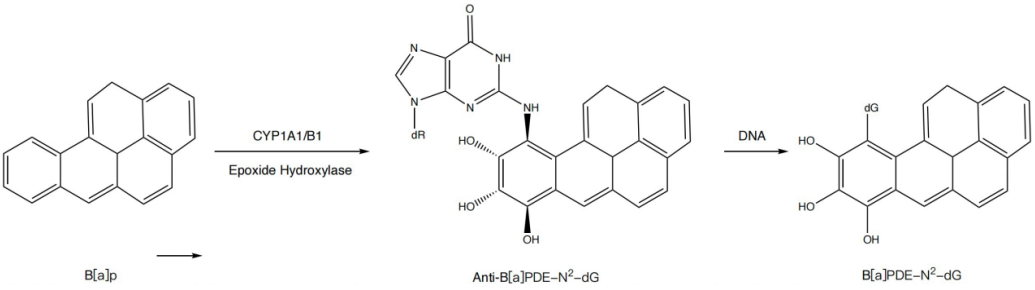

Figure 2. Activation of B[a]P by cytochrome P4501A1/1B1 (CYP1A1/B1) and epoxide hydrolase and subsequent reaction with deoxyguanosine (dG), forming anti-BPDE-N2-dG DNA adducts [16, 27].

In non-small cell lung cancer (NSCLC), cigarette smoking is significantly linked to an increase in BPDE DNA adduct levels, promoter hypermethylation of p16, and death-associated protein kinase (*DAPK*) gene expression [43]. To determine whether there were any variations in the susceptibility to exposure to CS in the creation of DNA adducts between cancer patients and controls, Chuang et al. [44] compared 158 oral cancer patients and 64 non-cancer individuals. The findings demonstrated a strong correlation between BPDE DNA adduct levels and smoking

status. Patients with high DNA adduct levels had a roughly 9.936-fold increased risk of oral cancer compared to those with low DNA adduct levels ($p < 0.001$).

Another PAH derivative, DB[a,l]P, has been shown to cause 31% of B6C3F1 mice to develop OSCC [45]. Through the generation of its dihydrodiol and subsequent conversion to the ultimate carcinogen DBPDE, DB[a,l]P can be metabolically activated [42, 46]. DBPDE can react with DNA to create deoxyadenosine (DB[a,l]PDE-N6-dA) and deoxyguanosine (DB[a,l]PDE-N2-dG) adducts (Figure 3).

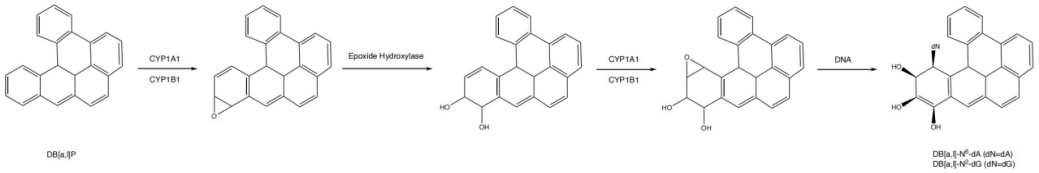

Figure 3. Conversion of polyaromatic hydrocarbon derivatives DB[a,l]P into deoxyadenosine (DB[a,l]PDE-N6-dA) and deoxyguanosine (DB[a,l]PDE-N2-dG) DNA adducts [30].

In animals, both of these adducts have been found in the oral cavity of mice treated with DB[a,l]P [28, 30], and DBPDE-N6-dA adducts contribute to the initiation of DB[a,l]P-induced oral carcinogenesis [28]. While in humans, BPDE-N2-dG and DBPDE-N6-dA are significantly higher in the buccal cells of cigarette smokers than non-smokers [29]. Based on these findings, it is evident that PAHs in tobacco smoke can contribute to the development of oral cancer in humans.

2.3. DNA Adducts Formed by Acrolein

The amount of AcrdG DNA adducts discovered in smokers' oral tissue has been reported to be a few micromoles per mole of guanine, which is much higher than the amount of PAH DNA adducts found in smokers' oral or lung tissue [32, 47, 48]. Acrolein can be absorbed by human cells with a fair amount of efficiency. Upon direct interaction with DNA's guanine residues, without the need for metabolic activation, it produces the mutagenic exocyclic DNA adducts 6-hydroxy-1, N2-propanodeoxyguanosine and 8-hydroxy-1, N2-propanodeoxyguanosine adducts (AcrdG) [49] (Figure 4).

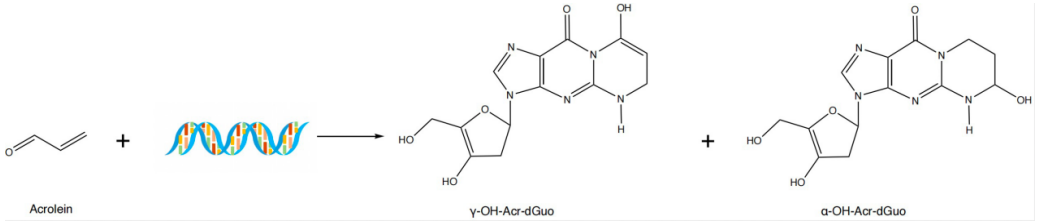

Figure 4. Formation of mutagenic AcrdG DNA adducts from interaction of acrolein with DNA [31].

These primarily cause G:C to T:A transversion mutations that resemble PAHs [50]. The preferential sites of Acr–dG binding are guanines within cytosine-guanine (CpG) sites. DNA damage and repair inhibition are two adverse effects of Acr that lead to lung tumorigenesis [51]. In addition, a report demonstrated for the first time that e-cigarette users had elevated levels of acrolein DNA adducts in their oral cells [52]. This recent finding is substantial evidence since e-cigarette usage has gained momentum in several countries, and some advocate this to replace the conventional smoking habit.

3. Genes Mutation in CS Induced Oral Carcinogenesis

OSCC is a heterogeneous tumour with many events. The tumour suppressor gene, *p53* mutations were commonest in tobacco related OSCC, in combination with other mutations such as *CDKN2A*, *HRAS*, *KIT*, *PIK3CA*, *STK11*, *SMARCB1, ABL1*, and *RB1* [53], and are also strongly associated with oral potentially malignant disorders [54].

Tobacco related mutations in *p53* and *CDKN2A* are a hallmark of HPV negative HNSCC [55]. Nearly half of OSCC patients had *p53* mutations at exons 5–9, and G to A or G to T mutations are the most common variants that have been seen and linked to CS [56–59]. In addition, it has been shown that nicotine treatment decreased retinoblastoma (pRb) and *p53* expression in oral cancer cells [60], whereas NNK may increase the likelihood of lung cancer progression and worse outcomes in patients with *p53* mutations by interfering with normal mitotic progression and chromosome integrity [61].

It is found that methylated CpG dinucleotides are the preferred substrates for BPDE DNA adduct induced G to T transversion mutations of *p53* in mammalian cells, which shares parallels with the spectrum of *p53* mutations found in smoking associated lung malignancies [62]. Other related findings include high percentages (31%) of GC>TA and GC>AT substitutions produced by DB[a,l]P pertinent to oncogenic alterations in the *p53* gene in HNSCC [45].

A similar *p53* mutational spectrum was found among AcrDNA binding spectrum in Acr treated normal human bronchial epithelial (NHBE) cells, CS related lung cancer, and CS related OSCC [31, 51]. In normal human keratinocytes (NOK), acrolein boosted cell proliferation, anchorage independent activity, and cell migratory activity. They discovered elevated levels of phosphorylated epidermal growth factor receptor (pEGFR), downstream protein kinase B (PKB or also known as AKT) and extracellular signal regulated kinase (ERK) pathways, as well as elevated levels of cyclin D1 and cellular MYC (c-myc) in NOK cells that had undergone acrolein transformation. Transformed NOK cells formed tumours in xenografts nude mice [63].

Additionally, two pyridyloxobutyl DNA adducts, i.e. O(6)-pyridyloxobutyl-dG (O6-POB-dGuo) and O(6)-pyridylhydroxybutyl-dG (O6-PHB-dGuo) have been shown to inhibit DNA replication in E. coli cells to a relatively small or moderate amount. O6-POB-dGuo also caused G to T transversions, but preferentially produced G to A transitions [64].

Generally, a single carcinogen investigation on the mechanism of carcinogenesis in different cancers is useful in determining the specific carcinogenic mechanism. Still, it may be biased in light of the aforementioned findings. It is worth noting that the combination of DB[a, l]P and NNN resulted in a higher proportion of mutations in the lactose repressor protein (LacI) mice than would be predicted by the simple addition of each investigator's individual mutation fractions. In addition, the combined mice's mutational profile more closely resembled that of the *p53* gene in human head and neck cancers than did either of the individual agents [65]. The receptor binding and signalling pathways involved in CS induced oral carcinogenesis were discussed in the following sections.

4. Receptor Binding in CS Induced Oral Carcinogenesis

4.1. Nicotinic Acetylcholine Receptors (nAChRs) and Beta-Adrenergic Receptors (β-AR)

Nicotine regulates cellular processes through nicotinic acetylcholine receptors (nAChRs). In addition to nicotine, its tissue metabolite NNN and NNK can also have carcinogenic effects owing to binding to nAChRs on nonneuronal cells [66, 67]. The nAChR can activate several signalling pathways that can have tumorigenic effects, i.e. phospholipase C (PLC), protein kinase C (PKC) isoforms, phosphatidylinositol 3 kinase (PI3K), protein kinase B (AKT), JUN N terminal kinase (JNK), tyrosine kinase activation (SRC), janus kinase 2 (JAK2), ras homology (RHO), signalling GTPase protein (RAC), p38 mitogen activated protein kinases (MAPK), Wnt, Hippo/associated protein (YAP), epidermal growth factor receptor (EGFR), and nuclear factor kappa B (NF-κB) can all be activated by the downstream signalling from nAChRs (Figure 5) [68–76].

NNK can also bind to the beta-adrenergic receptor (β-AR) to exert its carcinogenicity, apart from its classic downstream effectors signalling protein which are protein kinase (PKA), cyclic AMP response element binding protein (CREB), beta-adrenergic receptor1 (ATF1), and EGFR [77] (Figure 5).

4.2. Epidermal Growth Factor Receptor (EGFR)

The expression of EGFR has been associated with a number of downstream pathways, which have been linked to cell proliferation, differentiation, division, survival, and cancer development.

In human studies, Sabbah, Hajjo and Sweidan [78] and Nishioka et al. [79] found that treatment with nicotine enhanced EGFR phosphorylation, cell motility, and proliferation of OSCC cell line human squamous carcinoma 2 (HSC2). Additionally, nicotine therapy stimulated the EGFR's downstream effectors PI3K/AKT and p44/42 MAPK (ERK) (Figure 5), which in turn aided in cell growth. Furthermore, nicotine stimulates cell growth in breast cancer

cells by triggering the EGFR pathway, which is linked to mitogenesis [75]. Another research demonstrated that in HNSCC cells, nicotine promoted pEGFR nuclear translocation, Akt phosphorylation, migration, invasion, and proliferation (Figure 5). And also, nicotine reversed the effects of cetuximab, which prevented HNSCC cell invasion, migration, and proliferation. It is also demonstrated that NNK transactivates the EGFR pathway, as well as phosphorylates ERK1/2 (downstream of EGFR) via β1-AR signalling by targeting specific tyrosine residues in human lung NSCLC NCIH322 cells and normal human airway epithelial (HPLD1) cells [77] (Figure 5). In addition, Wisniewski, Ma and Schneider [80] recently discovered that through a de novo lipogenic biosynthetic pathway, nicotine markedly increases oral dysplastic keratinocyte cell migration by activating EGFR signalling through a fatty acid synthase FASN dependent mechanism.

Whereas an in vivo investigation showed that nicotine increased the lymph node metastasis of xenografted tumours via its binding to the nAChR receptor. In contrast, a nAChR inhibitor decreased both the lymph node metastasis and nuclear localisation of pEGFR in xenografted tumours [81].

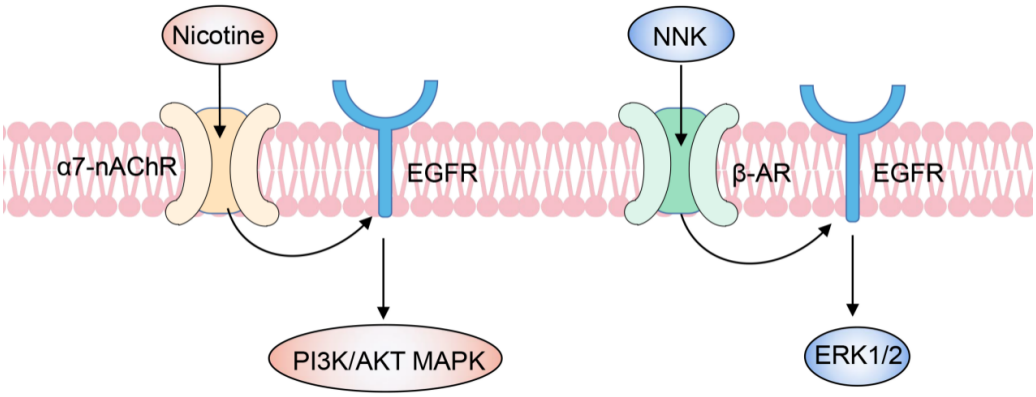

Figure 5. Nicotinic acetylcholine receptors (nAChRs) and beta-adrenergic receptors (β-AR) with EGFR.
Note: Nicotine regulates cellular processes through α7-nAChR. Nicotine enhanced EGFR phosphorylation and then stimulated the EGFR's downstream effectors PI3K/AKT and MAPK. NNK trans-activates the EGFR pathway, as well as phosphorylates ERK1/2 (downstream of EGFR) via β1-AR signalling by targeting specific tyrosine residues [77–79].

5. Signalling Pathways in CS Induced Oral Carcinogenesis

5.1. Signal Transducer and Activator of Transcription (STAT) Pathway

The STAT protein family comprises transcription factors that play a complex and crucial role in controlling physiological cell processes like proliferation, differentiation, apoptosis, and angiogenesis. It also helps to organise the epigenetic environment of immune cells [82]. Almost all immune regulatory systems, including those involved in tumour cell identification and tumour driven immune escape, are mediated by the Janus kinase signal transducer and activator of transcription (JAK/STAT) signalling [83]. Nicotine acts through the collaboration between the JAK2/STAT3 and rat sarcoma (Ras)/RAF kinase and (Raf)/mitogen-activated extracellular signal-regulated kinase (MEK)/ERK pathway (also known as Ras/Raf/MEK/ERK or MAPK/ERK) via alpha7-nAChR (α7-nAChR) binding (Figure 6). When the α7-nAChR has two complementary signalling pathways coupled to it, activation occurs, either by its physiological ligand acetylcholine (Ach) or by nicotine (Nic), causing changes in gene expression. As a result of the upregulated expression, the pathway mediated by Ras/Raf/MEK/ERK provides for an increased cytoplasmic concentration of STAT-3, whereas activation of the tyrosine kinase JAK2 results in STAT-3's phosphorylation and subsequent translocation of STAT3 dimers to the nucleus to modify gene expression (Figure 6) [68].

In human studies, a report indicated that NNK led to the activation of several signal transduction effectors, i.e., GATA binding protein 3 (GATA-3), NF-κB, and STAT-1, whereas NNN predominantly activated GATA-3 and STAT-1 by stimulating nAChRs in human bronchial epithelial BEP2D cells (a suitable model for studying the various stages of human bronchial carcinogenesis) [84]. They also reported that NF-κB and STAT-1 were raised in the immortalised oral epithelial (Het1A) cells when treated with nitrosamines. The increased gene expression that NNK and NNN caused was associated with the STAT-1 protein binding activity, which boosted Het1A cells' proliferative potential

and had an antiapoptotic impact [85]. Furthermore, NNK may contribute to STAT-3 activation in OSCC cells [86]. Both STAT-3 and STAT-5 are upregulated in OSCC. But STAT-5 positive cases were observed more in T3 and T4 higher stage (13/15) than in the initial stages (11/15) of oral cancer samples, in contrast to the level of STAT-3 which was maximum in T1 and T2 (74.4%), and its level decreased in T3 and T4 (47.1%) [87]. These findings indicate that activation of the tobacco related STAT-3 pathway is an early event in CS induced oral carcinogenesis [88]. Therefore, it is possible that the STAT-5/cyclin D1 pathway might also be one of the crucial events for oral cancer development [87]. However, whether the STAT-5/cyclin D1 pathway is related to receptor binding has not yet been clarified.

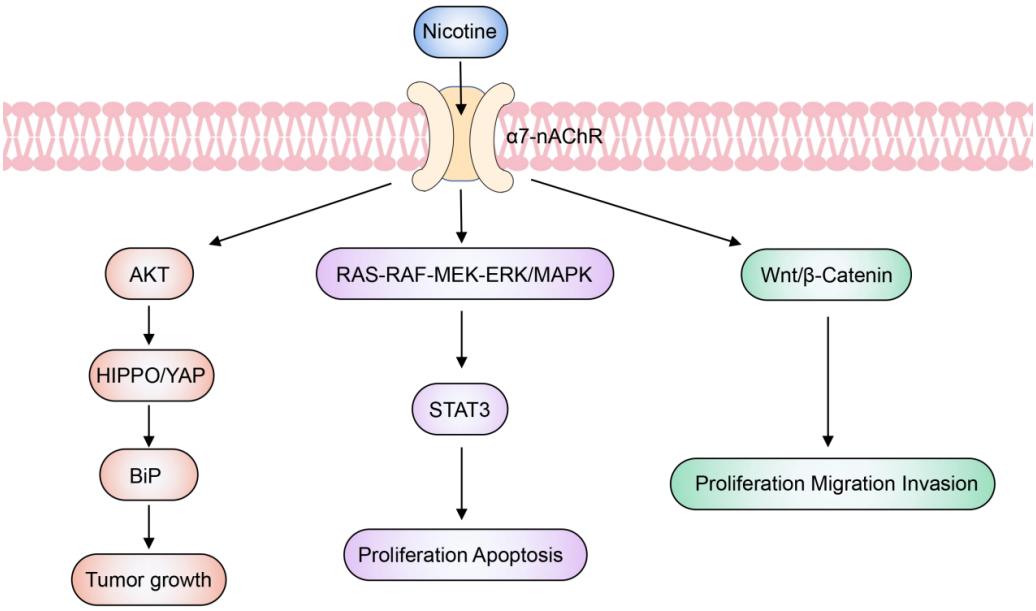

Figure 6. Role of nicotine and α7-nAChR in oral carcinogenesis.

Note: Left: nicotine, coupled with α7-nAChR-Akt signalling, the HIPPO/YAP pathway becomes activated, leading to nicotine-induced BiP expression then promoting tumor growth. Middle: nicotine acts through the RAS/RAF/MEK/ERK pathway (MAPK) via α7-nAChR binding, resulting in an increased cytoplasmic concentration of STAT-3, which regulates cell proliferation and apoptosis. Right: nicotine activates the Wnt/β-catenin pathway through α7-nAChR, then regulates proliferation, migration, and invasion [68, 73, 74].

5.2. Hippo (YAP/TEAD) Pathway

A potential driver of OSCC development has been identified as the Hippo/YES associated protein (YAP) transcriptional cofactor. To trigger the transactivation of downstream target genes, YAP is primarily linked to the TEA domain (TEAD) transcription factor. Shuttling between the nucleus and the cytoplasm of YAP, which is phosphorylation-dependent, is the primary mechanism regulating YAP/TEAD transcriptional activity. When upstream kinases phosphorylate YAP, it localises in the cytoplasm, therefore, is unable to interact with TEAD, and the underlying mechanisms of nicotine-induced binding immunoglobulin protein (Bip) production are triggered. Subsequently, coupled with α7-nAChR-Akt signalling, the YAP/TEAD transcriptional complex becomes activated, leading to nicotine-induced BiP expression [74] (Figure 6). BiP (also known as GRP78) is a family member of HSP70 and was expressed noticeably more in tissues of OSCC patients than in healthy oral tissues [89]. Its expression has been linked to tumour growth, invasion, metastasis, and resistance to cancer treatments [90–92].

5.3. Wnt and MAPK Pathways

In human studies, an in vitro study showed that nicotine stimulation could increase tongue squamous cell carcinoma (TSCC) cell proliferation, migration, and invasion, while downregulating E-cadherin and activating the Wnt/beta catenin (Wnt/β-catenin) and Wnt/planar cell polarity (Wnt/PCP) pathways, in which the α7-nAChR inhibitor BTX may be able to block [73]. On the other hand, another study reported that reactive oxygen species (ROS) caused by cigarette smoking activate the Wnt/β-catenin and MAPK signalling axis with oral cancer progression [93]. It is also demonstrated that nicotine and NNK significantly enhanced cell proliferation through MAPK/COX2 in gas-

tric cancer (AGS) cells, which expressed both α7-nAChR and β-AR (Figure 7) [94]. NNK stimulates DNA synthesis in NCI H322 cells via β-AR signalling (β1-AR predominantly) [77] (Figure 7). Moreover, pharmacological inhibition of the MAPK pathway by MK886 (an inhibitor of the 5-lipoxygenase activating protein (FLAP)) significantly reduced the proliferative response of these cells to NNK [95]. While in animals, there is also evidence which confirmed that (S)-N'nitrosonornicotine [(S)-NNN] exposure significantly alters Wnt 6 in Male F344 rats [96].

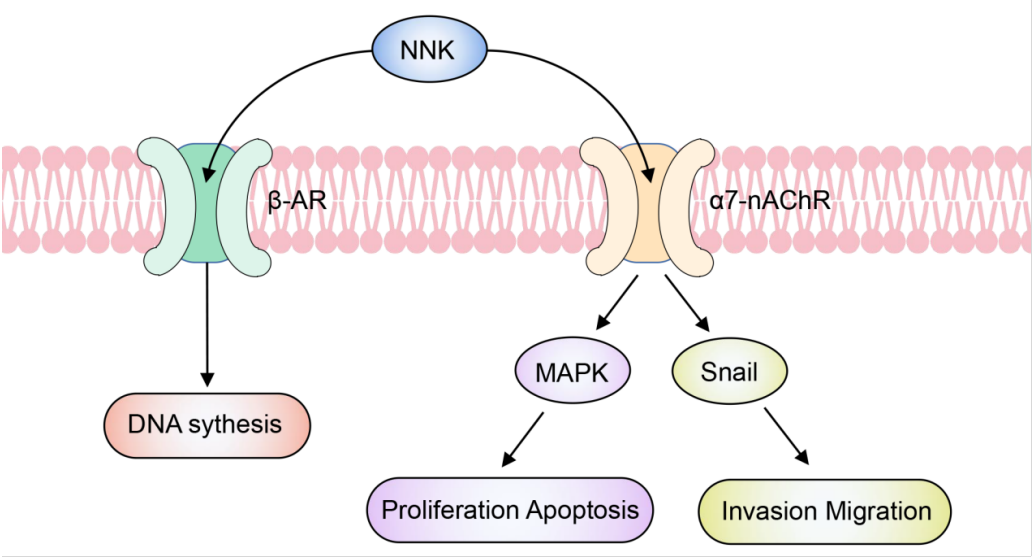

Figure 7. Role of NNK in oral carcinogenesis.
Note: Left: NNK stimulates DNA synthesis in NCI-H322 cells via β-AR signalling. Right: NNK regulated cell proliferation and apoptosis through α7-nAChR-MAPK signalling, whlie regulating cell invasion and migration through α7-nAChR-Snail signalling [77, 94, 97].

In oral cancer, the MAPK pathway is known to contribute to tumour angiogenesis, cell proliferation, apoptosis inhibition, invasion, and metastasis [98]. Rajagopalan et al. [99] have shown that immortalised human oral keratinocytes (OKF6/TERT1) cells exposed to cigarette smoke exhibit pronounced overexpression and activation of MAPK1. They also found that MAPK1 was activated in shisha treated OKF6/TERT1 cells [100]. As previously mentioned, nicotine can activate the MAPK pathway through α7-nAChR, but no prior work has looked into how βAR affects MAPK activation during oral carcinogenesis.

5.4. PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway controls cell growth, metabolism, and survival in healthy physiology. But changes in this system result in malignant changes, and PI3K was overexpressed in tumour samples of tobacco related OSCC [101, 102]. It is shown that the binding of NNK to the α7-nAChR induced DNA damage by activating the PI3K/AKT pathway in the human lung adenocarcinoma A549 cell line [103]. Nevertheless, no prior studies about such mechanisms have been carried out in OSCC cells.

Roy et al. [104] observed that the 24 h treatment of human tongue SCC (SAS) and laryngeal SCC (KB) cells with tobacco extract (TE), B[a]P, and nicotine increased the mRNA levels of Akt1 and two isoforms, and the treatment with TE for 24 h induces proliferation of SAS cells. Moreover, the knockdown of both Akt1 and two isoforms led to the reduction of Cox-2 protein expression. Nishioka et al. [105] demonstrated that nicotine and NNK stimulate the mitogenic signal transduction system by activating proteins like PKC, AKT, and p44/42 MAPK (ERK), which inhibit apoptosis and promote cell proliferation in normal lung epithelial cells and cancer cells derived from lung cancer.

In addition, nicotine and benzo(α)pyrene (BaP) were shown to regulate oral tumorigenesis through AKT/mTOR/STAT3 signalling cascade by modifying the tumour necrosis factor α-induced protein 8-like (TIPE) family proteins expression, which regulates cell growth, survival, proliferation, invasion, and migration through modulation of various cell signalling molecules such as COX2, survivin, Bcl2, cIAP1, XIAP, LC3B, CXCR4, MMP9, and VEGFA (Figure 8) [106]. However, the mechanism of BaP causing the above changes is still unclear, and more studies are needed to elucidate its exact mechanism.

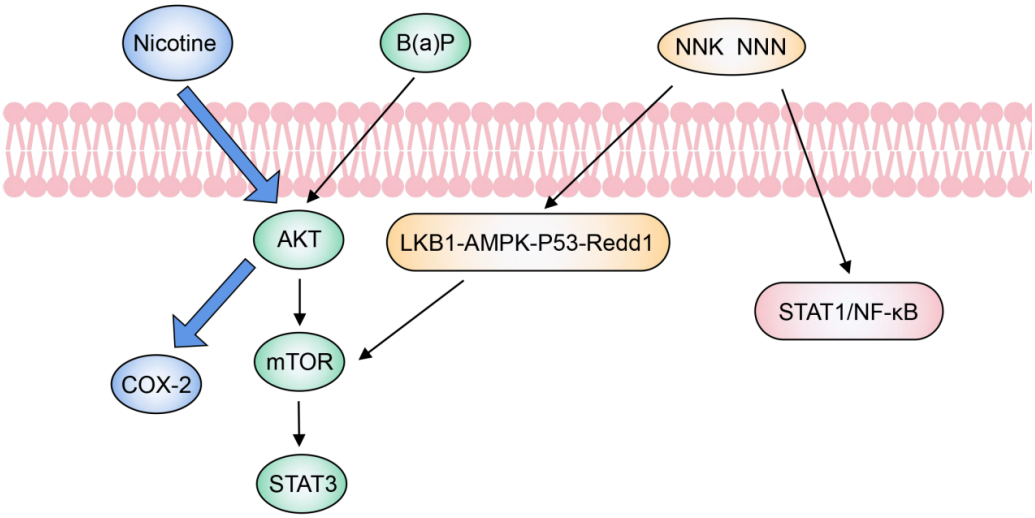

Figure 8. Pathways in CS related oral carcinogenesis without evidence about receptor binding.
Note: Nicotine and B[a]P regulated oral tumorigenesis through AKT/mTOR/STAT3 signalling. NF-κB and STAT-1 were raised in the Het-1A cells when treated with nitrosamines (NNK, NNN). NNK and NNN increased proliferation, survival, invasion, and migration of oral cancer cells via the LKB1-AMPK-p53-Redd1-mTOR axis [85, 104, 106, 107].

5.5. Epithelial Mesenchymal Transition (EMT)

Epithelial mesenchymal transition (EMT), invasion, and migration are all well known to be regulated by neutrophil gelatinase associated lipocalin (NGAL) protein. NGAL is downregulated in oral cancer tissues and cells.

In human studies, oral cancer cells treated with NNK and NNN downregulated NGAL in a dose dependent manner. Silencing of NGAL increased proliferation, survival, invasion, and migration of oral cancer cells via the LKB1/AMPK/p53/Redd1/mTOR axis [107]. It is demonstrated that OSCC exhibits aberrant mTOR activity, which is linked to a poor prognosis [108].

Snail is an EMT related transcription factor and the primary apoptotic regulator. Through the Snail-raf kinase inhibitor protein (Snail/RKIP) signalling pathway, Nieh et al. [97] demonstrated that long term NNK exposure contributes to HNSCC by raising anti-apoptosis and treatment resistance. Their findings also point to the possibility of stopping the evolution of HNSCC by inhibiting or targeting the α7-nAChR or Snail. In vitro, Snail RNA interference (RNAi) reversed long term nicotine induced oncogenic characteristics of OSCC oral epithelial (OE) cells; subsequent in vivo study showed that receivers of xenografts of long-term nicotine exposed OE cells that underwent administration of small interference RNA targeting (SiSnail) construct displayed decreased tumour growth [109].

6. Co-Carcinogenic Mechanisms and Their Signalling Pathway

6.1. CS Induced Inflammatory Response via NF-κB and IL-1β Activation

Nuclear transcription factor NF-κB, a hallmark of inflammatory responses, is a common protein whose activation has been linked to chemical carcinogenesis and plays a significant role in inflammation [110, 111]. Since NF-κB can be activated by physical, chemical, oxidative, and carcinogenic stressors, it may serve as the central dogma of stress responses [111]. Recent studies have demonstrated that the NNK activates NF-κB in normal human bronchial epithelial cells [112], lung cancer cells [113], and colon cancer cells [114]. Sawhney et al. [115] investigated the effect of NNK on oral cell systems in vitro. They demonstrated that NNK treatment of oral precancerous lesions (OPL) and oral cancer cells resulted in the activation of NF-κB and higher levels of COX-2 (NF-κB downstream's target), which is a protein related to inflammation. According to their findings, NNK is one of the carcinogenic elements of smokeless tobacco extracts (STE) that activates NF-κB and COX-2. The inflammatory response of epithelial cells to carcinogens and the start of the carcinogenic cascade appears to be activated by NNK. NNK and nicotine increase COX-2 expression in oral cells, which may aid cancer development [116, 117].

One of the essential proinflammatory cytokines implicated in the development of tumours is interleukin (IL)-

1β. Based on the study by Lee et al. [118], IL-1β promotes proliferation both in human dysplasia oral mucosa (DOK) and OSCC TW2.6 cell line. NNK treatment significantly increased IL-1β production in the OSCC TW2.6 cell line. It is shown that IL-1β activated ERK/MEK and AKT pathways and stimulated a protumorigenic cytokine network through significantly increased levels of granulocyte macrophage colony stimulating factor (GM-CSF), growth related oncogene alpha (GROα), IL-6, and IL-8, and marginally increased levels of RANTES, a β chemokine, potent chemotactic and potent leukocyte activator, along with increased in IL-1α, and monocyte chemoattractant protein 1 (MCP1) secretion. Additionally, IL-1β induces the EMT by activating Snail in the TW2.6 cells, and its expression level is associated with lymph node metastasis of OSCC [118].

6.2. CS Induced Oxidative Stress Damage

Guanine is the nucleobase that is most vulnerable to oxidative damage. 8-hydroxy-2-deoxyguanosine (8-oxo-dG) and 8-oxo-7,8-dihydro-2'deoxyguanosine (8-OHdG) are the two primary alterations that emerge from its damage [119]. It is possible to measure the 8-OHdG at low levels of DNA damage, so it is the most reliable biomarker for oxidative DNA damage [120]. Compared to similarly treated cells cultured in an acidic environment (pH 6.5), nicotine causes more DNA strand breaks in oral epidermal carcinoma cells grown in a basic (pH 8) environment. Additionally, 8-OH-dG/8-oxo-guanine and reactive oxygen radicals may contribute to the DNA strand breaks brought on by CS [121]. Salivary and urinary 8-OH-dG levels have been proven as a marker of oxidative stress induced DNA damage in oral cancer patients with a significant value for diagnosis [122, 123]. Furthermore, salivary 8-OH-dG level is significantly upregulated in tobacco related oral submucous fibrosis (OSMF) and even higher in OSCC [124].

6.3. CS Induced Gene Promoter Methylation

OSCC patients present a higher prevalence of methylation of *p16*, death associated protein kinase (*DAPK*), and methylation of O6-methylguanine DNA methyltransferase (*MGMT*) [125]. Compared to normal oral mucosa tissue without smoking habits, there is a higher frequency of promoter region hypermethylation observed in *p16*, *DAPK* and *MGMT* genes in oral cancer tissues and in corresponding adjacent normal mucosa [125, 126].

Hypermethylation, which leads to the inactivation of some tumour suppressor genes, such as *p16,* has been pointed out as an initial event in HNSCC. Hypermethylation of the *p16* gene can be detected in the smoker's oral cavity, meaning the inactivation of *p16* is an early event that might confer cell growth advantages contributing to the tumorigenic process. In rat liver, tumours induced by NNK also observed a high incidence of *p16* methylation [127].

Biologically, a DNA repair enzyme, DNA methyltransferase *(MGMT)*, can protect DNA from the formation of O6-alkylguanine adducts. However, with CS, tobacco specific nitrosamines (TSNAs) and O6-methylguanine built up over time and resulted in abnormal MGMT expression in cellular DNA. Its reduced expression may activate oncogenes or inactivate tumour suppressor genes, promoting the development of cancer or other diseases [128]. There is a significant association between reduced expression of MGMT and smokeless tobacco use in OSCC patients. Downregulation of MGMT expression is an early event in oral tumorigenesis, and loss of MGMT expression is found to be sustained during the development and progression of OSCC. Furthermore, it is also associated with reduced disease-free survival [129].

7. Conclusions

In conclusion, this review summarised the carcinogenic mechanisms of the main tobacco constituents, i.e., nicotine, nitrosamines, PAH, and acrolein in oral carcinogenesis. Some previous studies focused on summarising the mechanism of detailed carcinogens in specific pathways. In contrast, this review endeavours to provide a comprehensive update on the underlying mechanism of various components in tobacco induced oral carcinogenesis. The mechanism of the tobacco related oral carcinogenesis were clarified through three perspectives: DNA adduct, receptor binding, and cocarcinogenic pathway. However, the mechanism of mixed with carcinogens on OSCC is currently less understood. As the majority of the chemical constituents of tobacco smoke are combinations and most tobacco related OSCC were caused by the tobacco instead of specific ingredients of it. Therefore, the focus of the study may change in the future to examine the synergistic effect mechanisms by which existing combinations of carcinogens can cause cancer.

Author Contributions

Conceptualisation, Methodology, Validation, Writing – Review & Editing—Final Draft, N.A.R.; Data collection and analysis, Writing—Original Draft, J.L.; Validation, Writing—Review & Editing, S.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work in this paper.

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