Supplementary Material

Chemicals and reagents

All chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless indicated otherwise. The following antibodies were obtained from Cell Signaling Technologies (Danvers, MA, USA): p-S6 (S235/236), p-eukaryotic translation initiation factor 4E–binding protein 1 (p-4E-BP1,S65), p-Akt (S473), p-P70S6K(T389), PI3K p110, PI3K p85, p-mTOR (S2448), and survivin. Anti-mouse Ki-67 antibody was purchased from Dako (Carpentaria, CA, USA). Tgfbr1 antibodies (ab31013) were purchased from Abcam (Cambridge, MA, USA). Cyclin D1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Histology and Immunohistochemistry

To determine tumor multiplicity of tongue, tumor invasive depth and tumor size was counted using an Aperio CS scanscope digital image system (Vista, CA, USA). Antibody against p-Akt (S473,1:50), p-S6 (S235/236,1:200), Survivin (1:400), PI3K p110 (1:50), Ki-67 (1:400), and Cyclin D1 (1:500) were stained in sections of Tgfbr1/Pten 2cKO tongue SCC samples by immunohistochemistry using an appropriate biotin-conjugated secondary antibody and a Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA, USA), as previously reported in protocols (1, 2). Slices were scanned using an Aperio ScanScope CS scanner with background substrate for each slice, and quantified using Aperio Quantification software (Version 9.1) for membrane, nuclear, or pixel quantification. An area of interest was selected either in the epithelial or the cancerous area for scanning and quantification. Histoscore of membrane and nuclear staining was calculated as a percentage of different positive cells using the formula (3+)×3+(2+)×2+(1+)×1. Histoscore of pixel quantification was calculated as total intensity/total cell number. The threshold for scanning of different positive cells was set according to the standard controls provided by Aperio.

Western blot analysis

Cultured cells were lysed in T-PER (Pierce, Rockford, IL) containing a complete mini protease inhibitor cocktail and phosphate inhibitors (Roche, Branchburg, NJ). Tongue mucosa harvested from two individual *Tgfbr1*$^{flox/flox}$/*Pten*$^{flox/flox}$ mice and two *Tgfbr1/Pten* 2cKO mice, and five tumors harvested from *Tgfbr1/Pten* 2cKO mice, were used for Western blot analysis. Detailed procedures for immunoblotting were described as described previously (1, 2) .

Supplementary Table 1. SiRNA using in this study

Gene Symbol	Species	Resource	Catalog
AllStars Negative Control	Human	Qiagen	1027280
TGFBR1-5*	Human	Qiagen	SI00301903
TGFBR1-6	Human	Qiagen	SI02223627
PTEN-3	Human	Qiagen	SI00006909
PTEN-6*	Human	Qiagen	SI00301504
MAPK1	Human	Qiagen	SI00300755
AllStars Hs Cell Death	Human	Qiagen	1027298

*SiRNA used in the following experiment

Supplementary Reference

1. Bian Y, Terse A, Du J, et al. Progressive tumor formation in mice with conditional deletion of TGF-beta signaling in head and neck epithelia is associated with activation of the PI3K/Akt pathway. Cancer Res 2009; 69: 5918-26.

2. Bian Y, Hall B, Sun ZJ, et al. Loss of TGF-beta signaling and PTEN promotes head and neck squamous cell carcinoma through cellular senescence evasion and cancer-related inflammation. Oncogene 2011. doi: 10.1038/onc.2011.494.