A Role of Sphingosine Kinase 1 in Head and Neck Carcinogenesis

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Abstract

It is important to identify novel and effective targets for cancer prevention and therapy against head and neck squamous cell carcinoma (HNSCC), one of the most lethal cancers. Accumulating evidence suggests that the bioactive sphingolipids, such as sphingosine-1-phosphate (S1P) and its generating enzyme, sphingosine kinase 1 (SphK1) play pivotal roles in several important biological functions including promoting tumor growth and carcinogenesis. However, roles of SphK1/S1P in HNSCC development and/or progression have not been defined previously. Therefore, in this study, we first analyzed the expression of SphK1 in human HNSCC tumor samples and normal head & neck tissues (n = 78 and 17, respectively) using immunohistochemistry. The data showed that SphK1 is overexpressed in all of the HNSCC tumors tested (stages I–IV). We next investigated whether SphK1 is necessary for HNSCC development. To define the role of SphK1/S1P in HNSCC development, we utilized 4-nitroquinoline-1-oxide (4-NQO)-induced HNSCC model in wild-type mice compared with SphK1−/− KO mice. Remarkably, we found that the genetic loss of SphK1, which reduced S1P generation, significantly prevented 4-NQO–induced HNSCC carcinogenesis, with decreased tumor incidence, multiplicity, and volume when compared with controls. Moreover, our data indicated that prevention of 4-NQO–induced HNSCC development in SphK1−/− KO mice might be associated with decreased cell proliferation, increased levels of cleaved (active) caspase 3, and downregulation of phospho (active) AKT expression. Thus, these novel data suggest that SphK1/S1P signaling may play important roles in HNSCC carcinogenesis, and that targeting SphK1/S1P might provide a novel strategy for chemoprevention and treatment against HNSCC.

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world and a major cause of significant morbidity. In the United States, more than 48,000 new cases and 11,000 deaths of HNSCC were estimated in 2009 (1). In spite of the recent advances in surgical procedures and other treatment modalities, the 5-year survival rate for HNSCC patients, approximately 50%, has improved only marginally in the past 3 decades. The available therapeutic options are dependent on the site, size, and the stage of the lesions. Current modalities include surgery and/or chemoradiation therapy, but prognosis is often complicated by poor control of locoregional disease and significant morbidity and loss of quality-of-life. Clearly, new strategies are needed to treat and aid in the management of HNSCC, which mainly depend on identifying novel targets that are involved in the development and/or progression of HNSCC.

Sphingolipids are a family of molecules that have a long-chain (sphingoid) base backbone and include free-sphingoid bases (sphingosine and sphinganine), ceramides, sphingomyelins, cerebrosides, sulfatides, and gangliosides. Multiple studies have shown that sphingolipids, in addition to being structural constituents of cell membranes, play key roles as signaling molecules. In particular, 2 of these sphingolipid metabolites, ceramide and sphingosine 1-phosphate (S1P), have been investigated as important effecter molecules that regulate cell proliferation, differentiation, angiogenesis, and survival in opposite directions: S1P promotes cell proliferation and survival, whereas ceramide generally inhibits cell proliferation and stimulates apoptosis (2, 3). Thus, the balance between ceramide and S1P is important to determine faith of cancer cells to undergo growth inhibition or survival. This balance is critically regulated by sphingosine kinase (SphK), which converts sphingosine to S1P by phosphorylating sphingosine. Two
SphK isoforms, SphK1 and SphK2, have been cloned and characterized to date. Although roles of SphK2 in cancer pathogenesis and/or development remain enigmatic, SphK1, the agonist-inducible isoform that can be activated by a variety of growth factors, cytokines, and mitogens, has been implicated in cell transformation and tumor growth. In recent studies, SphK1 was shown to be critically involved in the colon carcinogenesis through at least cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) pathway regulation (4, 5). Alterations of ceramide synthesis, especially defects in the generation of C18-ceramide, a product of ceramide synthase 1 (CerS1), also known as longevity assurance gene 1, were reported to play important roles in the pathogenesis and/or progression of HNSCC (6). Interestingly, recent reports suggest an unexpected role for increased levels of CerS6/C16-ceramide in stimulating the growth of HNSCC tumors as opposed to CerS1/C18-ceramide, and that regulation of CerS6-generated C16-ceramide production resulted in ER-stress–mediated apoptosis (7). In addition, a recent study reported that SphK1 is upregulated in HNSCC and the positive expression of SphK1 is associated with shorter patient survival time (8). These studies suggest that alterations of sphingolipid metabolism, especially SphK1-related metabolism and signaling play important roles in HNSCC pathogenesis.

The compound 4-nitroquinoline 1-oxide (4-NQO), a carcinogen of tobacco smoke-related heterocyclic amines, induces tongue squamous cell carcinoma (SCC) after treatment in drinking water in mice and rats. 4-NQO, a water-soluble quinoline derivative, causes formation of DNA adducts, resulting in adenosine substitution for guanosine (9, 10). 4-NQO also undergoes redox cycling to produce reactive oxygen species that result in mutations and DNA strand breaks (11). Administration of 4-NQO produces a temporal carcinogenesis progression model with multiple dysplastic, preneoplastic, and neoplastic lesions after treatment (12). These sequential changes in the epithelial cells mimic human oral cavity neoplastic transformation. Additionally, 4-NQO–induced mouse oral cavity cancer is similar to human HNSCC in the expression of many genes related to tumorigenesis (13–15). Recent studies suggest that 4-NQO–induced tongue cancers show activation of the mTOR pathway, a potential target for human HNSCC chemotherapy (16, 17).

In this study, in order to test our hypothesis that SphK1/SIP pathway may play a role in HNSCC development, we show (1) SphK1 is overexpressed in human HNSCC and rodent preneoplastic and cancer tissues of tongue induced by 4-NQO using immunohistochemistry, (2) SphK1 deficiency significantly inhibits 4-NQO–induced tongue carcinogenesis, (3) its inhibitory effects are due to reducing cell proliferation and inducing apoptosis in tumors, (4) with reduction of S1P and C16-ceramide levels in blood, and (5) through reduction of Akt-mTOR pathway. These findings support for the first time the involvement of the SphK1/SIP metabolism in HNSCC development and/or tumor growth and further suggest that SphK1 represents a novel and potential target for HNSCC chemoprevention and/or treatment.

Materials and Methods

Human HNSCC samples

Human HNSCC tissue microarray slides (HN811 & HN803) were purchased from the Biomax.us. These tissue microarray slides include 78 cases of HNSCC, 7 cases of adjacent normal mucosa, and 10 cases of normal tongue tissues.

Animals, chemicals, and carcinogen

SphK1 KO mice, a kind gift from Dr. Richard L. Proia, National Institute of Diabetics and Digestive and Kidney Diseases (NIDDK)/NIH, Bethesda, MD, USA, have been backcrossed to C57BL/6 mice at least 10 times. Male C57BL/6 mice at 5 weeks old were purchased from the Charles River Laboratories as wild-type mice (Wilmington, MA, USA). Mice were housed and handled in the division of laboratory animal facility under MUSC guideline. All animals were maintained under controlled conditions of humidity (50 ± 10%), light (12/12 hour light/dark cycle) and temperature (23 ± 2°C). They were allowed free access to diet and water. All animal experiments were approved by the institutional animal care and use committee at MUSC. A carcinogen, 4-NQO (CAS 56–57–5), and 5'-bromodeoxuridine (BrdU) were purchased from Sigma-Aldrich.

Experimental protocol (Figure 2A)

All male animals including SphK1 KO and wild-type C57BL/6 mice were treated with 4-NQO at 40 μg/ml in drinking water for a period of 16 weeks starting at 6 weeks of age. After carcinogen treatment, all animals were exchanged to normal drinking water for 8 weeks. The experiment was terminated at 24 weeks after the start of the experiment, and all animals were euthanatized by CO2 suffocation. All animals received BrdU (Sigma, 50 mg/kg body weight, i.p.) 1 hour before sacrifice. Blood samples were collected through heart puncture. After careful autopsy, tongues were excised and all tongue tumors were carefully examined for number and size. The length (L), width (W), and depth (D) of each tumor were measured with calipers, and tumor volume (V) was calculated using the formula \( V = L \cdot W \cdot D / 6 \). Tongue tumors and normal tissues were fixed in 10% buffered formalin and embedded in paraffin blocks for histological evaluation. Diagnosis of tongue tumors using hematoxylin and eosin (H&E) stained sections was conducted according to the criteria described by Kramer et al. (18). Serial sections of tongue tumor and normal mucosa were used for immunohistochemical staining.

Immunohistochemistry

All immunohistochemical analyses were carried out with the avidin–biotin complex immunoperoxidase technique. Tissue sections were mounted on glass slides, deparaffinized with xylene, and rehydrated with graded alcohol. After blocking endogenous peroxidase with 3% hydrogen peroxide in
methanol for 10 minutes, slides were immersed in 10-mmol/L citrate buffer (pH 6) and placed in a steamer for 30 minutes to enhance antigen exposure. Then, slides were incubated with the primary antibody at 1:100 (1:40 for phospho–Akt) dilution in PBS for 1 hour at RT or overnight at 4°C after incubation with 2% normal host serum of secondary antibody in PBS for 30 minutes. Rabbit polyclonal antibodies against human SphK1 (19) and cleaved caspase-3 (Cell Signaling Technology), a rabbit monoclonal antibody against phospho–Akt (Ser[173]) (Cell Signaling Technology) and a rat monoclonal antibody (Abcam Inc.) against BrdU were used as the primary antibodies. Immunostaining was developed using Vectastain ABC reagents (Vector Laboratories), 3,3′-diaminobenzidine, and hydrogen peroxide after incubation with the secondary antibody for 30 minutes. Tissue sections were counterstained with hematoxylin. As a control, duplicate sections were stained without primary antibodies. Immunohistochemical expression was measured by calculating score for SphK1 immunohistochemical staining in human head & neck samples: 0, negative; 0.5, weak staining in <50% of cells; 1, weak staining >50% of cells; 1.5, moderate staining <50% of cells; 2, moderate staining in >50% of cells; 2.5, strong staining ≤50% of cells; 3, strong staining in ≥50% of cells.

Levels of sphingolipids in blood samples
Sphingolipids in blood samples were analyzed by sphingolipid profiling using a tandem mass spectrometry (LC-MS/MS) with positive mode electrospray ionization (EMS) in the Lipidomics Core Facility, MUSC as described previously (2). Results were expressed as picomoles sphingolipids per 100 μL.

Cell proliferation and apoptosis analyses
Cell proliferation and apoptosis were analyzed by BrdU incorporation and cleaved caspase-3 positive cells, respectively in cancer cells in wild type and SphK1 KO mice using immunohistochemistry. Immunohistochemically BrdU-positive cells were considered to be S-phase cells and cleaved caspase-3–positive cells to be apoptotic. All cancer cells in each slide were counted. For analyses of BrdU and apoptosis, only well-defined and darkly stained cells were counted using a microscope. The percentage of labeled cells (labeling index) was determined by calculating the labeled cell number: total cell number × 100. Two scientists counted the positive cells and total cell number in the slides in blind manners.

Statistical analysis
Human SphK1 expression measurements at different stages of head and neck tumor development were analyzed by 1-way ANOVA and Bonferroni’s multiple comparison tests among groups. In the 4-NQO–induced tongue carcinogenesis model, tumor incidence, expressed as the percentage of tumor-bearing animals, was analyzed with the χ² probability test, whereas tumor multiplicity, expressed as the mean number of tumors per mouse, and tumor volume (mm³) were analyzed with the unpaired Student’s t-test between SphK1 KO and wild-type mice. Levels of sphingolipids in blood samples, and BrdU and apoptotic indices were analyzed with the unpaired Student’s t-test between SphK1 KO and wild-type mice. Differences were considered statistically significant at P < 0.05.

Results

SphK1 expression in human HNSCC
We first determined whether SphK1 is expressed in human HNSCC samples (n = 78) and normal head and neck tissues (n = 17) using immunohistochemistry carried out with human SphK1 antibody (5). The results are summarized in Figure 1A and representative figures of SphK1 immunohistochemistry are shown in Figure 1B and C. SphK1 immunohistochemistry reveals that SphK1 is strongly stained in cytoplasm, especially plasma membrane of HNSCC cells as well as in inflammatory cells including macrophages and fibroblasts (Fig. 1B and C). We found that normal mucosa shows very low or no expression of SphK1. Interestingly, SphK1 is slightly higher expressed in normal mucosa adjacent to SCC than normal mucosa, but this did not reach statistically significant differences. All HNSCC samples showed very high expression of SphK1 even in stage I (P < 0.001 vs. normal or normal adjacent HNSCC, Fig. 1A) and there were no differences of SphK1 expression levels among stages I to IV, suggesting that SphK1 expression may be mainly involved in early stages of malignant transformation from normal mucosa to HNSCC. These results are consistent with the previous report (8).

HNSCC induced by 4-NQO in mice
The next question was whether SphK1 plays a role in HNSCC development. In order to answer this question, we conducted 4-NQO–induced tongue carcinogenesis model using SphK1 KO and wild-type mice (Fig. 2A). First, the data showed that exposure to 4-NQO for 16 weeks was sufficient to induce tongue tumors in 26 out of 27 wild-type mice (96%), as expected (Table 1). Representative macroscopic photos of tongue tumors in wild type and SphK1 KO mice are shown in Figure 2B. There were 2 or 3 white and elastic hard tumors found in tongues taken from wild-type mice. SphK1 KO mice showed smaller tumors than wild-type mice or no tumors. Figure 2C shows H&E staining and SphK1 immunohistochemistry on HNSCC induced by 4-NQO in wild-type mice. Histological examination using H&E staining and SphK1 immunohistochemistry on HNSCC indicated that SphK1 KO were diagnosed as dysplastic lesion (a) and SCC (b). Both dysplastic lesion (a) and well-differentiated SCC (b) in tongue showed high expression of SphK1, indicating that SphK1 is overexpressed in preneoplastic lesions and HNSCC. These results suggest that SphK1 may play a role in 4-NQO–induced HNSCC development.

SphK1 deficiency inhibits 4-NQO–induced HNSCC
Then, we determined whether SphK1 is necessary for 4-NQO–induced HNSCC development using SphK1+/− KO
compared with wild-type mice. To answer this important question, SphK1 KO and wild-type mice were treated with 4-NQO, and tongue cancer development and progression in these mice were examined. As summarized in Table 1, although treatment with 4-NQO–induced tongue SCC development in 96% (26/27) wild-type mice, only 72% of SphK1 KO mice (18 of 25) developed tongue SCC in response to 4-NQO, indicating that HNSCC was significantly reduced in SphK1 KO when compared with wild-type mice ($P < 0.05$). In addition, SphK1 KO mice developed only 1.2 tumors per mouse whereas wild-type mice developed 2.1 tumors per mouse ($P < 0.01$). Importantly, SphK1 KO mice developed significantly smaller tongue tumors than wild-type mice (Fig. 2B, 4.85 mm$^3$ vs. 27.02 mm$^3$, respectively, $P < 0.05$). Taken together, these results suggest that genetic loss of SphK1 significantly prevents 4-NQO–induced tongue SCC with reducing tumor development including incidence and multiplicity as well as size when compared with wild-type mice.

**Evaluation of ceramide and S1P levels in 4-NQO-mediated HNSCC in SphK1 KO versus wild-type mice**

It has been reported that the sphingolipid profile is modified during HNSCC development in patients (6, 20). The previously published data suggest that although the levels of C$_{18}$-ceramide are generally lower, C$_{16}$-ceramide is significantly upregulated in the majority (about 80%) of tumor tissues of HNSCC patients as compared with their adjacent normal tissues. Therefore, we hypothesized that the sphingolipid profile should be altered in carcinogen-induced HNSCC. To test this hypothesis, we analyzed sphingolipid profiles in the blood samples obtained from wild type and SphK1 KO mice after 4-NQO exposure using LC-MS/MS. As expected, levels of S1P were significantly reduced (more than 50%) in SphK1 KO mice as compared with wild-type mice ($P < 0.001$, Fig. 3A). Interestingly, levels of sphingosine were also significantly decreased in SphK1 KO mice as compared with those in wild-type mice ($P < 0.001$, Fig. 3B). Levels of total ceramide in SphK1 KO were slightly lower than those in wild-type mice (Fig. 3C), however, levels of C$_{16}$-ceramide were significantly reduced in SphK1 KO mice as compared with those in wild-type mice ($P < 0.05$, Fig. 3D), which is consistent with the preventive role of loss of SphK1 in 4-NQO–induced tongue SCC. Levels of C$_{20:1}$-ceramide in SphK1 KO mice were significantly higher than those in wild-type mice ($P < 0.05$, data not shown), however, the other ceramide species levels in SphK1 KO mice showed not so much differences as compared with those in wild-type mice (data not shown). Levels of...
dihydro-S1P, dihydro-sphingosine, and dihydro-C16-ceramide in SphK1 KO mice were significantly lower than those in wild-type mice ($P < 0.001$, data not shown).

Examining the mechanisms by which SphK1 deficiency modulates 4-NQO–induced HNSCC development

To investigate the mechanism by which loss of SphK1 inhibits carcinogen-induced HNSCC development, we examined cell proliferation and apoptosis using the BrdU incorporation assay and cleaved caspase-3 detection by immunohistochemistry, respectively, in wild type compared with SphK1 KO mice after 4-NQO exposure. The results of a BrdU labeling index (Fig. 4A) in HNSCC showed that more than 20% of cancer cells (23.38%) entered S-phase in wild-type mice, whereas only 17% of cancer cells (17.66%) in SphK1 KO mice, indicating that SphK1 KO mice show slightly but significantly lower cell proliferation in HNSCC tumors than controls (wild-type mice) ($P < 0.05$).
Detection of cleaved caspase-3 levels, which indicated the presence of active caspase and apoptosis, by immunohistochemistry in HNSCC tumors in SphK1 KO versus wild-type mice revealed that in SphK1 KO mice, more than 50% of HNSCC tumor cells (52.17%) were positively stained for cleaved caspase-3, whereas, in wild-type mice, only 23.77% of HNSCC tumor cells were positively stained with cleaved caspase-3 (Fig. 4B). Therefore, these data indicate that the genetic loss of SphK1 results in significantly higher (about 60%) apoptotic cell death in HNSCC tumors than grown in wild-type control mice ($P < 0.01$).

Collectively, these findings suggest that although SphK1 deficiency slightly decreases HNSCC cell proliferation, it significantly induces apoptosis in HNSCC tumors when compared with controls, suggesting that SphK1 may play a role in carcinogen-induced HNSCC pathogenesis and/or development via controlling mainly caspase 3 activation and apoptosis, and to a lesser extend via regulation of tumor proliferation.

It has been reported that the phosphatidylinositol 3-kinase-Akt-mammalian target of rapamycin (mTOR) pathway was activated in 4-NQO–induced HNSCC and treatment with rapamycin, an mTOR inhibitor, significantly reduced the tumor burden in the 4-NQO–induced oral carcinogenesis (16). To examine whether the genetic loss of SphK1 is associated with the regulation of this pathway in 4-NQO–induced HNSCC, we first analyzed the expression of phospho-Akt in HNSCC tumors developed in wild-type mice versus SphK1 KO mice using immunohistochemistry (Fig. 5A and B, respectively). Interestingly, data showed that 4-NQO–induced HNSCC tumors in SphK1 KO mice expressed decreased levels of active (phospho)-Akt when compared with that of wild-type mice. These data indicate that prevention of 4-NQO–induced HNSCC development in response to the genetic loss of SphK1 might be associated with the inhibition of Akt-signaling, which is known to play significant roles in carcinogen-induced HNSCC.

### Discussion

In this study, we show that SphK1 protein is overexpressed in the majority of HNSCC tumor tissues obtained from patients at stages I–IV compared with normal head and neck tissues. These data suggest that overexpression of SphK1 may be an early event that can be detected during the early stages of HNSCC (stage I) whereas its expression levels were very low in normal head and neck tissues. More

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<th>Table 1. Results of SphK1 deficiency on 4-NQO–induced tongue carcinogenesis</th>
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<td>Multiplicity and volume values are mean ± SE.</td>
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<td>Significantly different from wild-type mice by $\chi^2$ test ($^P &lt; 0.05$).</td>
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<td>Significantly different from wild-type mice by Student t-test ($^{**P} &lt; 0.01$ and $^{***P} &lt; 0.05$).</td>
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Figure 3. Sphingolipid profile in blood samples from SphK1 KO and wild-type mice. The mice were treated with 4-NQO at 40 µg/mL in drinking water for 16 weeks and observed for another 8 weeks with normal drinking water. Blood samples were collected via heart puncture after complete euthanasia. Levels of sphingolipids were analyzed by sphingolipid profiling using a tandem mass spectrometry with positive mode electrospray ionization in the Lipidomics Core Facility, MUSC. Levels of S1P A, Sphingosine B, total ceramide C, and C16-ceramide D, in blood samples from SphK1 and wild-type mice. Levels of S1P ($^{*P} < 0.001$), Sphingosine ($^{**P} < 0.001$), and C16-ceramide ($^{*P} < 0.05$) are significantly decreased in blood from SphK1 KO mice as compared with those in wild-type mice.
importantly, the role of SphK1/S1P signaling in the development of HNSCC was established using the 4-NQO–induced HNSCC model in wild type and SphK1 KO mice. SphK1 is already overexpressed in dysplastic lesions, preneoplastic lesions of HNSCC. Specifically, the data showed for the first time that the genetic loss of SphK1 significantly prevented carcinogen-induced HNSCC tumor development with reduced tumor incidence, multiplicity, and volume when compared with control wild-type mice. Lipidomics analyses showed that S1P levels were significantly decreased in the blood of SphK1 KO compared with wild-type mice following 4-NQO treatment, consistent with reduced 4-NQO–induced HNSCC in SphK1 KO compared with wild-type mice. Mechanically, the data also indicated that reduced HNSCC development in SphK1 KO mice was associated with increased cleaved (active) caspase-3 levels, decreased cell proliferation and lower levels of active (phospho)-Akt expression when compared with wild-type mice.

It is becoming increasingly clear that SphK1 acts as an oncogene and overexpression of SphK1/S1P induces tumor growth and inhibits ceramide-mediated apoptosis, resulting in drug resistance in various solid tumors or leukemias (21–24). On the other hand, inhibition of S1P generation by knockdown of SphK1 or S1P antibody prevents tumor growth and/or proliferation, and results in apoptosis (5, 25, 26). Indeed, in this study, we found that SphK1 is already activated in the early stages, dysplastic lesions, in HNSCC carcinogenesis, indicating that SphK1 may play a role in the early stages of HNSCC development. Thus, SphK1 may be a good candidate of cancer prevention against HNSCC. Recent data also suggested that ceramides with different chain lengths might play distinct and sometimes opposing roles in the regulation of HNSCC tumor growth. For example, it was previously shown that although CerS1/C18-ceramide suppresses HNSCC tumor growth, increased C16-ceramide generation by CerS6 promotes HNSCC tumor proliferation (6). To this end, it was interesting that in addition to decreased S1P levels, we also detected decreased C16-ceramide levels in the HNSCC tumors developed in SphK1 KO compared with wild-type mice. These data are consistent with reduced tumor development in response to knockdown of CerS6/C16-ceramide in HNSCC xenografts grown in SCID mice (6), indicating that C16-ceramide might support HNSCC tumor growth as S1P, and their decreased generation might be associated with the prevention of 4-NQO–induced HNSCC development in SphK1 KO mice.

Accumulating evidence indicates that COX-2 is overexpressed in both oral premalignant lesions and HNSCC, and that this is correlated with decreased apoptosis, increased angiogenesis, and invasiveness (27). Our previous data in colon carcinogenesis suggest that SphK1 mediates COX-2 expression and PGE2 production (4, 5). Taken together, it is expected that SphK1 deficiency may inhibit 4-NQO–induced HNSCC through inhibition of the COX-2/PGE2 pathway. However, recent clinical trials have not been very successful in chemoprevention of oral premalignant lesions.
and HNSCC [Reviewed in (17)]. Ketorolac, a COX-1 and COX-2 inhibitor, failed to show any significant reduction in oral leukoplakia. Celecoxib, a specific COX-2 inhibitor, was ineffective in controlling oral premalignant lesions. Thus, we hypothesized that SphK1 may be involved in HNSCC development via different mechanisms from the COX-2/PGE2 pathway. Indeed, our data indicated that COX-2 protein was not highly expressed in 4-NQO-induced HNSCC tested using immunohistochemistry (data not shown), although several previous reports using male F344 rats indicated that 4-NQO–induced HNSCC tumors overexpressed COX-2 and COX-2 inhibitors showed inhibitory effects on this rat model (28, 29). Another study using mouse reported that increased COX-2 expression was detected in the early dysplastic lesions and SCC induced by 4-NQO (16). A very recent study indicates that SphK1 activation is required to activate the transcription factor nuclear factor κB (NF-κB) and to induce the secretion of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and the proinflammatory protein high-mobility group protein B1 (HMGB1) in LPS model (30). These proinflammatory cytokines and protein play a key role in inflammation and cancer. Thus, we hypothesized that the SphK1/S1P pathway may mediate inflammation-related cancer development. In fact, we showed that this pathway mediates colon carcinogenesis and colitis (5, 31). Further experiments to confirm this hypothesis in HNSCC development are also required.

Another line of evidence suggests that Akt-mTOR pathway is activated in 4-NQO-induced HNSCC development (16). It is shown that the activation of Akt-mTOR pathway is an early event during HNSCC development. Indeed, our data indicated that loss of SphK1 and prevention of HNSCC development in response to 4-NQO might be associated with decreased levels of active (phospho)–Akt. These findings suggest that one of the inhibitory mechanisms by which SphK1 deficiency prevents 4-NQO–induced HNSCC development may be involved in the inhibition of the Akt-mTOR pathway. Accumulating evidence suggests that mTOR pathway may regulate lipid biosynthesis through activation of the transcription factor sterol regulatory element-binding protein-1 (SREBP-1) [Reviewed in (32)]. A significant body of evidence suggests that the activation of SREBP-1 facilitates cancer progression by providing the lipids required for membrane synthesis [Reviewed in (33)]. Thus, inhibition of mTOR may reduce cancer cell growth/proliferation by blocking both protein and lipid biosynthesis. The potential link between mTOR/AKT and SphK1/S1P signaling in the development of HNSCC, however, is still not clear, and needs to be investigated further in future studies.

In conclusion, we show here that SphK1 is overexpressed in the majority of human HNSCC tumors obtained from patients with stages I–IV. Importantly, our novel data revealed that the genetic loss of SphK1, which significantly reduces S1P generation, prevents 4-NQO–induced HNSCC development. SphK1 is already activated in the early stages of HNSCC. Moreover, it appears that reduced carcinogen-induced HNSCC development in SphK1 KO mice might be associated with inhibition of cell proliferation, increased cleaved (active) caspase-3, and decreased levels of phospho (active)–Akt. Thus, taken together, these data suggest that the SphK1/S1P pathway may play a pivotal role in HNSCC development, and targeting SphK1/S1P may present a novel approach for HNSCC chemoprevention and/or treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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