Effects of the Integrin-Linked Kinase Inhibitor QLT0267 on Squamous Cell Carcinoma of the Head and Neck

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**Objective:** To study the expression of integrin-linked kinase (ILK) in human squamous cell carcinoma of the head and neck (SCCHN) tumor specimens and cell lines and the efficacy of the novel small molecule QLT0267.

**Design:** Immunohistochemical analysis of 17 SCCHN tumor tissue specimens and 3 normal tongue tissue specimens for ILK expression and in vitro analysis of the effectiveness of QLT0267 on SCCHN cells.

**Setting:** Academic medical center.

**Main Outcome Measures:** Expression levels of ILK in SCCHN tumor specimens and cell lines and the efficacy of QLT0267 in inhibiting cell growth and inducing apoptosis in SCCHN cell lines.

**Results:** Most SCCHN tumor specimens stained for ILK, whereas none of the 3 normal tongue tissue specimens stained for ILK. Integrin-linked kinase was expressed in all 6 SCCHN cell lines tested. In 4 pairs of normal and SCCHN tumor specimens, ILK expression and activity were higher in most tumor samples tested. A kinase assay showed that QLT0267 inhibited the ILK activity of 2 SCCHN cell lines (TU167 and MDA1986). Modified tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, DNA fragmentation ladder, and TUNEL (terminal deoxynucleotidyl transferase-mediated biotin–deoxyuridine triphosphate nick-end labeling) assays showed that QLT0267 inhibited cell growth and induced apoptosis in these 2 cell lines. A dose-dependent decrease in Akt phosphorylation was observed for these 2 cell lines on treatment with QLT0267.

**Conclusions:** Integrin-linked kinase is overexpressed in SCCHN tumor specimens. Targeting ILK with the small-molecule ILK inhibitor QLT0267 inhibits cell growth and induces apoptosis in SCCHN cell lines by reducing ILK activity and Akt phosphorylation. Integrin-linked kinase may be an attractive target for molecular therapy with which to enhance treatment of SCCHN.

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directly phosphorylates GSK-3β, thereby inhibiting its activity. It has recently been established that conditional knockout of ILK results in almost complete inhibition of phosphorylation of Akt on Ser473, significant inhibition of Akt activity, and suppression of phosphorylation of GSK-3β on Ser9 and cyclin D1 expression.

Our group previously showed that ILK is overexpressed in anaplastic thyroid carcinoma. To determine whether ILK plays a role in squamous cell carcinoma of the head and neck (SCCHN) pathogenesis and progression and whether it may be a useful target for cancer therapy, we investigated the expression of ILK in SCCHN human tumor specimens and cell lines and in an orthotopic nude mouse model for tongue cancer. We also tested the efficacy of a newly identified small-molecule inhibitor of ILK activity, QLT0267, against SCCHN cells. We hypothesized that ILK is overexpressed in SCCHN and that QLT0267 induces tumor growth inhibition and apoptosis.

**METHODS**

**ANTIBODIES**

Integrin-linked kinase antibodies were purchased from Upstate Biosciences (Beverly, Mass). Integrin-linked kinase antibody body 06-550 was used at a dilution of 1:5000 for Western blotting, whereas for immunoprecipitation, ILK antibody 06-559 was used. Antibody against phosphorylated Akt S473 was purchased from Cell Signaling Technology Inc (Beverly) and was used at a dilution of 1:2000. For secondary antibodies, we used goat anti–mouse antibody (BioRad Laboratories, Hercules, Calif) used at a dilution of 1:2000 and goat anti–rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, Calif) at a dilution of 1:3000.

**QLT0267**

The ILK inhibitor QLT0267 was obtained from QLT Inc (Vancouver, British Columbia). This reagent has been described previously. In brief, QLT0267 was developed through the optimization of a lead compound identified in the high-throughput screening of a rationally designed small-molecule library against the target ILK. QLT0267 inhibits the kinase activity of ILK in cell-free assay at 26 nmol/L and has an approximately 1000-fold selectivity over some kinases tested under similar conditions (including CK2, CSK, DNA-PK, PIM1, protein kinase B [or Akt kinase], and PKC) and approximately 100-fold selectivity over other kinases (including GSK-3β). For the present study, QLT0267 was prepared as a 10-mg/mL solution by adding PTE vehicle (66.6% polyethylene glycol 300/8.2% Tween 80/25% ethanol [9%/0.2%/citric acid [w/v] to the powder, sonicating the mixture for 10 minutes, and vortexing the mixture until the powder was fully dissolved. The dosing solutions were prepared in 1 batch and stored at −80°C, to be thawed on the day of dosing. For in vitro administration, QLT0267 was dissolved in dimethylsulfoxide to a 20 mM concentration.

**CELL LINES**

Human SCCHN cell lines TU686 (SCC from the base of tongue), MDA1986 (cervical nodal metastasis of tongue cancer), TU167, and TU1139 (SCC of the floor of the mouth) were obtained from Gary Clayman, MD, of The University of Texas M.D. Anderson Cancer Center. Cell lines HN5 (SCCHN) and FaDu (pharyngeal carcinoma) were obtained from Luka Milas, MD, of The University of Texas M.D. Anderson Cancer Center. The less aggressive cell line TU167 and the more aggressive cell line MDA1986 were later used for comparison. All the cells were maintained in Dulbecco modified eagle medium (DMEM) supplemented with 5% fetal bovine serum (FBS), sodium pyruvate, nonessential amino acids, L-glutamine, and a 2-fold vitamin solution (Life Technologies Inc, Grand Island, NY).

**IMMUNOHISTOCHEMICAL ANALYSIS**

Seventeen specimens of human SCCHN tumors with adjacent normal mucosal tissue and 3 specimens of normal human tongue tissue were obtained from the Department of Pathology at The University of Texas M.D. Anderson Cancer Center. All the specimens were obtained before surgery with the consent of the patients and in accordance with Health Insurance Portability and Accountability Act of 1996 guidelines and regulations and were fixed in formalin and embedded in paraffin. For immunohistochemical staining, 3-μm sections of the specimens were deparaffinized and hydrated as follows: xylene, 3 minutes; 100% ethanol, 2 minutes twice; 95% ethanol, 1 minute twice; 80% ethanol, 1 minute; and phosphate-buffered saline (PBS), 2 minutes twice. Antigen retrieval was performed using DakoTarget retrieval solution (Dako, Carpinteria, Calif). After incubation with 0.3% peroxide in methanol and incubation with normal blocking serum, the sections were incubated with primary anti–ILK antibody 06-550 at a dilution of 1:100 at 4°C overnight. Immunodetection was performed using peroxidase-labeled secondary antibody (Santa Cruz Biotechnology Inc) diluted in blocking solution for 1 hour at room temperature using diaminobenzidine as the chromogen. Positive controls for ILK immunostaining were formalin-fixed, paraffin-embedded, human prostate surgical specimens. Negative controls were sections stained with nonspecific rabbit IgG at the same protein concentration as the primary anti–ILK antibody. All the sections were counterstained with Gill hematoxylin (Sigma-Aldrich Corp, St Louis, Mo). Staining intensity was scored as 0 (none), 1+ (weak), 2+ (moderate), or 3+ (strong).

**ILK ASSAY FOR HUMAN TISSUE SAMPLES**

Normal and SCCHN tumor tissues were homogenized and lysed, 250 µg of the lysate from each sample was preclarified with 23 µL of protein A beads (Upstate Biotechnology, Lake Placid, NY) for 1 hour at 4°C, and then 2 µg of anti–ILK antibody 06-559 was added for immunoprecipitation overnight at 4°C. The next day, the beads were washed twice with lysis buffer and then added to the samples and allowed to rotate for 1 hour at 4°C. At the end of the hour, the samples were centrifuged, the supernatant was discarded, and the beads were washed twice with lysis buffer and once with kinase buffer. Then, 25 µL of the kinase reaction buffer, along with myelin basic protein (Upstate Biotechnology) and γ32P-labeled adenosine triphosphate, was added to each sample. The reaction ran for 30 minutes at 30°C and was terminated using 12 µL of 3× sodium dodecyl sulfate (SDS) buffer dye. Samples were vortexed, centrifuged for 2 minutes, and separated on a 12% SDS–polyacrylamide gel. The images were read using a phosphoimager for quantification.

**WESTERN IMMUNOBLOTTING**

Cells were grown in 10-cm culture flasks (Corning Inc, Corning, NY). Dulbecco modified eagle medium with 10% FBS was used. After the cells reached 70% to 80% confluency, the medium was discarded, and adherent cells were washed twice with ice-cold PBS. For recombinant epidermal growth factor Western
blot analysis, cells were serum starved overnight and treated with QLT0267 the following morning at different times. Later on, recombinant epidermal growth factor was added for 15 minutes. Cells were later washed with ice-cold PBS and then solubilized in lysis buffer (1× concentration: 20mM Tris [pH 7.5], 150mM sodium chloride, 1mM ethylenediaminetetraacetic acid [EDTA], 1mM ethyleneglycoltetraacetic acid [EGTA], 1% Triton, 2mM sodium pyrophosphate, 1mM α-glycerolphosphate, 1mM sodium orthovanadate, 1-µg/mL leupeptin, and 1mM phenylmethylsulphonylfluoride) for 15 minutes on ice. Lysates were clarified by centrifugation at 10 000 rpm for 15 minutes at 4°C, and protein concentrations were quantified using the BioRad assay kit. Equal amounts of protein (30-50 µg) were resolved by using 10% SDS–polyacrylamide gel electrophoresis and were transferred onto a nitrocellulose membrane (Millipore, Billerica, Mass). The membrane was blocked with 1% milk in Tris-buffered saline–Tween (TBS-T) for 30 to 45 minutes at room temperature. Afterwards, the primary antibody was added at the proper dilution (see the “Antibodies” subsection) for 1 hour at 4°C. After the membrane was washed 3 times with TBS-T for 10 minutes each, the corresponding secondary antibody was added at the proper dilution (see the “Antibodies” subsection) for 1 hour at room temperature. The membrane was washed 3 times with TBS-T, and protein detection was performed using horseradish peroxidase–conjugated anti–rabbit IgG (Santa Cruz Biotechnology) or anti–mouse IgG (Sigma-Aldrich Corp) and an enhanced chemiluminescence kit (Amersham Pharmacia, Buckinghamshire, England).

ILK ASSAY FOR SCCHN CELL LINES

A total of 250 µg of lysate was immunoprecipitated overnight with 1 µg of rabbit polyclonal anti–ILK antibody (Upstate Biotechnology). We used the nonradioactive Akt kinase kit from Cell Signaling Technology Inc. In brief, 30 µL of protein A beads was added to the immune complexes and was allowed to rotate for 2 to 3 hours at 4°C. Afterwards, the beads were microcentrifuged for 30 seconds at 4°C, and the pellet was washed twice with 500 µL of the same lysis buffer used for Western immunoblotting and twice with 500 µL of kinase buffer (1× concentration). 25mM Tris (pH 7.5), 5mM α-glycerolphosphate, 2mM dithiothreitol, 0.1mM sodium orthovanadate, and 10mM magnesium chloride. For the cold kinase assay, the pellet was suspended in 40 µL of 1× kinase buffer supplemented with 200µM adenosine triphosphate and 1 µg of GSK-3 fusion protein (Cell Signaling Technology Inc). After 30 minutes of incubation at 30°C, the reaction was terminated using 5 µL of 3X SDS sample buffer, and the suspension was vortexed and microcentrifuged for 2 minutes. The supernatant was transferred to a new tube, and the sample was boiled for 5 minutes and then loaded (15-30 µL) on 10% SDS–polyacrylamide gel. Phosphorylation of the substrate was detected by Western blot analysis with anti–GSK-3 Ser21/9 antibody at a dilution of 1:1000 (Cell Signaling Technology Inc).

ORTHOTOPIC IMPLANTATION OF TUMORS

To produce tumors, TU167 and MDA1986 cells were seeded on Laboratory-TeK II Chamber slides from Nalge Nunc Inc (Naperville, Ill) and were fed with DMEM media with 10% FBS. On day 2, the serum was replaced with DMEM with 2% FBS containing either dimethylsulfoxide alone or 5µM QLT0267. After 48 hours of treatment, cells were washed twice with PBS, fixed with paraformaldehyde (4%) in PBS, and processed for TUNEL (terminal deoxynucleotidyl transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling) staining by using a fluorometric TUNEL system kit (DeadEnd; Promega, Madison, Wis). In brief, after fixation with 4% paraformaldehyde, the slides were washed twice with PBS for 5 minutes and then incubated with 0.2% Triton X-100 for 15 minutes at room temperature. After two 5-minute washes with PBS, the samples were incubated with equilibration buffer for 10 minutes at room temperature. The equilibration buffer was drained, and the reaction buffer containing 44 µL of equilibration buffer, 5 µL of nucleotide mix, and 1 µL of terminal deoxynucleotidyl transferase (Promega kit) was added to the tissue sections and incubated in a humid atmosphere at 37°C for 1 hour, avoiding exposure to light. The reaction was terminated by immersing the samples in 2X standard saline citrate for 15 minutes. Samples were then washed 3 times for 5 minutes to remove unincorporated fluorescein–2′-deoxyuridine 5′-triphosphate. For total TUNEL expression, the cells were counted in 10 random fields using a light microscope.

GROWTH INHIBITION ASSAY

A growth inhibition assay was performed to assess the effect of the inhibition of ILK by QLT0267 on tumor cell growth. Cells were plated in 0.2 mL of medium (DMEM with 10% FBS) in 96-well Corning plates with escalating doses of QLT0267 (up to 11µM) for 72 hours. At the end of the incubation, growth inhibition was measured using the modified tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay (Sigma-Aldrich Corp). A total of 25 µL of modified tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide solution (3 mg/mL in PBS) was added to each well. After 2 hours of incubation at 37°C, 0.1 µL of dimethylsulfoxide was added for 5 minutes. Optical density at 570 nm was measured using a 96-well multispecimen autoreader (MR 5000 fluorescence; Dynatech, Burlington, Mass) with DMEM as a blank.

DNA FRAGMENTATION ASSAY

To determine the effect of QLT0267 treatment on apoptosis in SCCHN cells, a DNA fragmentation assay using low-molecular-weight DNA was prepared as described previously.14 Briefly, TU167 and MDA1986 cells (3×10^6 per plate) were seeded onto 100-mm plates and treated with 5µM QLT0267 for 12, 24, or 48 hours. Both floating and attached cells were scraped and collected in medium, washed 3 times with PBS, and resuspended in 1 mL of lysis buffer (20mM Tris [pH8], 10mM EDTA [pH8], and 0.5% Triton X-100). After incubation on ice for 30 minutes, the lysates were spun at 12 000 rpm in a microcentrifuge for 10 minutes. Low-molecular-weight DNA in the supernatant was extracted with equal volumes of phenol and chloroform for 1 hour at 4°C. Ammonium acetate (2M) was added to the aqueous phase, and the DNA was precipitated with 2 volumes of ethanol at −20°C overnight. The DNA was treated with 1-mg/mL RNase A at 37°C for 1 hour, and total DNA was analyzed using 1.5% agarose gel and was visualized using ethidium bromide staining.

TUNEL ASSAY

TU167 and MDA1986 cells were seeded on Laboratory-TeK II Chamber slides from Nalge Nunc Inc (Naperville, Ill) and were fed with DMEM media with 10% FBS. On day 2, the serum was replaced with DMEM with 2% FBS containing either dimethylsulfoxide alone or 5µM QLT0267. After 48 hours of treatment, cells were washed twice with PBS, fixed with paraformaldehyde (4%) in PBS, and processed for TUNEL (terminal deoxynucleotidyl transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling) staining by using a fluorometric TUNEL system kit (DeadEnd; Promega, Madison, Wis). In brief, after fixation with 4% paraformaldehyde, the slides were washed twice with PBS for 5 minutes and then incubated with 0.2% Triton X-100 for 15 minutes at room temperature. After two 5-minute washes with PBS, the samples were incubated with equilibration buffer for 10 minutes at room temperature. The equilibration buffer was drained, and the reaction buffer containing 44 µL of equilibration buffer, 5 µL of nucleotide mix, and 1 µL of terminal deoxynucleotidyl transferase (Promega kit) was added to the tissue sections and incubated in a humid atmosphere at 37°C for 1 hour, avoiding exposure to light. The reaction was terminated by immersing the samples in 2X standard saline citrate for 15 minutes. Samples were then washed 3 times for 5 minutes to remove unincorporated fluorescein–2′-deoxyuridine 5′-triphosphate. For total TUNEL expression, the cells were counted in 10 random fields using a light microscope.
0.159-mm² fields at ×100 magnification. Quantification of apoptotic endothelial cells was expressed as the mean of the ratio of apoptotic endothelial cells to the total number of endothelial cells in 10 random 0.011-mm² fields at ×400 magnification.

STATISTICAL ANALYSIS

All statistical computations were performed on a 600-mHz personal computer (Dell Inc, Austin, Tex) using a statistical software program (SAS; SAS Institute Inc, Cary, NC). The Fisher exact test was used to assess the difference in ILK staining intensity scores between normal tissue and SCCHN tumor tissue. *P* < .05 was set to indicate statistical significance.

RESULTS

ILK PROTEIN EXPRESSION IN HUMAN SCCHN VS NORMAL TISSUE SAMPLES

Using immunohistochemical techniques, we assessed the level of ILK expression in 17 human SCCHN tumor specimens with adjacent normal mucosal tissue and 3 specimens of normal human tongue. Fifteen of the 17 tumor specimens stained for ILK, whereas none of the normal tongue specimens did (Figure 1). Six samples (35.3%) stained 1+, 7 (41.2%) stained 2+, and 2 (11.8%) stained 3+ for ILK. Only 11.8% of the SCCHN tumors had nega-
tive (zero score) ILK staining. The difference in ILK staining intensity scores between SCCHN tumor tissue and normal tissue was significant \((P = .009)\).

**ILK PROTEIN EXPRESSION AND KINASE ACTIVITY IN HUMAN SCCHN TISSUE SAMPLES**

To confirm the results of the immunohistochemical evaluation for ILK expression, we evaluated the level of ILK expression and kinase activity in 4 pairs of normal tissue and SCCHN tumor tissue by subjecting them to Western blotting. In 3 of the pairs, ILK expression was higher in the cancerous tissue than in normal tissue (Figure 2A). In contrast, in all 4 pairs, ILK activity was higher in tumor tissue than in normal tissue (Figure 2B).

**ILK PROTEIN EXPRESSION IN HUMAN SCCHN CELL LINES AND EFFECTS OF QLT0267 ON ILK ACTIVITY**

To determine the expression level of ILK in SCCHN cell lines in vitro, Western blotting was performed on lysates of 6 SCCHN cell lines; the protein was expressed in all 6 lines (Figure 3A). The less aggressive TU167 cell line had the highest ILK expression level. The TU167, TU159, HN5, and FaDu cell lines had higher ILK expression levels than did the Tu686 and MDA1986 cell lines. To study whether the relative levels of ILK expression were similar in vivo, we assessed ILK expression immunohistochemically using an athymic nude mouse model for oral tongue cancer. The TU167 tumor xenografts maintained a higher expression of ILK than did MDA1986 xenografts (Figure 3B, bottom row).

On the basis of these in vivo and in vitro results, we used the high-ILK–expressing cell line TU167 and the low-ILK–expressing cell line MDA1986 to assess the efficacy of the novel ILK inhibitor QLT0267. In an in vitro kinase assay, the TU167 cell line had higher baseline ILK activity than did the MDA1986 cell line (Figure 3C). Furthermore, 1µM QLT0267 reduced the in vitro ILK activity in both cell lines.

**QLT0267 REDUCES Akt PHOSPHORYLATION IN SCCHN CELLS IN VITRO**

One of the major downstream targets for ILK activity in cells is Akt protein. We determined whether inhibiting ILK...
activity by QLT0267 leads to inhibition of Akt phosphorylation. Western blotting revealed that QLT0267 inhibited the epidermal growth factor–induced phosphorylation of Akt at the Ser473 residue in the TU167 and MDA1986 cell lines in a dose-dependent manner (Figure 4). A marked reduction in Akt phosphorylation was evident within 2 hours of treatment with 2µM QLT0267 in both cell lines. Phosphorylation of Akt was completely inhibited with 4µM QLT0267 in the TU167 cell lines, whereas residual levels of phosphorylated Akt were still evident at that dose in the MDA1986 cell line.

QLT0267 INHIBITS THE GROWTH OF SCCHN CELLS IN VITRO

To test the effect of the inhibition of ILK by QLT0267 on tumor cell proliferation, a modified tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bro-

Figure 3. Integrin-linked kinase (ILK) expression in vitro and in vivo and the effect of QLT0267 on ILK activity in squamous cell carcinoma of the head and neck (SCCHN) cells in vitro. A, Western blot analysis of ILK expression in a panel of SCCHN cell lines in vitro. β-Actin was used as an internal loading control. B, In vivo immunohistochemical analysis of TU167 and MDA1986 SCCHN cell lines injected into the tongues of athymic nude mice (1 million cells per mouse) and stained for ILK. Normal mouse tongue was used to detect baseline levels of ILK in normal tongue tissue (upper row: hematoxylin-eosin staining; lower row: ILK staining; original magnification ×100). C, An in vitro kinase assay was used to determine GSK phosphorylation levels in TU167 and MDA1986 cells treated with or without 1µM QLT0267. The images were quantified using a phosphoimager.

Figure 4. Effect of integrin-linked kinase inhibitor QLT0267 on Akt phosphorylation in squamous cell carcinoma of the head and neck cells in vitro. Western blotting for phosphorylated Akt (p-Akt) Ser473 in TU167 (A) and MDA1986 (B) cells treated with up to 4µM QLT0267 for 2 hours. Phosphorylation of Akt was induced with epidermal growth factor (EGF). The blots shown are representative of 3 independent experiments. β-Actin was used as an internal loading control.
mide growth inhibitory assay was performed. After 72 hours of treatment with escalating doses of QLT0267, TU167, MDA1986, TU159, TU686, FaDu, and HN5 cells showed reduced cell growth that was dose dependent (Figure 5). The inhibitory concentration at 50% for TU167 and MDA1986 cells was 2µM, for MDA1986 was 5µM, for TU159 and HN5 was greater than 8µM, and for FaDu was 3µM. Values are given as means. Error bars represent SDs.

Figure 5. Effect of integrin-linked kinase inhibitor QLT0267 on squamous cell carcinoma of the head and neck (SCCHN) cellular growth. Six different SCCHN cells were incubated in triplicate with medium (Dulbecco modified eagle medium plus 2% fetal bovine serum) or with up to 8µM QLT0267 for 72 hours and then assayed for cell growth inhibition using the modified tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Cell growth at 0µM QLT0267 served as the control and was set at 1.0 for both cell lines. The inhibitory concentration at 50% for TU167 and TU686 was 2µM, for MDA1986 was 5µM, for TU159 and HN5 was greater than 8µM, and for FaDu was 3µM. Values are given as means. Error bars represent SDs.

Finally, we assessed the effect of QLT0267 treatment on apoptosis in SCCHN cells. As demonstrated by DNA fragmentation and TUNEL assay, 5µM QLT0267 induced a time-dependent induction of apoptosis in the TU167 and MDA1986 cell lines. After 48 hours of treatment, QLT0267 induced a higher level of apoptosis in the TU167 cell line than in the MDA1986 cell line (Figure 6).

COMMENT

In this study, we found that ILK protein is expressed in human SCCHN cell lines and overexpressed in the SCCHN tumor specimens in vitro. Activity of ILK is increased in SCCHN specimens and 2 of the tested cell lines. The inhibition of ILK using the novel ILK inhibitor QLT0267 effectively reduces cell growth and induces apoptosis in the SCCHN cell lines TU167 and MDA1986 in vitro. This inhibitor also reduces the in vitro kinase activity of ILK and the phosphorylation of its substrate, Akt, at Ser473 in these 2 cell lines.

Integrin-linked kinase has previously been shown to be overexpressed in human colon,3 prostate,2 and stomach13...
cancer. We found that ILK is also highly expressed in human SCCHN tumor specimens and in a panel of human SCCHN cell lines, and we previously reported that other head and neck tumors, such as thyroid carcinoma, overexpress ILK in human tissue samples and cell lines. Integrin-linked kinase may also be overexpressed in other types of human cancer, and targeting ILK may be an effective means by which to treat various cancer types.

In contrast to a study that linked increased ILK expression with advanced tumor, we found that ILK was strongly expressed in the less aggressive SCCHN cell line TU167 and that the more aggressive SCCHN cell line MDA1986 had a lower level of ILK expression. This discrepancy can be due to chance (we used only 6 SCCHN cell lines). A larger sample of tumor specimens is needed to accurately correlate the level of ILK expression with tumor grade.

There is a small but growing body of literature on the use of small-molecule inhibitors in treating ILK-expressing tumors. Tan et al found that the ILK inhibitor KP-392 significantly reduced the tumor volume of prostate cancer xenografts. Similarly, our group previously reported that the ILK inhibitor QLT0267 reduced the tumor volume of anaplastic thyroid cancer xenografts. Those observations agree with the present finding of a large reduction in cell growth and an induction of apoptosis in SCCHN cell lines treated with QLT0267. In addition, several groups of researchers have investigated the effect of reducing ILK expression by using antisense and small interfering RNA constructs on tumor cell growth and survival.

Integrin-linked kinase has been found to play a key role in phosphorylating and thus activating Akt, which is a major protein involved in cell survival. In the present study, we found that inhibiting ILK by using QLT0267 substantially reduced Akt phosphorylation in the SCCHN cell lines TU167 and MDA1986. Phosphorylated Akt expression in oral tongue tumors was recently correlated with poor patient prognosis. This may explain the reduced cell growth and the induction of apoptosis in response to QLT0267 treatment in this study.

In summary, ILK is expressed in SCCHN tumors and cell lines, and this protein may play an important role in promoting tumor progression by regulating tumor cell growth and survival. Use of the novel ILK

Figure 6. Effect of integrin-linked kinase inhibitor QLT0267 on squamous cell carcinoma of the head and neck cell apoptosis in vitro. A, TU167 and MDA1986 cells were treated with 5µM QLT0267 for 12, 24, or 48 hours. DNA fragmentation was visualized by ethidium bromide staining of agarose gel. B, TU167 and MDA1986 cells were treated with 5µM QLT0267 for 48 hours and then processed for TUNEL (terminal deoxynucleotidyl transferase–mediated biotin–deoxyuridine triphosphate nick-end labeling) staining using the apoptosis detection kit. HPF indicates high-power field. Error bars represent SDs. C, Apoptotic cells were stained in green using TUNEL staining. More apoptotic (green) cells are shown after treatment with QLT0267 in both TU167 and MDA1986 cells.
inhibitor QLT0267 on the tested cell lines was associated with cell growth inhibition and induction of apoptosis. Thus, use of an ILK inhibitor, when added to conventional cancer therapy, may increase the chemosensitization and radiosensitization of tumors in patients with SCCHN.

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Author Contributions: Drs Younes, Yigitbasi, Yazici, Bucana, El-Naggar, Mills, and Myers and Ms Jasser had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Younes, Jasser, Mills, and Myers. Acquisition of data: Younes, Yigitbasi, Yazici, and Myers. Analysis and interpretation of data: Younes, Yigitbasi, Bucana, and El-Naggar. Drafting of the manuscript: Younes. Critical revision of the manuscript for important intellectual content: Younes, Yazici, Jasser, El-Naggar, Mills, and Myers. Obtained funding: Bucana and Myers. Administrative, technical, and material support: Younes, Jasser, and El-Naggar. Study supervision: Younes, Yigitbasi, Yazici, and El-Naggar.

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REFERENCES