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Chemoprevention of Head and Neck Squamous Cell Carcinoma through Inhibition of NF- κ B Signaling

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Abstract

Nuclear factor-kappa B (NF- κ B) transcription factors regulate cellular processes such as inflammation and cell survival. The NF- κ B pathway is often activated with development and progression of head and neck squamous cell carcinoma (HNSCC). As such, NF- κ B represents an attractive target for chemoprevention. HNSCC involves progression of lesions from premalignant to malignant, providing a window of opportunity for intervention with chemopreventive agents. Appropriate chemopreventive agents should be inexpensive, nontoxic, and target important pathways involved in the development of HNSCC. Several such agents that inhibit the NF- κ B pathway have been investigated in HNSCC. Retinoids have been studied most extensively but have shown limited potential in human trials. Epidermal growth factor receptor inhibitors and PI3K-mTOR inhibitors may benefit a subset of patients. Other agents such as green tea extract and curcumin are appealing because they are generally regarded as safe. In contrast, there is evidence that Vitamin E supplementation may actually increase mortality of cancer patients. Repurposed drugs such as cyclooxygenase (COX) inhibitors and antidiabetic drugs are an emerging area of interest. Future research to develop agents with lower toxicity and higher specificity for the NF- κ B pathway, and to target these therapies to individual patient genetic signatures should help to increase the utility of chemoprevention in HSNCC.

Keywords

nuclear factor-kappa B (NF- κ B); head and neck cancer; chemoprevention; epidermal growth factor receptor (EGFR); phosphatidylinositol 3-kinase (PI3K); retinoids; mammalian target of rapamycin (mTOR); curcumin; cyclooxygenase (COX) inhibitors; green tea extract (GTE)

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Conflict of interest

None declared.

Introduction

Chemoprevention of cancer was first described by Sporn, et al. in 1976 using Vitamin A and its analogues (retinoids) to control the differentiation of preneoplastic epithelial tissues¹. Squamous cell epithelial tissues, such as those of the head and neck, present an ideal model for the study of chemoprevention because they follow a histopathological progression from normal tissue to hyperplasia to severe dysplasia to carcinoma in situ to invasive carcinoma². This progression can, in many instances, be easily biopsied at any stage and followed clinically by the naked eye. For this reason, oral premalignant lesions (OPL) serve as prime targets for chemopreventive agents³. Moreover, the phenomenon of field cancerization is well understood in head and neck squamous cell carcinoma (HNSCC), having been characterized first in oral cancer by Slaughter, et al. in 1953⁴. Field cancerization explains the observation whereby an area of epithelium exposed to carcinogens undergoes key genetic and chromosomal alterations or clonal spread of cells with such alterations⁵. Though only one part of this carcinogen-exposed tissue may initially advance toward neoplasia, the surrounding tissue remains at increased risk for locoregional recurrence based on the genomic aberrations which it has acquired in parallel with the neoplastic process⁵. Because of this retained risk for cancer development in the epithelium adjacent to primary disease, second primary tumors (SPT) act as a possible target for secondary chemoprevention in patients previously diagnosed and treated for HNSCC.

When developing or repurposing compounds for use in the prevention of cancers, it is important that they be relatively inexpensive and nontoxic. Given that many of the individuals who are targeted for the use of chemopreventive agents do not have a diagnosis of cancer, compliance rates may already be low⁶ and study dropout rates high⁷. This underscores the need for clinical trial designs using synthetic or natural compounds directed at the most frequently dysregulated and deleterious pathways in malignancy⁸. Since nuclear factor-kappa B (NF- κ B) and the signaling pathways which control its activity are such significant regulators of HNSCC development and progression, it is practical to focus chemoprevention studies on agents which are able to block NF- κ B activity.

NF- κ B activation and signaling pathways

NF- κ B transcription factors play a crucial role in a variety of normal cellular processes including inflammation and cell survival, and are implicated in the molecular pathogenesis of cancer^{9–11}. These transcription factors were first identified by David Baltimore's laboratory in 1986 as regulators of immunoglobulin κ light chain expression in B lymphocytes¹². Five NF- κ B family members, which can homo- or heterodimerize, are expressed in mammalian cells. RelA (p65), RelB, and c-Rel are synthesized as mature proteins and possess transactivation domains (TADs) responsible for initiating transcription of target genes. NF- κ B1 (p50) and NF- κ B2 (p52) are generated by processing of the precursor proteins p105 and p100, respectively, and lack TADs but can positively regulate transcription by interacting with other proteins or negatively regulate transcription by competing for DNA binding^{13–15}. In cells in a basal state, inhibitors of κ B (I κ Bs), a family of proteins with ankyrin repeat domains, bind to NF- κ B dimers, disguising the nuclear localization signal and maintaining the NF- κ B dimers in the cytoplasm^{16,17}. Activation of

NF- κ B signaling involves phosphorylation of I κ B by I κ B kinases (IKKs). Subsequently, I κ B ubiquitination and degradation by the proteasome exposes the nuclear localization sequence and DNA-binding sites of the NF- κ B dimers, allowing dimers to translocate to the nucleus¹⁸. Normally, nuclear NF- κ B activates resynthesis of I κ B, which mediates inactivation and export to the cytoplasm. However, chronic activation by a variety of signal stimuli, carcinogens, or viruses that promote hyperplasia and inflammation, can lead to premalignancy and cancer^{10,11} (Figure 1).

NF- κ B activation can occur via the classical (canonical) or alternative (non-canonical) pathways (Figure 1). The classical pathway is activated by a variety of stimuli including cellular injury, pathogens, and proinflammatory cytokines such as tumor necrosis factor (TNF)¹⁸. Ligand binding to TNF receptors (TNFR) or toll like receptors (TLR) stimulates IKK β (IKK2), which is in complex with IKK α (IKK1) and the regulatory protein IKK γ (NEMO). IKK β -mediated phosphorylation and proteasomal degradation of I κ Bs, particularly I κ B α , leads to activation of p50/RelA and p50/c-Rel dimers¹⁹. The alternative pathway is activated by a specific subset of TNF family cytokines and other factors including CD40L, BAFF, and lymphotoxin- β (LT β)²⁰. Alternative pathway signaling occurs through TNFR-associated factors (TRAF) complex and NF- κ B-inducing kinase (NIK), which activates IKK α leading to processing of p100 to p52 and nuclear translocation of p52/RelB complexes^{21,22}.

Upon translocation to the nucleus, NF- κ B dimers bind to promoter or enhancer regions of DNA and act as transcription factors. NF- κ B target genes play a normal physiological role in immune response, cellular stress response, and inhibition of apoptosis, but they can also lead to cancer cell proliferation, survival, metastasis, and resistance to anticancer therapies^{10,23,24}. One such target gene is *cyclin D1*, which mediates progression from G₁ to S phase of the cell cycle. Its overexpression in HNSCC correlates with a poor clinical outcome^{25–27}. NF- κ B target genes involved in protection of cells from apoptosis include *BCL-X_L* and *A1/Bfl-1*, which inhibit cytochrome c release from mitochondria. In addition, *TRAF1* and *TRAF2*, as well as caspase inhibiting factors, *c-IAP1* and *c-IAP2*, protect cells from apoptosis following TNF α stimulation^{28,29}.

A variety of inputs upstream of NF- κ B can activate or inhibit signaling in different contexts. TRAFs function downstream of various cytokine receptors to activate NF- κ B signaling (Figure 1). Specifically, TRAF1, 2, 5, and 6 can mediate the canonical pathway, while TRAF3 and 6 associate with NIK and modulate NF- κ B alternative pathway activation^{30,31}. Receptor-interacting proteins (RIPs), which is involved in cell entry into survival, apoptosis, or necroptosis pathways, can induce NF- κ B activation following TNFR stimulation or DNA damage^{32,33}. Many different kinases can phosphorylate IKKs leading to NF- κ B activation, including mitogen-activated protein kinases (MAPKs) and the serine-threonine kinase, Akt, which is activated by phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) and is constitutively active in many cancers³⁴. Given the wide variety of target genes of NF- κ B signaling and the multiple pathways that cross-talk with NF- κ B, the role of NF- κ B signaling in cancer depends on the specific cellular context.

Role of NF- κ B in development and progression of HNSCC

HNSCC includes cancers arising in the oral cavity, nasal cavity, larynx, and pharynx and is one of the six most common cancers in the world³⁵. More than 35,000 new cases and 7,800 deaths occurred in the US in 2010³⁶. HNSCC can be divided into 2 major subsets: carcinogen-associated and viral pathogen-associated. Carcinogen-associated HNSCC is caused by tobacco use and betel nut (areca) chewing. Chronic exposure to cigarette smoke carcinogens causes inflammation, which contributes to the aberrant activation of NF- κ B signaling and development of HNSCC. Cigarette smoke contains polyaromatic hydrocarbons and reactive oxygen species (ROS), which damage DNA and induce production of proinflammatory cytokines such as TNF α and IL-1 in airway epithelial cells^{37,38}. DNA damage and binding of TNF and IL-1 to their receptors stimulates IKK-NF- κ B signaling, leading to increased transcription of proinflammatory cytokines such as IL-6 and proangiogenic cytokines such as IL-8 and vascular endothelial growth factor (VEGF)^{39,40}. NF- κ B proinflammatory cytokines can promote tumor growth and metastasis, and serum levels have been shown to correlate with response to treatment and survival^{41,42}. Cigarette smoke has been shown to cause I κ B α phosphorylation and degradation leading to activation of NF- κ B signaling in HNSCC and other cancer cell lines⁴³. Oral keratinocytes exposed to betel nut extract show a decrease in cytosolic I κ B α and an increase in NF- κ B and MAPK signaling⁴⁴. PI3K and epidermal growth factor receptor (EGFR), which is overexpressed in about 90% of HNSCC, can also activate NF- κ B signaling, leading to IL-8 and VEGF production⁴⁵.

Ultimately, carcinogen and ROS related genetic and epigenetic alterations affecting upstream signaling leads to aberrant activation of IKK and NF- κ B (Figure 1). As a transcription factor for anti-apoptotic genes, NF- κ B can promote tumor survival in a variety of cancers, including HNSCC. Matrix metalloprotease-9 (MMP-9), which correlates with nodal metastasis and decreased survival, is synergistically upregulated by proinflammatory cytokines and growth factors in a NF- κ B-dependent manner^{46–48}. Chemokine receptor 7, also implicated in metastasis, is upregulated by NF- κ B^{49,50}. Given the role of NF- κ B activation in tumor development, cell survival, and malignant progression in carcinogen-associated HNSCC, this pathway is a logical target for chemoprevention.

NF- κ B in pathogen-associated HNSCC

Human papillomavirus (HPV) and Epstein-Barr virus (EBV) are implicated in the pathogen-associated subset of HNSCC. The high-risk HPV type 16 is most frequently associated with cancer of the oropharynx and larynx, while EBV is most often linked to nasopharyngeal carcinoma (NPC). The incidence of HPV-associated HNSCC is increasing in Western countries, and patients typically present at a younger age and have a better overall survival compared to carcinogen-associated HNSCC^{51,52}. Cancer development in HPV-associated tumors involves inactivation of tumor suppressor proteins by viral proteins. HPV E6 protein binds p53 and targets it for degradation leading to inhibition of apoptosis. HPV E7 protein binds and inactivates retinoblastoma family proteins leading to increased cell cycle progression^{53,54}. In airway epithelial cells, E6 has been shown to induce NF- κ B activation via a mechanism that is independent of p53 degradation and involves interaction with proteins containing PDZ (PSD95/Dlg/ZO-1) domains⁵⁵. Progression of HPV-infected cells

from premalignant to malignant is accompanied by an increase in NF- κ B protein levels and constitutive activation^{56,57}.

NPC is rare in the Western hemisphere compared to southern Asia, where EBV is believed to cause almost 100% of cases⁵⁸. NF- κ B proteins are commonly activated in NPC and overexpression correlates with a worse prognosis^{59,60}. The EBV oncoprotein latent membrane protein 1 (LMP1) contains signaling domains known as carboxyl-terminal activating regions (CTARs), which induce NF- κ B, PI3K-Akt and MAPK signaling^{61,62}. CTAR2 activates the canonical NF- κ B pathway by TRAF6-mediated I κ B α phosphorylation, while CTAR1 activates the alternative pathway by inducing processing of p100 to p52^{63,64}. In contrast, the EBV protein LMP2A inhibits NF- κ B pathway activity, which may suppress the immune response to NPC⁶². The role of the NF- κ B pathway in HPV-or EBV-associated HNSCC is not yet as well-defined as the role in carcinogen-associated HNSCC, and additional research would help to clarify the optimal way to target the NF- κ B pathway in these pathogen-associated cancers.

NF- κ B-targeted chemoprevention

Over 700 compounds have been shown to be capable of inhibiting NF- κ B activity in over 9,000 published studies describing them⁶⁵. While the NF- κ B signaling pathways have been targeted for cancer therapy in various clinical studies^{8,65–69}, many of these inhibitors have not been investigated thoroughly for cancer chemoprevention because they are either too expensive for widespread use, too novel in discovery, or too pleiotropic in function and unacceptable in safety profile. As such, this review concentrates on those NF- κ B-inhibiting agents which have been studied for the prevention of HNSCC (Tables 1 and 2) and explains at which steps in the NF- κ B pathway they are known to act (Figure 2).

Retinoids

Retinoids are precursors, derivatives, and analogues of Vitamin A and are the most extensively studied compounds for the prevention of HNSCC^{70,71}. They are understood to regulate many processes such as proliferation, differentiation, and apoptosis⁷² by binding to two types of nuclear ligand-gated receptors: retinoic acid receptors (RAR's) and retinoid X receptors (RXR's)⁷³. The most commonly investigated forms of retinoids are 4-hydroxyphenylretinamide (4-HRP or fenretinide), 13-cis-retinoic acid (13-cRA or isotretinoin), and β -carotene^{74–76}. These compounds have been used in a variety of preclinical and clinical trials for the chemoprevention of head and neck cancer and exert effects on NF- κ B through a range of different mechanisms.

Of the most widely used retinoid compounds, the synthetic retinoid, fenretidine, seems to have a favorable toxicity profile and effectiveness in reversing OPL progression into invasive carcinoma^{74,77,78}. In an in vitro system of HNSCC, 4-HRP suppresses constitutive NF- κ B activity⁷⁹. When combined with TNF stimulation, 4-HRP decreases NF- κ B activation and inhibits TNF-dependent I κ B α degradation, phosphorylation, and kinase activation⁷⁹. In further elucidating the mechanism through which fenretinide acts on NF- κ B, Shishodia, et al. showed that 4-HRP suppresses TNF-induced Akt activation and cyclooxygenase-2 (COX-2) promoter activity. While fenretinide was not able to directly

affect the binding of NF- κ B to DNA, it does inhibit the phosphorylation and nuclear translocation of the NF- κ B subunit, p65, thereby repressing TNF-induced NF- κ B reporter gene activity⁷⁹.

13-cRA also inhibits inflammatory cytokine production and angiogenesis both in vitro and in vivo by inhibiting activation and nuclear translocation of NF- κ B subunits p65, p50, and c-Rel in epithelial cancer⁸⁰. Bexarotene, a RXR ligand, inhibits NF- κ B-mediated transcription through histone deacetylation by releasing CBP/p300 from NF- κ B target genes in thyroid cancer⁷³. Finally, using a SCC xenograft model in *NF- κ B-RE-luc* mice, treatment with all-trans-retinoic acid suppressed NF- κ B activation while simultaneously inhibiting tumor growth⁸¹.

Although the ability of retinoids to debilitate NF- κ B pathway activity is well established in a variety of cancers, including HNSCC, they have shown limited chemopreventive potential in human trials^{82–88}. One explanation for this is the heterogeneity of HNSCC tumors⁸⁹ and resistance to certain types of retinoids⁷⁰. In a step toward personalized chemoprevention, Hildebrandt, et al. show that genetic variants of the PI3K/PTEN/Akt/mTOR pathway can identify patients at high risk for SPT and predict response to 13-cRA. Future studies investigating NF- κ B pathway variations could provide more insight about patient sensitivity or resistance to retinoid-based chemopreventive regimens.

EGFR inhibitors

EGFR amplification or phosphorylation is detected in over 90% of human HNSCC tumors⁹⁰. Additionally, EGFR overexpression or phosphorylation in HNSCC is associated with malignant transformation⁹¹ and poor clinical prognosis⁹². It is well-established that EGFR phosphorylation can stimulate NF- κ B activity in head and neck cancer^{45,93}. Previously, our group provided the first evidence that EGF induces the activation of NF- κ B reporter genes in HNSCC through phosphorylation of EGFR⁴⁵. Conversely, using either an EGFR-directed tyrosine kinase inhibitor (TKI) or an EGFR-directed antibody, we showed that EGF-induced and, to a lesser extent, basal NF- κ B reporter gene expression is repressed when EGFR is inhibited⁴⁵. This mechanism was further characterized in HNSCC by testing the effects of recombinant EGF and another EGFR TKI, called gefitinib, on the whole protein and phosphorylated protein expression of NF- κ B subunit p65 (S536). S536 is considered the most important site for p65 transactivation by I κ B kinase β of the classical I κ B kinase complex²⁴. Gefitinib treatment partially inhibited constitutive p65 (S536) activation and significantly inhibited EGF-induced NF- κ B p65 (S536) activation in two out of three HNSCC cell lines studied⁹⁴.

However, in a pilot phase I study of gefitinib for treatment of locally advanced head and neck cancer, only one patient out of seven was determined a “molecular responder” to gefitinib treatment⁹⁵. Even though EGFR was phosphorylated in six out of seven pretreatment biopsies, limited effects on molecular pathways such as EGFR, NF- κ B, STAT-3, ERK, and Akt were observed in all but one of the tumor specimens after gefitinib treatment^{94,95}. This suggests that while EGFR is overexpressed in the vast majority of HNSCC tumors, EGFR activation most likely plays a dominant role in NF- κ B activation and HNSCC progression in only a small subset of patients because of multiple other

compensatory pathways upstream of NF- κ B. As such, the potential for EGFR inhibitors as effective chemopreventive agents may be limited to combinatorial treatment strategies for patients with relevant underlying genetic signatures. Several clinical trials are ongoing using the EGFR TKI, erlotinib, alone or in combination with other compounds for the prevention of head in neck cancer (Table 2). It will be important to consider tissue genotype and biomarker expression when interpreting the results of these studies.

Green Tea Extracts (GTE)

Plant polyphenols, such as those extracted from the leaves of the green tea plant, *Camellia sinensis*, are understood to exhibit an array of anti-HNSCC properties like inhibition of tumor growth, invasion, metastasis, angiogenesis, and enhancement of apoptosis^{96–99}. Four main polyphenols comprise the majority of GTE: epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG)¹⁰⁰. EGCG is the most abundant and best studied polyphenol in GTE and is speculated to hold the most potent anticancer activity^{70,101}.

EGCG acts on many proteins and genes, including NF- κ B, p53, EGFR, Akt, STAT3, AP-1, MAPK, and TNF, involved in HNSCC development and progression^{102–105}. In EBV-driven NPC, LMP-1-induced phosphorylation of I κ B α is prevented by EGCG treatment. As a result, NF- κ B (p65) nuclear translocation is greatly suppressed and cell survival is inhibited¹⁰⁶. Amin, et al. showed that EGCG inhibits HNSCC growth synergistically with erlotinib through a p53-dependent NF- κ B inhibitory mechanism¹⁰⁷. Their results show that while EGCG alone does not significantly affect expression of cell cycle regulatory proteins, when combined with erlotinib, EGCG treatment is able to decrease expression of p65 and many of its transcriptional targets. Functionally, this combination also inhibits cell growth in an in vitro viability assay¹⁰⁷. Another study in HNSCC has shown that EGCG treatment strongly inhibits constitutive NF- κ B activation and resultant production of VEGF¹⁰².

Several clinical trials have recently been conducted to investigate whether GTE can be used safely and efficaciously in humans for the prevention of HNSCC (Table 1). When taken systemically, GTE provided a clinical OPL regression response rate of 50% compared to 18.2% for placebo¹⁰⁸. This led to a clinical trial currently underway in which EGCG is being administered with erlotinib for treatment of OPL (NCT 01116336). The use of EGCG to inhibit NF- κ B-mediated proliferation and angiogenesis merits continued investigation for chemoprevention in HNSCC.

Curcumin

Curcumin is the yellow component of the spice turmeric which is derived from the plant, *Curcuma longa*. It has been consumed as a dietary supplement for thousands of years and has long been implicated in the prevention of tumor formation, growth, and metastasis¹⁰⁹. In HNSCC, many studies have shown that curcumin acts to inhibit the NF- κ B pathway, prompting investigations using it in vitro and in vivo as a chemopreventive agent.

In HNSCC cell lines expressing constitutively active NF- κ B and IKK, treatment with curcumin inhibits NF- κ B activity through abrogation of IKK^{109–111}. Additionally, curcumin treatment potently inhibits NF- κ B-regulated target gene expression and cellular

proliferation, while inducing apoptosis, G1 phase arrest and DNA fragmentation^{110,112,113}. This result was corroborated in a study which demonstrated that curcumin downregulates smokeless tobacco-induced NF- κ B and COX-2 activity in both oral premalignant and cancer cells¹¹⁴. Functionally, the NF- κ B inhibition seen with curcumin treatment is negatively correlated with the migratory phenotype and propensity for local invasion of HNSCC cells both in vitro and in vivo^{115–117}. Conversely, NF- κ B p65 cDNA overexpression reduces the effects of curcumin on HNSCC growth and invasion¹¹⁵. Through its regulation of proteolytic enzymes such as MMPs¹¹⁸ and cellular adhesion molecules like cadherins, curcumin alters HNSCC cell motility in a NF- κ B-dependent manner^{116,117}.

Suppression of HNSCC growth, phosphorylation of IKK, and expression of NF- κ B and its targets has been further validated in mice xenograft tumors treated with a curcumin topical paste or i.v. formulation^{111,119}. This was replicated using a synthetic curcumin analogue called EF31, which was shown to significantly inhibit HNSCC human xenograft tumor growth and phosphorylation of IKK and p65¹²⁰. However, the usefulness of curcumin as a chemopreventive agent may be limited to HPV-negative tissues, as preliminary evidence indicates that HPV-positive tumors are not as sensitive to its effects¹²¹. It is also poorly absorbed from the digestive tract, limiting its systemic bioavailability, and topical application is associated with short term exposure and tissue discoloration. Regardless, the pharmacologically-favorable toxicity profile of curcumin¹²¹ has led to its use in several completed and ongoing human trials for HNSCC chemoprevention (Tables 1 and 2).

COX inhibitors

OPL are often driven to progress into HNSCC through pathways of chronic inflammation associated with enhanced prostaglandin synthesis. Accordingly, HNSCC tumors express high levels of COX^{122–125}. COX-2 inhibition can decrease proliferation and invasion of HNSCC cells and modulate the expression of proteins integral to angiogenesis and apoptosis in favoring chemoprevention¹²⁶. As such, several animal studies have investigated whether COX-2 inhibitors show HNSCC antitumor effects, concluding with positive results^{127–130}. One small clinical trial published in 1981 showed potential for indomethacin, a NSAID prostaglandin synthesis inhibitor, in stabilization and regression of late-stage HNSCC's¹³¹. However, the results of the in vivo animal studies and this early chemotherapy trial have not translated particularly well in human HNSCC chemoprevention trials (Table 1).

One explanation for the unpredictability of COX-2 inhibition in HNSCC by its pharmacologic inhibitors is continuous compensation by redundant activators of the prostaglandin synthesis pathway, such as NF- κ B^{126,132}. Indeed, multiple studies have shown that high NF- κ B expression parallels COX-2 overexpression in both oral precancer and cancer^{133,134}, which directs squamous cells toward tumorigenesis. Another reason COX-2 inhibitors have not found great acceptance in the clinic for HNSCC chemoprevention is that they are associated with adverse cardiovascular events, which has led to the suspension or discontinuation of several clinical trials¹³⁵. Since COX-1 may also play a role in HNSCC tumorigenesis, the use of nonselective COX inhibitors could be just as effective in cancer chemoprevention, without the cardiovascular risks associated with selective COX-2 agents¹³⁶.

Celecoxib, a selective COX-2 inhibitor, abrogates NF- κ B activity by suppressing I κ B phosphorylation and degradation^{113,137}. Celecoxib also inhibits constitutive NF- κ B activity¹³⁸, p65 phosphorylation and nuclear translocation, and cyclin D1, MMP-9, and COX-2 induction by cigarette smoke condensate¹³⁷. But even though COX inhibition has been directly linked to consequential NF- κ B inhibition, it has been established that COX inhibitors alone are not enough to suppress HNSCC development (Table 1). Since the anticancer effects of COX inhibition may be mediated by both COX-dependent and COX-independent pathways, and since NF- κ B is a key transcriptional regulator of pro-inflammatory mediators such as COX, targeting both COX and NF- κ B simultaneously with drug combinations might improve the efficacy of COX inhibitors in HNSCC chemoprevention.

Vitamin E

Vitamin E is composed of two classes of compounds, tocopherols and tocotrienols, each with four isomers (α , β , γ , δ)¹³⁹. Tocotrienols are the unsaturated forms of Vitamin E and are known to inhibit the growth and survival of multiple cancers¹⁴⁰. γ -tocotrienol inhibits angiogenesis, metastasis, proliferation and invasion through suppression of constitutive NF- κ B and its downstream targets in gastric cancer¹⁴¹. δ -tocotrienol shows antitumor activity in pancreatic cancer through inhibition of NF- κ B in vitro and in vivo¹⁴⁰. Vitamin E supplementation protects against early-stage esophageal carcinogenesis in rats, but not late-stage esophageal cancer development. The decrease in early-stage tumor growth is mirrored by decreased NF- κ B activation, protein and mRNA levels¹⁴². Similar studies have shown that these compounds act through decreasing NF- κ B-mediated signaling pathways in lung, breast, bladder, colon, and prostate cancers^{143–147}.

α -tocopherol is the most biologically active and best studied form of vitamin E in HNSCC. It has been used alone or in combination with retinoids for several HNSCC chemoprevention trials since the early 1990's^{84,148–152}. While all of these trials have reported positive results, the most recent randomized trial suggests that vitamin E supplementation is actually harmful¹⁵³. In that study, 540 HNSCC patients took α -tocopherol or placebo for three years for SPT chemoprevention, and those individuals in the supplement arm had increased all-cause and cause-specific mortality rates¹⁵³. Further, the effects of α -tocopherol and the other forms of vitamin E on NF- κ B activity have not been investigated whatsoever in HNSCC.

PI3K-mTOR inhibitors

PI3K-mTOR has been shown to promote IKK-NF- κ B activation and transcription and translation of target genes and proteins^{154,155}. Targeting mTOR by rapamycin or analogs was found to potently inhibit tumorigenesis of HPV-positive and HPV-negative human HNSCC tumor xenografts^{156,157}. Rapamycin also inhibited development of chemically induced skin and oral SCC^{158,159}, and HNSCC that develop spontaneously in genetically engineered *Kras*^{G12D}/*p53*^{-/-}, *Pten*^{-/-}, or *Pten*^{-/-}/*Tgfbr1*^{-/-} mice in vivo^{160,161}. In a pilot pharmacodynamic clinical study using the rapalog, temsirolimus, for 3 weeks, evidence for tumor reduction was observed in 8/14 patients¹⁶². Together, these findings indicate that the

PI3KAkt-mTOR axis serves as a promising target for chemoprevention and therapy in HNSCC.

Antidiabetic drugs

Based on retrospective epidemiologic data analysis of diabetic patients treated with certain classes of hypoglycemic drugs called thiazolidinediones (TZDs), these drugs are associated with decreased risk of both lung and head and neck cancers^{163,164}. In accordance with these findings, two peroxisome proliferator-activated receptor gamma (PPAR- γ) agonists used for treatment of diabetes, pioglitazone and troglitazone, have been tested in rat 4-NQO-induced oral cancer models, and have been shown to prevent the occurrence of tongue cancers^{165,166}. PPAR- γ ligands block angiogenesis and tumor growth of non-small cell lung cancer by inhibiting NF- κ B transcriptional activity¹⁶⁷. In breast cancer, PPAR- γ agonists reduce tumor survival in concert with suppression of NF- κ B activity¹⁶⁸. While the mechanisms whereby TZDs hamper NF- κ B activity have been teased out in colorectal, prostate, leukemia, lung, and breast cancers^{167–172}, there is currently little to no evidence to show the effects on NF- κ B in HNSCC. Metformin, a first-line treatment for type II diabetes, has also been implicated to have antineoplastic effects in HNSCC¹⁷³. Metformin inhibits HNSCC cell growth and mTOR signaling in vitro and in vivo, and prevents the malignant transformation of OPL into HNSCC's¹⁷⁴. Since there is preliminary evidence for reduction of OPL with pioglitazone^{174,175}, larger studies and further elucidation of its mechanism of action will be important research aims heading into the future.

Other natural compounds

Berry extracts contain high levels of antioxidants, such as ellagic acid, which are known chemopreventive agents. Black raspberry extracts (BRE), in particular, have been shown to prevent esophageal and colorectal tumors in rats^{176,177}, and to inhibit I κ B α phosphorylation in mouse epidermal cells¹⁷⁸. BRE was initially used in clinical trials for patients at high risk of developing esophageal and colon cancers¹⁷⁹, and currently holds promise for its application in the treatment of OPL¹⁸⁰ (Table 2).

Genistein is a natural isoflavonoid found in soybeans. It has been shown to inhibit cancer growth by inactivating NF- κ B via the Akt and Notch-1 pathways in breast cancer, hepatocellular carcinoma, osteosarcoma, lung, pancreatic, and prostate cancers^{181–187}. Inactivation of NF- κ B by soy isoflavones has also been reported in squamous cell carcinoma, including those of the head and neck¹⁸⁸. A clinical trial using soy isoflavones is ongoing at the University of Michigan Cancer Center to assess HNSCC biomarker expression of p53, p16, EGFR, VEGF, Bcl-xL and COX-2, and risk of SPT (NCT01028001).

Resveratrol is a natural phenol commonly known for its presence in grape skins. It holds anticancer properties in breast, esophageal, prostate, stomach, colon, pancreas, thyroid, ovarian, cervical, and head and neck cancers¹⁸⁹. In HNSCC, resveratrol inhibits proliferation and DNA damage¹⁹⁰. Resveratrol suppresses NF- κ B activity in leukemia, pheochromocytoma, prostate, and lung cancers^{191–195}, but this activity has not been specifically shown in head in neck cancer. Though resveratrol can be delivered safely at up

to 5g/day with minimal side effects, its systemic bioavailability is limited at even high doses by rapid phase II metabolism¹⁹⁶. As such, resveratrol may prove to be more efficacious for prevention of HNSCC than many other malignancies because it can be applied topically or otherwise come into direct contact with the oral epithelium⁷⁰. Given its auspicious safety profile, effectiveness toward cancer inhibition in vitro and in vivo, and ability to curb NF- κ B activity in many cancers, it would be worthwhile to further investigate resveratrol's ability to prevent HNSCC in humans.

Many other natural compounds, other than those discussed above, have been shown to inhibit NF- κ B activity in a variety of cancers. These NF- κ B-inhibitory compounds are also currently being tested for their chemopreventive potential and include: pomegranate juice, luteolin, lycopen, and other fruit and vegetable extracts.

Future Directions

One of the primary drawbacks of many of the agents that have been used to date for HNSCC chemoprevention is their dose-limiting toxicities. While many inventive approaches have been implemented to overcome the risks associated with systemic delivery of certain agents, local application of chemopreventive compounds may be the easiest strategy to limit these risks, especially for highly accessible OPL and cancers like HNSCC⁷¹. Topical gels¹⁹⁷, epithelial patches¹⁹⁸, oral lozenges and mouthrinses¹⁹⁹, and intraepithelial injections^{200,201} have all been investigated for reducing malignant transformation of OPL in humans. Formulating compounds with NF- κ B-inhibitory potential for local delivery to head and neck epithelia could serve as a way of improving drug activity in high-risk tissues and reducing the risk of HNSCC tumorigenesis. Another way of avoiding the side effects associated with many chemopreventive agents is to identify compounds with higher specificity for the desired molecular targets. The agents discussed in this paper have many biological actions besides those exerted on NF- κ B.

An area yet largely unexplored for the chemoprevention of head and neck cancer is in HPV-positive cells or patients. Though vaccination of uninfected individuals can surely help to prevent HNSCC, there remains a relative paucity of knowledge about how to prevent HNSCC development in people already infected with and unvaccinated for HPV. Future studies will be necessary in HPV-positive cell lines and patients, as the incidence of HPV-positive head and neck cancers continues to increase.

Conclusion

Considering the great heterogeneity in pathogenesis among HNSCC patients, developing personalized molecular monitoring based on genetic traits, managing early head and neck lesions, and inhibiting inflammation could prove to be the most effective strategies for preventing HNSCC on an epidemiologic level. NF- κ B is known to be a dysregulated driver of HNSCC, as are several other important inflammation- and cancer-associated transcription factors, tumor suppressors, and oncogenes. As researchers continue to identify and characterize biomarkers which can act as surrogates for disease outcomes, clinicians will be

better poised to offer effective care based on the underlying genetic and proteomic manifestations of patients' diseases.

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Figure 1. NF-κB activation in head and neck squamous cell carcinoma (HNSCC)

In carcinogen-associated HNSCC, chronic exposure to reactive oxygen species and repeated DNA damage lead to NF-κB activation. Activation can also occur via aberrant signaling through cytokine and growth factor receptors, such as TNFR, TLR, and EGFR, or activation by intermediate kinases, such as IKKs, PI3K/Akt, and MAPKs. In the pathogen-associated subset of HNSCC, the HPV E6 protein induces degradation of the p53 tumor suppressor protein and activates NF-κB via interactions with proteins containing PDZ domains. The EBV LMP1 protein activates the canonical NF-κB pathway by TRAF6-mediated IκBα phosphorylation and the alternative pathway by inducing processing of p100 to p52. Activation of the canonical pathway by TNF family members or other cytokine receptors, such as TLR, causes phosphorylation of IκB by an IKKα/IKKβ/IKKγ complex, leading to ubiquitination and proteasomal degradation of IκB. p105 is processed to p50, and p50/RelA or p50/cRel heterodimers translocate to the nucleus and bind to promoters to regulate gene expression. The alternative pathway is activated by CD40L, BAFF, and LTβ, and involves signaling through TRAF3/6 complex and NIK to IKKα/IKKα homodimers, which activate processing of p100 into p52. Subsequently, p52/RelB heterodimers translocate to the nucleus and act as a transcription factor. Both pathways cooperatively regulate a variety of

processes such as proliferation, apoptosis, migration, inflammation, and angiogenesis. Abbreviations: EGFR= epidermal growth factor receptor, GFRs= growth factor receptors, TNFR=tumor necrosis factor receptor, TLR= toll like receptor, PTEN= phosphatase and tensin homolog, PI3-K= phosphatidylinositol 3-kinase, MAPK= mitogen activated protein kinase, ROS= reactive oxygen species, IKK= I κ B kinase, I κ B= inhibitor of κ B, HPV= human papillomavirus, EBV= Epstein-Barr virus, LT β = lymphotoxin β , TRAF= TNFR associated factor, BAFF= B-cell activating factor, NIK= NF- κ B inducing kinase.

Figure 2. Sites of action of chemopreventive agents on canonical NF-κB pathway

Extracellular signals such as EGF, TNF, IL-1, and other growth factors (1) bind to their respective receptors (2), and induce phosphorylation cascades through signaling pathways, such as PI3K-Akt-mTOR, MAPK, and IKK (3). Upon activation of these intermediary kinases, IκB becomes phosphorylated and ubiquitinated (4), leading to degradation via the 26S proteasome (5). NF-κB subunits then translocate to the nucleus (6) where they regulate transcription of many genes (7) and subsequent translation of proteins that promote HNSCC (8). Several HNSCC chemoprevention agents are able to inhibit this process at a number of different steps, as depicted. Abbreviations: EGFR= epidermal growth factor receptor, GFRs= growth factor receptors, TNFR=tumor necrosis factor receptor, TLR= toll like receptor, IKK= IκB kinase, IκB= inhibitor of κB, PI3K= phosphatidylinositol 3-kinase, mTOR= mammalian target of rapamycin, TRAF= TNFR associated factor, GTE= green tea extract, TKI= tyrosine kinase inhibitor, COX= cyclooxygenase, PPARγ= peroxisome proliferator-activated receptor gamma.

Table 1
Completed head and neck cancer chemoprevention clinical trials using compounds shown to suppress NF- κ B

Clinical trials with published results in the last 13 years are represented in this table, showing study authors, phases, and sizes, in addition to the chemoprevention target (primary or secondary), drug dosing schedule, and clinical outcome. Abbreviations: OPL= oral premalignant lesion, SPT= second primary tumor, FBR= freeze-dried black raspberry, GTE= green tea extract, RP= retinyl palmitate, BC= β -carotene, 13-cRA= 13-cis-retinoic acid, COX= cyclooxygenase, PPAR- γ = peroxisome proliferator-activated receptor gamma.

Compound	Study Author (Year)	Phase	n	Target	Regimen	Clinical Outcome
Black raspberry extract	Shumway, et al. (2008) ¹⁸⁰	II	29	OPL	FBR 0.5 g 10% gel qid for 6 weeks	positive
	Mulshine, et al. (2004) ¹²⁴	IIb	57	OPL	Ketorolac 10 ml 0.1% rinse 30s bid vs placebo for 3 mo	negative
COX inhibitors	Papadimitrakopoulou, et al. (2008) ²⁰²	II	50	OPL	Celecoxib 100 mg bid vs 200 mg bid vs placebo for 3 mo	negative
	Wirth, et al. (2008) ²⁰³	I	22	OPL	Celecoxib 400 mg bid for 3–12 mo	undetermined
Curcumin	Cheng, et al. (2001) ²⁰⁴	I	7	OPL	Curcumin 500–12,000 mg qd for 3 mo	positive
	Rai, et al. (2010) ²⁰⁵	I	100	OPL	Curcumin 1g/day for 130–190 days	positive
	Pisters, et al. (2001) ²⁰⁶	I	19	SPT	GTE 0.5–5 mg/m ² qd or 1–2 mg/m ² tid for 6 mo	undetermined
Green tea extracts	Schwartz, et al. (2005) ²⁰⁷	I	6	chemopreventive biomarkers	GTE 400–500 mg 5 $\times$ daily for four weeks	positive
	Tsao, et al. (2009) ¹⁰⁸	II	41	OPL	GTE 500, 750, or 1000 mg/m ² daily vs placebo for 12 weeks	positive
	Rhodus, et al. (2011) ¹⁷⁵	IIa	44	OPL	Pioglitazone 45 mg qd for 3 mo	positive
PPAR- γ agonists	Gaeta, et al. (2000) ⁸²	III	21	OPL	Acitretin 20 mg qd for 4 weeks	positive
Retinoids	Van Zandwijk, et al. (2000) ⁸⁸	III	2,592	SPT	RP 300k IU/day for 1 year followed by 150k IU/day vs no treatment for 1 year	negative
	Mayne, et al. (2001) ⁸³	III	264	SPT	BC 50 mg/day vs placebo for 90 mo	negative
	Toma, et al. (2003) ⁸⁷	III	214	SPT	BC 75 mg qd vs no treatment for 3 years	negative
	Toma, et al. (2004) ⁸⁶	III	267	SPT	13-cRA 0.5 mg/kg/day vs control for 3 years	negative
	Chiesa, et al. (2005) ⁷⁴	III	170	OPL	Fenretinide 200 mg qd vs no treatment for 1 year	positive
	Perry, et al. (2005) ⁸⁵	III	151	SPT	13-cRA 1 mg/kg/day vs 0.5 mg/kg/day vs placebo for 3 years	negative
	Khuri, et al. (2006) ²⁰⁸	III	1,190	SPT	13-cRA 30 mg/day vs placebo for 3 years	negative
	Lippman, et al. (2006) ⁷⁷	II	35	OPL	Fenretinide 200 mg/day for 3 mo	limited
	Papadimitrakopoulou, et al. (2009) ⁸⁴	III	162	OPL	13-cRA 0.5 mg/kg/day, or RP 25k IU/day, or BC 50 mg/day + RP 25k IU/day for 3 years	negative

Compound	Study Author (Year)	Phase	n	Target	Regimen	Clinical Outcome
Vitamin E	William, et al. (2009) ⁷⁸	II	15	OPL	Fenretinide 900 mg/m ² bid for 12 weeks	limited
	Shin, et al. (2001) ¹⁵¹ , Seixas-Silva, et al. (2005) ¹⁵⁰	II	45	SPT	α-tocopherol 1200 IU/day + Interferon-α 3 MU/m ² q2d + 13-cRA 50 mg/m ² /day for 1 year	positive
	Bairati, et al. (2006) ¹⁵³	III	540	SPT	α-tocopherol 400 IU/day vs placebo for 3 years	negative

Table 2
Ongoing head and neck cancer chemoprevention clinical trials using agents proven to inhibit NF-κB

HNSCC chemoprevention clinical trials that are currently underway in the United States and India were found by searching <http://www.clinicaltrials.gov> and <http://www.charakinternational.com>. Studies are reported by academic institution, study reference number and phase, drug chemoprevention target (primary or secondary) and dosing regimen. Abbreviations: OPL= oral premalignant lesion, SPT= second primary tumor, LBR= lyophilized black raspberry, FBR= freeze-dried black raspberry, EGCG= epigallocatechin-3-gallate, COX= cyclooxygenase, PPAR-γ= peroxisome proliferator-activated receptor gamma.

Compound	Sponsor	Study ID/Reference	Phase	Target	Regimen
Black raspberry extracts	Ohio State University Comprehensive Cancer Center	NCT01469429 ^a	Ib	SPT	LBR lozenge vs placebo qid for 2 years
		NCT01504932 ^a	I	SPT	LBR lozenge qid vs no treatment for 6 mo
		NCT01465776 ^a	I	chemopreventive biomarkers	LBR lozenge ×3 tid from biopsy until surgery
COX inhibitors	Ohio State University	NCT01192204 ^a	I/II	OPL	FBR 10% gel 0.5 g qid for 3 mo
		NCT00299195 ^a	I	OPL	Sulindac 150 mg bid vs placebo for 24 weeks
		NCT01160302 ^a	0	chemopreventive biomarkers	Curcumin 4 g bid for 21–28 days
Curcumin	LSU Health Sciences Center Shreveport	See ^b	II/III	OPL	Curcumin 4 g bid for 28 days
	Tata Memorial Cancer Center, India		II/III	OPL	Curcumin 3.6 g bid
	Anrita Institute, Kochi, India		II	OPL	Curcumin gel tid for 6 mo
	Regional Cancer Center, Trivan, India		II	SPT	Erlotinib 150 mg qd for 1 year
EGFR inhibitors	University of Alabama Birmingham	NCT00570232 ^a	II	SPT	EGCG 200 mg tid + erlotinib 50, 75, or 100 mg qd for 6 mo
	Emory University	NCT01116336 ^a	I	OPL	Celecoxib 400mg qd + Erlotinib 100 mg qd for 6 mo
		NCT00400374 ^a	I/II	SPT	Cetecoxib 400 mg bid + erlotinib 50, 75, or 100 mg qd for 6 mo
		NCT00314262 ^a	I/II	OPL	Erlotinib 150 mg qd or placebo for 1 year
PPAR-γ agonists	M.D. Anderson Cancer Center	NCT00402779 ^a	III	OPL	Pioglitazone 15 mg ×3 qd vs placebo for 24 weeks
Soy isoflavones	National Cancer Institute	NCT00951379 ^a	II	OPL	PTI G-2535 300 mg daily for three weeks
	University of Michigan Cancer Center	NCT01028001 ^a	II	chemopreventive biomarkers	

^a <http://www.clinicaltrials.gov>

^b http://www.charakinternational.com/pdfs/clinic_trial.pdf