

The Expression and Biological Function of DKK1 in Oral Squamous Cell Carcinomas by Bioinformatics Analysis

Huijie Yu

The People's Hospital of Shandong

Tianhua Li

The People's Hospital of Dongying

Xuemei Mao (✉ maoxuemei123@yandex.com)

The People's Hospital of Dongying

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**The expression and biological function of DKK1 in oral squamous cell
carcinomas by bioinformatics analysis**

Huijie Yu*, Tianhua Li*, Xuemei Mao

Department of stomatology, The People's Hospital of Dongying, Shandong, China

Corresponding authors:

Xuemei Mao, Department of stomatology, The People's Hospital of Dongying,
Shandong, China.

Email: maoxuemei123@yandex.com

* Huijie Yu and Tianhua Li contribute equally to this work

ABSTRACT

Background To investigate the expression of transcription factor Dickkopf-1 (DKK1) in oral squamous cell carcinomas (OSCC) by bioinformatics analysis and to clarify the connection between expression of DKK1 and clinicopathological features of OSCC, so as to elucidate the early diagnosis and prognostic significance of OSCC by DKK1.

Methods This study used the GEPIA database in conjunction with the TCGA database to analyze the expression level of DKK1 in OSCC tissues and then verify it by QRT-PCR and Western-blot analysis *in vitro*. The LinkedOmics database was used to describe the correlation between DKK1 expression and clinical pathological parameters of OSCC and its impact on prognosis. DKK1 was knocked down by RNA interference approach in SCC-4 and SCC-25 OSCC cell lines. In addition, the proliferation ability was assessed by MTT assay.

Results DKK1 was highly expressed in OSCC and positively correlated with OSCC pathological grade and T stage. The results of TCGA showed that high DKK1 mRNA expression was associated with overall survival in OSCC. Besides, both DKK1 mRNA and protein expression was confirmed increased significantly in oral squamous cell carcinomas SCC-25 and SCC-4. Furthermore, MTT analysis investigated that knockdown of DKK1 caused reduced proliferation ability of OSCC cells.

Conclusions The TCGA database analysis found that DKK1 is highly expressed in OSCC and is associated with multiple pathological indicators of OSCC, which will provide important theoretical guidance for subsequent oral squamous cell carcinoma research.

KEY WORDS

DKK1; Oral squamous cell carcinomas (OSCC); bioinformatics analysis; biomarker

1. Background

Oral squamous cell carcinomas (OSCC) is a common oral malignant tumor, accounting for 90% of the incidence and ranking the 6th place among systemic tumors [1]. The etiology of OSCC is complex. At present, many scholars believe that the disrupted balance between oncogene activation and tumor suppressor gene suppression may be one of the important causes of OSCC [2, 3]. It has brought difficulties to the clinical treatment of OSCC because of the insidious onset, high degree of malignancy, rapid disease progression, high rate of relapse, and hardly to diagnose in the early stage[4, 5]. Therefore, the exploration of oncogenes closely related to OSCC is expected to provide a new direction for tumor gene-targeted therapy.

Dickkopf-1 (DKK1) is part of the DKK family of proteins that includes DKK2, DKK3 and DKK4. This family of secreted proteins share similar conserved cysteine domains and inhibits the Wnt/ β -catenin pathway [6, 7]. DKK1 is involved in cell apoptosis through the Wnt signaling pathway[8]. DKK1 dysregulation has been implicated in the pathogenesis of a variety of cancers. Many lines of evidence show that upregulation of DKK1 contributes to the development of cancer in prostate tissue and in non-small cell lung carcinoma [9-13]. On the other hand, in gastric and colorectal cancer, DKK1 has been shown to be under-expressed [14]. In chronic lymphocytic leukemia (CLL) it is expressed at normal levels, but unable to affect Wnt/ β -catenin pathway, while in multiple myeloma, DKK1's role is shown to change from tumor suppressor to a stress responsive gene involved in the JNK pathway [15, 16]. As all these studies have shown, the activity and expression levels of DKK1 varies in different cancers. But the role of DKK1 in OSCC remains unclear and needs to be further investigated.

In this study, the role of DKK1 in oral squamous cell carcinoma was analyzed through the database website and verified by Quantitative real-time PCR and Western-blot analysis, so as to provide a theoretical basis for determining whether its mechanism and possible use as a predictive marker for the prognosis of OSCC patients.

2. Methods

2.1 GEPIA database

GEPIA (Gene Expression Profiling Interactive Analysis) database is dynamic Analysis of Gene Expression data, developed by Beijing university online database (<http://gepia.cancer-pku.cn>) combined with TCGA GTEx and analyze Gene Expression in different tumors in the database. In this study, the expression of DKK1 and its correlation with pathological analysis were analyzed in OSCC tissues and normal tissues.

2.2 Linkedomics database

Linkedomics database is third-party online tools for analyzing TCGA database (<http://linkedomics.org/login.php>). In this study, the website was used to analyze the RNAseq data in TCGA to understand the relationship between the mRNA level of DKK1 and the clinicopathological characteristics of OSCC. Using this site to analyze data requires only 5 steps: (1) select the type of tumor to be analyzed, oral squamous cell carcinoma was selected in this study; (2) RNAseq data of oral squamous cell carcinoma were selected; (3) Input the name of the gene to be analyzed, and fill in DKK1 here;(4) Select the data content of joint analysis, and “Clinical data” is selected in this step;(5) Select statistical method and non-parametric test. After submitting, wait for the analysis result, and click the corresponding option to view.

2.3 String-DB database

String database (<https://string-db.org/>) is a database for analyzing the interaction between genes or proteins, including direct physical interaction between proteins and indirect functional correlation between proteins. In addition to containing experimental data, PubMed abstracts, and other database data, it also contains results predicted using bioinformatics methods. In this study, "DKK1" was input, "human" was selected for species, "Medium0.4" for confidence, and 20 for maximum number interaction.

2.4 Quantitative real-time PCR

Quantitative real-time PCR (QRT-PCR) was used to inoculate HOK cells from normal oral epithelial cells, SCC-25 cells from oral squamous cell lines and SCC-4 cells into 6-well plates at a density of 1.5×10^5 cells per well (grown in RPMI 1640 medium at 37 °C under 5% CO₂). After 24h, cell mRNA was extracted by Trizol (Invitrogen Carlsbad, USA) method according to the instructions. RNA was quantitated with a NanoDrop spectrophotometer (Thermo, USA). The mRNA samples were reverse transcribed into cDNA using a commercial RT-PCR Kit according to the manufacturer's instructions (Thermo scientific, USA). Relative PCR quantification was performed using a commercial RT-PCR Kit according to the manufacturer's instructions (TaKaRa, Japan). Expression data were normalized to the amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA using the $-\Delta\Delta CT$ method. Primer sequences of DKK1: forward: AACGCTATCAAGAACCTGC, reverse: GATGACCGGAGACAAACA, target fragment of 460 bp. Primer sequences of GAPDH: forward: GGGAGCCAAAAGGGTCATCATCTC, reverse: CCATGCCAGTGAGCTTCCCGTTC, target fragment of 353 bp. Primers synthesized by AUGCT (Beijing, China).

2.5 Western-blot analysis

Total proteins of the cells were dissolved in lysis buffer and extracted following the manufacturer's protocol (Beyotime Institute of Biotechnology, Haimen, China). Protein concentration was determined using the bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Wilmington, DE, USA). Equal amounts of protein were separated by SDS-PAGE using 10% horizontal gels. Proteins were transferred to a polyvinylidene difluoride membrane (EMD Millipore Corp., Billerica, MA) in a wet blotting system. Membranes were blocked with 5% nonfat dry milk in TBS containing 0.1% Tween for 1 h at room temperature and incubated with the appropriate primary antibodies overnight at 4°C. After being washed, membranes were incubated with a secondary horseradish peroxidase (HRP)-coupled antibody and processed for enhanced chemiluminescence detection using Immobilon HRP substrate (EMD Millipore Corp., Billerica, MA). Signals were visualized and analyzed on a VisionWorks LS (UVP,

BioSpectrum Imaging System, USA). The integrated density of bands was quantified using ImageJ Software (National Institute of Health, Bethesda, MD, USA). The ratio of the integrated density of the target protein to that of GAPDH as the loading control was calculated to represent the expression level of protein. Antibodies were used as follows: anti-DKK1-1 diluted 1:1,000, and anti-GAPDH diluted 1:1,000, all applied from Santa Cruz (Santa Cruz Biotechnology Inc., CA).

2.6 MTT assay

The effect of DKK1 silencing on the proliferation of SCC-4 and SCC-25 cells was assessed using an MTT assay. Cells were seeded into 96-well plates at 3,000 cells per 100 μ l culture media per well. Transfections were performed the following day. A total of 10 μ l MTT reagent (5 mg/ml) was added to every well at various time points following transfection (24, 48 and 72 h), and 150 μ l dimethyl sulfoxide was added 4 h later. Cell viability of samples was measured at 490 nm using a FLUOstar OPTIMA microplate reader (BMG). Following transfection with si-DKK1 or its control, the cells were further cultivated for an additional 1-3 days. Each experiment contained three replicates and was repeated at least twice.

2.7 Statistical Analysis

All the experiments were performed at least three times, and data were expressed as means \pm S.E.M. Unpaired two-tailed Student's t-test was used to determine statistical significance depending on the normality of the data. All the statistical analyses were carried out using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA) considering a *P* value <0.05 as significant.

3. Results

3.1 DKK1 mRNA was highly expressed in OSCC and normal oral epithelial tissues

GEPIA database was used to analyze the expression of DKK1 in different tumors, and the results showed that the expression level of DKK1 was increased in most tumors, such as esophageal cancer, squamous cell carcinoma, pancreatic adenocarcinoma, etc., including head and neck squamous cell carcinoma (Figure 1). The expression of DKK1 mRNA in OSCC (n=519) and normal head and neck epithelial tissue (n=44) was further analyzed. And the results showed that compared with normal head and neck epithelial tissue, DKK1 mRNA was highly expressed in OSCC, and the difference was statistically significant ($P < 0.05$) as shown in Figure 2. These results suggest that DKK1 may play an important role in the occurrence and development of OSCC.

3.2 Correlation between DKK1 mRNA expression and the clinicopathological characteristics of OSCC

Given that the mRNA expression of DKK1 exhibited high level in OSCC compared to the normal tissues, we investigated the correlation between DKK1 mRNA expression and the clinicopathological characteristics of OSCC using LinkedOmics. And it was found that in terms of pathological stage ($P = 0.004537$, n=448) and T stage ($P = 0.0006259$, n=458), the differences between the DKK1 high expression group and DKK1 low expression group were statistically significant, as shown in Figure 3. The results above suggest that the high expression of DKK1 is related to the malignant degree and disease progression of OSCC.

3.3 Relationship between DKK1 mRNA expression and prognosis of OSCC

Moreover, to determine the relationship between DKK1 mRNA expression and the prognosis of OSCC, we used GEPIA database to analyze the correlation between DKK1 mRNA level and the survival rate of OSCC patients. The results showed that the expression level of DKK1 mRNA was negatively correlated with the overall survival rate of OSCC patients. The overall survival rate of patients with high DKK1 expression was significantly lower than that of patients with low DKK1 expression ($P < 0.001$) as

shown in Figure 4. The results above suggest that the high expression of DKK1 strongly affect the survival of HNCS patients, and is related to the early postoperative death and early recurrence of OSCC patients.

3.4 QRT-PCR and Western-blot analysis of the DKK1 expression in OSCC

In order to further verify the high expression of DKK1 in OSCC, we screened SCC-25 and SCC-4 as two oral squamous cell lines, then used QRT-PCR and Western-blot analysis to determine the expression of DKK1 in the above cell lines. As shown in Figure 5A, DKK1 mRNA was highly expressed in SCC-25 and SCC-4 of oral squamous cell lines compared with HOK of normal oral epithelial cell lines, and the expression was statistically significant ($P < 0.05$).

To extend our observations at the genetic level, we characterized the expression level of DKK1 protein in different cell lines using western-blot. Consistent with the gene expression, western-blot analysis demonstrated that DKK1 protein was increased obviously in SCC-25 and SCC-4 cell lines compared to the HOK of normal oral epithelial cell lines (Figure 5B), and the difference was statistically significant ($P < 0.05$) (Figure 5C).

3.5 Suppressing DKK1 expression inhibited the growth of OSCC cells.

To examine the effects of DKK1 on the growth of OSCC cells, we designed the siRNA for DKK1 (si-DKK1), which induced 60% decrease of DKK1 expression both at the protein and RNA levels in OSCC cells. MTT assay showed that downregulation of DKK1 suppression of the SCC-4 and SCC-25 cells growth at 48 and 72 h. This finding suggested that DKK1 may promote the proliferation of OSCC cells.

4. Discussion

OSCC is the most common tumor in the world, and its incidence has been younger in the past 30 years, which has seriously endangered human survival and health [17, 18]. Although the current treatment plan for OSCC is constantly improving, unfortunately the 5-year survival rate of OSCC patients is still less than 50%. Therefore, it is urgent to understand the occurrence and development of OSCC and discover effective gene-targeted treatment methods [19].

The DKK1 is frequently overexpressed in prostate cancer, non-small cell lung carcinoma, breast cancer, gastric and colorectal cancer [20-23]. It is suggesting that aberrant DKK1 expression contribute to progression of malignancies[24]. However, few reports have evaluated DKK1 expression in OSCC [25]. Here, we studied expression and prognosis of DKK1 in OSCC samples by analyzing the TCGA database. Through GEPIA data analysis, we found that compared with normal oral epithelial tissues, the expression of DKK1 in OSCC was significantly higher, and it was positively correlated with clinical staging. Besides, the relationship between DKK1 and OSCC clinicopathological characteristics through the LinkedOmics database showed that DKK1 expression was related to pathological staging and T staging. At the same time, the effect of DKK1 expression level on the overall survival rate of OSCC through GEPIA found that patients with high DKK1 expression have a poorer prognosis trend. Therefore, combining the DKK1 expression status with the tumor stage is useful to predict the prognosis of OSCC.

Hence, to confirm that DKK1 served as an oncogene in OSCC, we conducted cell line verification. PCR and Western blot analysis in Figure 5 revealed that DKK1 was up-regulated in OSCC cell lines. Knockdown of DKK1 inhibited the proliferation of OSCC cells. These findings indicated that DKK1 promoted the proliferation of OSCC cells. However, the mechanism of DKK1 becomes an oncogene in OSCC remains unknown, but accumulating evidence reveals that up-regulation of DKK1 is related to the accumulation of β -catenin. For example, Jing et al. reported that DKK1 promotes migration and invasion of non-small cell lung cancer via β -catenin signaling pathway[26].

5. Conclusions

In conclusion, we demonstrate that DKK1 is overexpressed in OSCC. Moreover, knockdown of DKK1 suppresses the cell growth of OSCC. DKK1 may play a potential therapeutic strategy for predicting the prognosis of patients in early disease stage.

Abbreviations

DKK1: Dickkopf-1; OSCC: oral squamous cell carcinomas; CLL: chronic lymphocytic leukemia; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; BCA: bicinchoninic acid; HRP: horseradish peroxidase; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PVDF: Polyvinylidene difluoride; qRT-PCR: Quantitative real-time polymerase chain reaction

Declarations

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of College Shandong University (approval number No. 2019-134).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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This current work was not supported by any sources of findings.

Authors' contributions

Huijie Yu and Tianhua Li are responsible for experimental design, experiments, data analysis and interpretation, and writing of the manuscript. Xuemei Mao is the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and given final approval of this version of manuscript to be published.

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References

1. Petersen PE: **Oral cancer prevention and control--the approach of the World Health Organization.** *Oral Oncol* 2009, **45**(4-5):454-460.
2. Funk GF, Karnell LH, Robinson RA, Zhen WK, Trask DK, Hoffman HT: **Presentation, treatment, and outcome of oral cavity cancer: a National Cancer Data Base report.** *Head Neck* 2002, **24**(2):165-180.
3. Xu Z, Jiang P, He S: **Identification for Exploring Underlying Pathogenesis and Therapy Strategy of Oral Squamous Cell Carcinoma by Bioinformatics Analysis.** *Med Sci Monit* 2019, **25**:9216-9226.
4. Liu T, David M, Ellis O, Hubert Low TH, Palme CE, Clark J, Batstone M: **Treatment for oral squamous cell carcinoma: Impact of surgeon volume on survival.** *Oral Oncol* 2019, **96**:60-65.
5. Kondoh N, Mizuno-Kamiya M, Umemura N, Takayama E, Kawaki H, Mitsudo K, Muramatsu Y, Sumitomo S: **Immunomodulatory aspects in the progression and treatment of oral malignancy.** *Jpn Dent Sci Rev* 2019, **55**(1):113-120.
6. Krupnik VE, Sharp JD, Jiang C, Robison K, Chickering TW, Amaravadi L, Brown DE, Guyot D, Mays G, Leiby K *et al*: **Functional and structural diversity of the human Dickkopf gene family.** *Gene* 1999, **238**(2):301-313.
7. Mao B, Niehrs C: **Kremen2 modulates Dickkopf2 activity during Wnt/LRP6 signaling.** *Gene* 2003, **302**(1-2):179-183.
8. Zhang F, Cao K, Du G, Zhang Q, Yin Z: **miR-29a promotes osteoblast**

- proliferation by downregulating DKK-1 expression and activating Wnt/beta-catenin signaling pathway.** *Adv Clin Exp Med* 2019, **28**(10):1293-1300.
9. Aguilera O, Fraga MF, Ballestar E, Paz MF, Herranz M, Espada J, Garcia JM, Munoz A, Esteller M, Gonzalez-Sancho JM: **Epigenetic inactivation of the Wnt antagonist DICKKOPF-1 (DKK-1) gene in human colorectal cancer.** *Oncogene* 2006, **25**(29):4116-4121.
 10. Wang J, Shou J, Chen X: **Dickkopf-1, an inhibitor of the Wnt signaling pathway, is induced by p53.** *Oncogene* 2000, **19**(14):1843-1848.
 11. Liu JT, Guo WB, Sun JY: **Serum Dickkopf-1 acts as a new biomarker in human breast cancer.** *Minerva Med* 2017, **108**(4):334-340.
 12. Yi N, Liao QP, Li ZH, Xie BJ, Hu YH, Yi W, Liu M: **RNA interference-mediated targeting of DKK1 gene expression in Ishikawa endometrial carcinoma cells causes increased tumor cell invasion and migration.** *Oncol Lett* 2013, **6**(3):756-762.
 13. Mao B, Wu W, Li Y, Hoppe D, Stannek P, Glinka A, Niehrs C: **LDL-receptor-related protein 6 is a receptor for Dickkopf proteins.** *Nature* 2001, **411**(6835):321-325.
 14. Jia X, Li N, Peng C, Deng Y, Wang J, Deng M, Lu M, Yin J, Zheng G, Liu H *et al*: **miR-493 mediated DKK1 down-regulation confers proliferation, invasion and chemo-resistance in gastric cancer cells.** *Oncotarget* 2016, **7**(6):7044-7054.
 15. Colla S, Zhan F, Xiong W, Wu X, Xu H, Stephens O, Yaccoby S, Epstein J, Barlogie B, Shaughnessy JD, Jr.: **The oxidative stress response regulates DKK1 expression through the JNK signaling cascade in multiple myeloma plasma cells.** *Blood* 2007, **109**(10):4470-4477.
 16. Filipovich A, Gandhirajan RK, Gehrke I, Poll-Wolbeck SJ, Kreuzer KA: **Evidence for non-functional Dickkopf-1 (DKK-1) signaling in chronic lymphocytic leukemia (CLL).** *Eur J Haematol* 2010, **85**(4):309-313.
 17. Thomson PJ: **Perspectives on oral squamous cell carcinoma prevention-proliferation, position, progression and prediction.** *J Oral Pathol Med* 2018, **47**(9):803-807.
 18. Omura K: **Current status of oral cancer treatment strategies: surgical treatments for oral squamous cell carcinoma.** *Int J Clin Oncol* 2014, **19**(3):423-430.
 19. Taghavi N, Yazdi I: **Prognostic factors of survival rate in oral squamous cell carcinoma: clinical, histologic, genetic and molecular concepts.** *Arch Iran Med* 2015, **18**(5):314-319.
 20. Igbini E, Guo F, Jiang SW, Kelley C, Li J: **Dkk1 involvement and its potential as a biomarker in pancreatic ductal adenocarcinoma.** *Clin Chim Acta* 2019, **488**:226-234.
 21. Niu J, Li XM, Wang X, Liang C, Zhang YD, Li HY, Liu FY, Sun H, Xie SQ, Fang D: **DKK1 inhibits breast cancer cell migration and invasion through suppression of beta-catenin/MMP7 signaling pathway.** *Cancer Cell Int* 2019,

- 19:168.
22. Lu W, Ni Z, Tong M, Jiang S, Zhang J, Feng C, Han C, Yuan T, Wang N, Zhao J *et al*: **DKK1 is epigenetically downregulated by promoter methylation and inhibits bile acid-induced gastric intestinal metaplasia**. *Biochem Biophys Res Commun* 2020, **523**(3):780-786.
 23. Jumpertz S, Hennes T, Asare Y, Schutz AK, Bernhagen J: **CSN5/JAB1 suppresses the WNT inhibitor DKK1 in colorectal cancer cells**. *Cell Signal* 2017, **34**:38-46.
 24. Zhuang X, Zhang H, Li X, Li X, Cong M, Peng F, Yu J, Zhang X, Yang Q, Hu G: **Differential effects on lung and bone metastasis of breast cancer by Wnt signalling inhibitor DKK1**. *Nat Cell Biol* 2017, **19**(10):1274-1285.
 25. Wang Z, Wang J, Chen Z, Wang K, Shi L: **MicroRNA-1-3p inhibits the proliferation and migration of oral squamous cell carcinoma cells by targeting DKK1**. *Biochem Cell Biol* 2018, **96**(3):355-364.
 26. Zhang J, Zhang X, Zhao X, Jiang M, Gu M, Wang Z, Yue W: **DKK1 promotes migration and invasion of non-small cell lung cancer via beta-catenin signaling pathway**. *Tumour Biol* 2017, **39**(7):1010428317703820.

Figure Legends

Fig. 1 Expression of DKK1 in different kind of tumors

The expression levels of DKK1 in different tumors, and the type of tumor with red at the top indicates that DKK1 is highly expressed in this tumor compared with normal tissues.

Fig. 2 Expression of DKK1 mRNA in OSCC and normal oral epithelial tissues

Quantitative PCR analysis showed that DKK1 mRNA expression significantly increased in OSCC (n=519) as compared with in normal oral epithelial tissues (n=44) (* $P < 0.05$).

Fig. 3 Correlation between DKK1 mRNA expression and clinicopathological features of OSCC

Association of DKK1 mRNA level and pathological features in OSCC. (A) DKK1 mRNA level with pathologic stage ($P = 4.537e-04$, n=448) and (B) DKK1 mRNA level with T stage ($P = 6.259e-04$, n=458). Box plots were produced using LinkedOmics (<http://www.linkedomics.org/login.php>), and statistically tested using the Kruskal–Wallis test.

Fig. 4 Overall survival of OSCC patients related to different DKK1 status (mRNA

level) based on TCGA data

The expression of DKK1 was inversely correlated with overall survival of all 518 OSCC patients ($***P < 0.001$) as revealed by Kaplan-Meier analysis.

Fig. 5 Expression of DKK1 in SCC-25 and SCC-4 of oral squamous cell lines

(A) Quantitative PCR analysis of DKK1 genes expression in SCC-25 and SCC-4 of oral squamous cell lines and HOK of normal oral epithelial cell lines. The data show the fold changes of the expression for DKK1 in SCC-25 and SCC-4 of oral squamous cell lines compared to HOK of normal oral epithelial cell lines ($*P < 0.05$) (n=8-10 for each group). Values are mean \pm S.E.M. and expression of genes is corrected for the housekeeping gene GAPDH. (B) Western blot analysis for DKK1 in SCC-25 and SCC-4 of oral squamous cell lines compared with HOK of normal oral epithelial cell lines ($*P < 0.05$) (n=8-10 for each group) Western blots of the target proteins were cropped as indicated, and full blot images are presented in Supplemental Fig.1. (C) Quantification of Western-blot analysis. Protein content is expressed relative to the control and represents three independent experiments with triplicate observations in each experiment. Volume is the sum of all pixel intensities within a band. All data are normalized to GAPDH and are expressed as mean \pm S.E.M..

Fig. 6 Silencing of DKK1 reduces OSCC cells growth

(A) qRT-PCR and Western blot assays analysis of DKK1 expression by silencing of DKK1 using si-DKK1 in SCC-25 and SCC-4 cells. Western blots of the target proteins were cropped as indicated, and full blot images are presented in Supplemental Fig.2. (B) The proliferation capacity of OSCC cells was measured by MTT assay at 24, 48 and 72 h post-transfection with si-DKK1 or its control. The data corresponds to the results of 3 independent experiments. $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$.

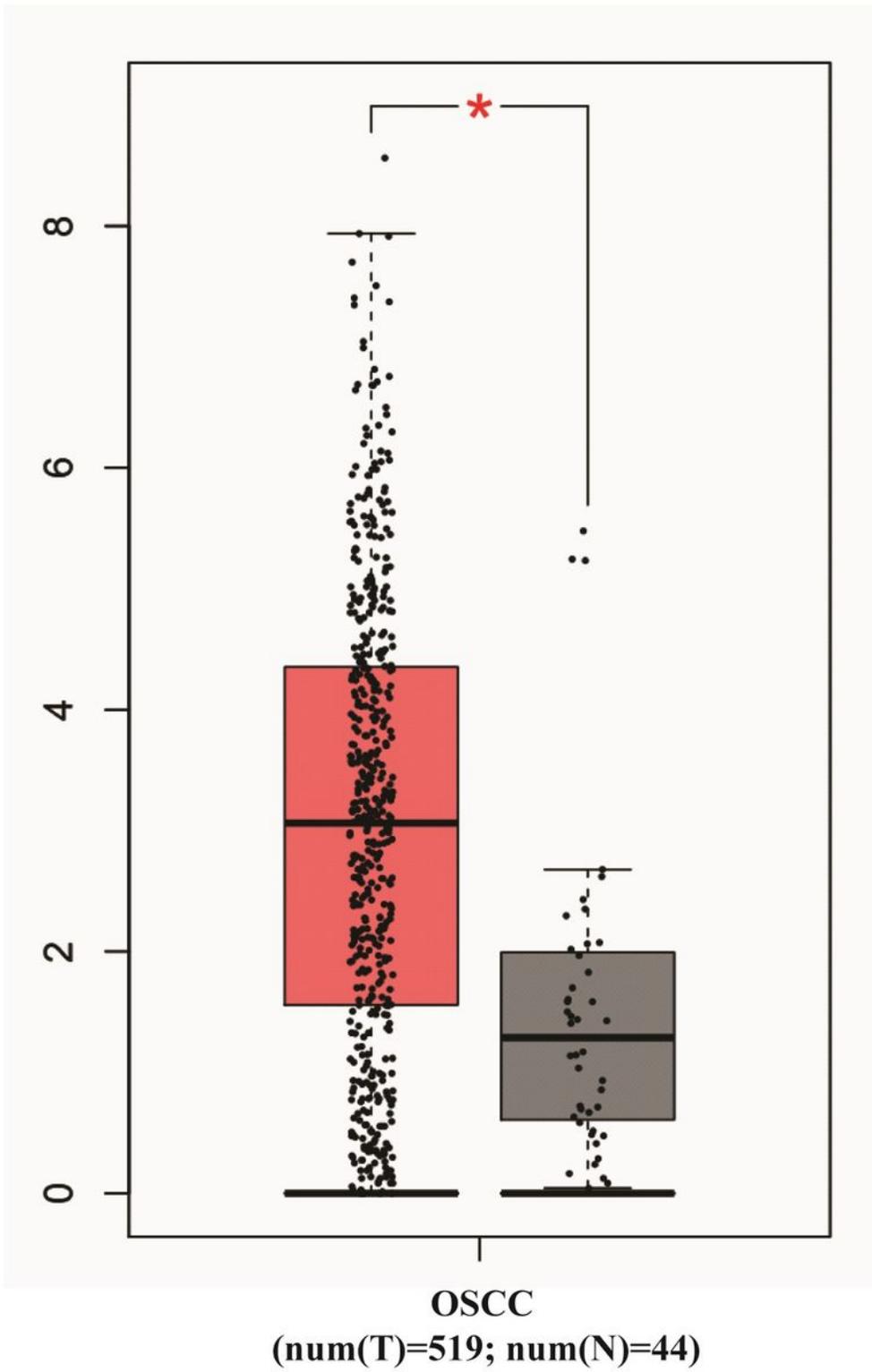


Figure 2

Expression of DKK1 mRNA in OSCC and normal oral epithelial tissues Quantitative PCR analysis showed that DKK1 mRNA expression significantly increased in OSCC (n=519) as compared with in normal oral epithelial tissues (n=44) (*P < 0.05).

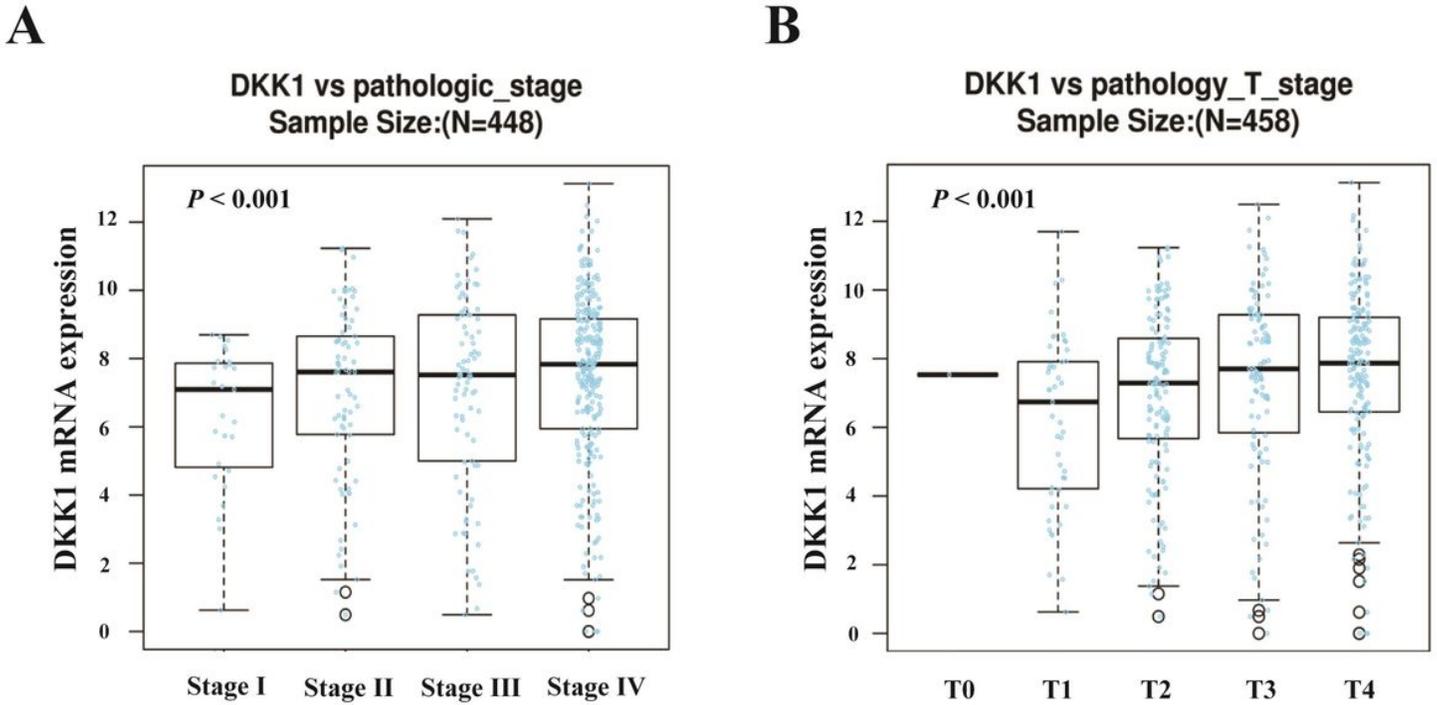


Figure 3

Correlation between DKK1 mRNA expression and clinicopathological features of OSCC Association of DKK1 mRNA level and pathological features in OSCC. (A) DKK1 mRNA level with pathologic stage ($P = 4.537e-04$, $n=448$) and (B) DKK1 mRNA level with T stage ($P = 6.259e-04$, $n=458$). Box plots were produced using LinkedOmics (<http://www.linkedomics.org/login.php>), and statistically tested using the Kruskal–Wallis test.

Overall Survival

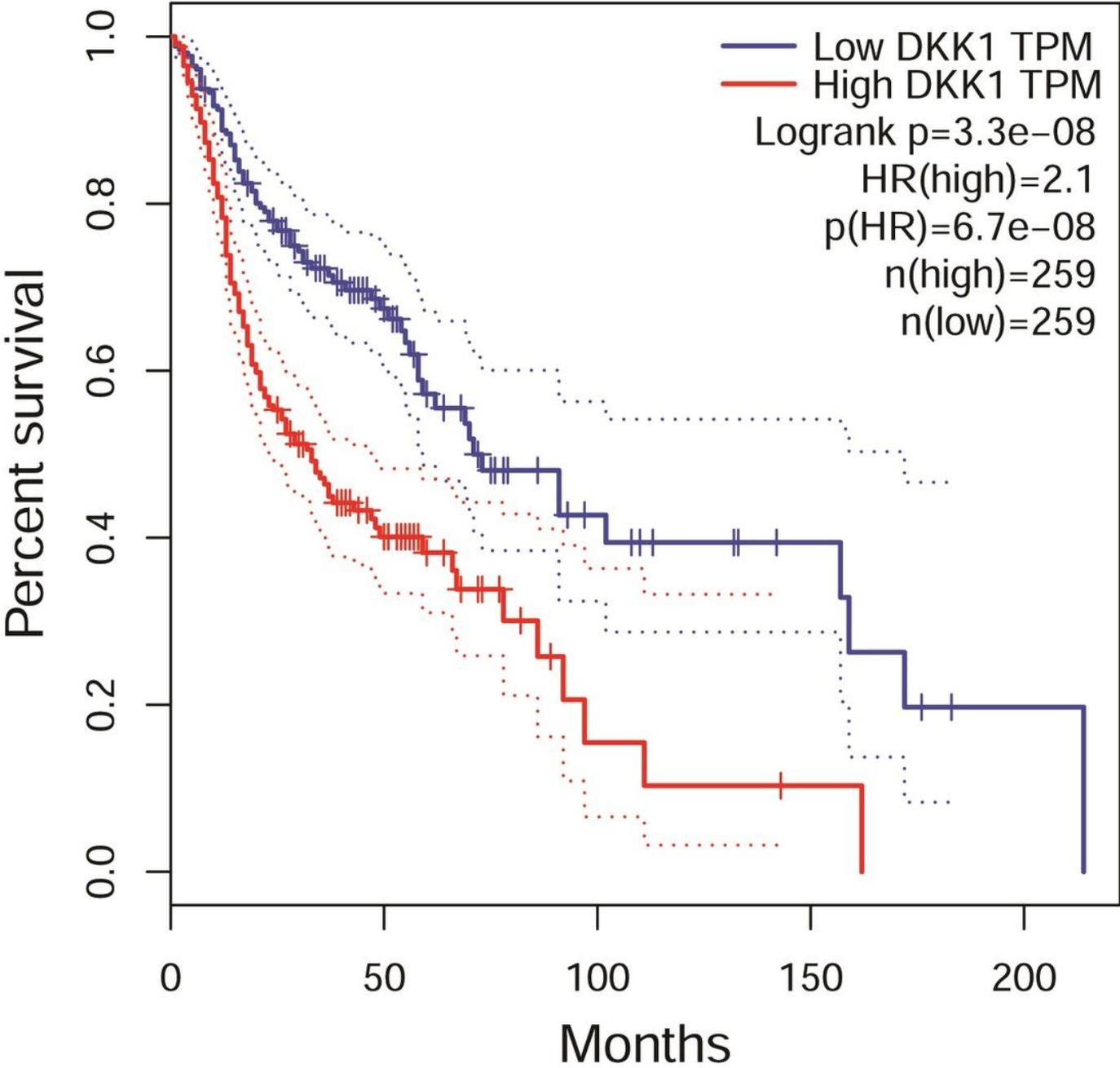


Figure 4

Overall survival of OSCC patients related to different DKK1 status (mRNA level) based on TCGA data. The expression of DKK1 was inversely correlated with overall survival of all 518 OSCC patients (**P < 0.001) as revealed by Kaplan-Meier analysis.

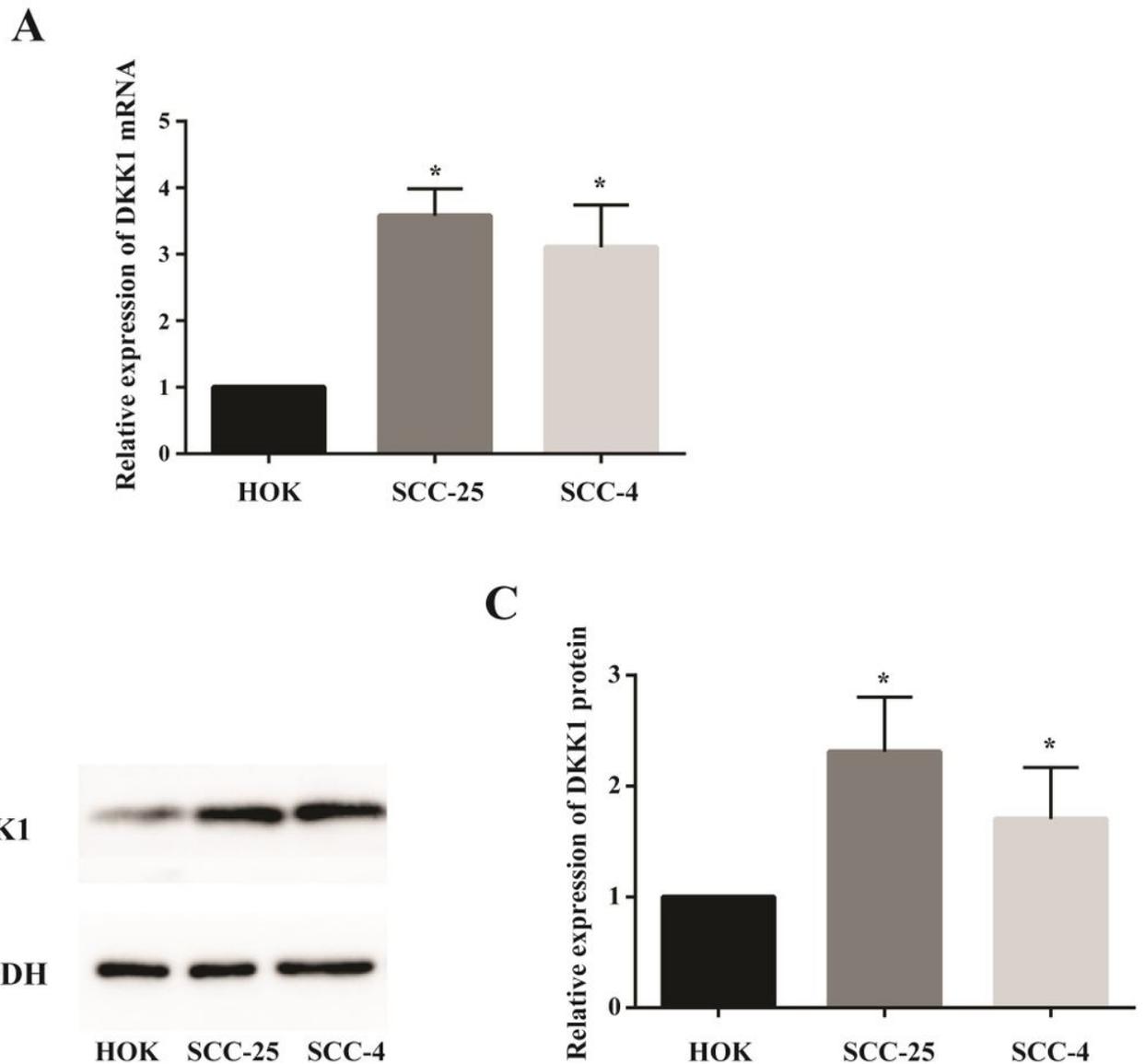
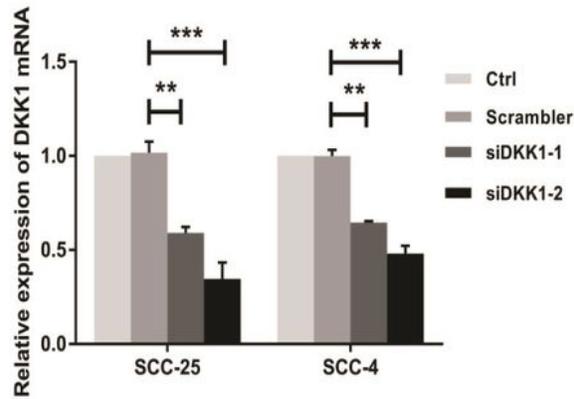
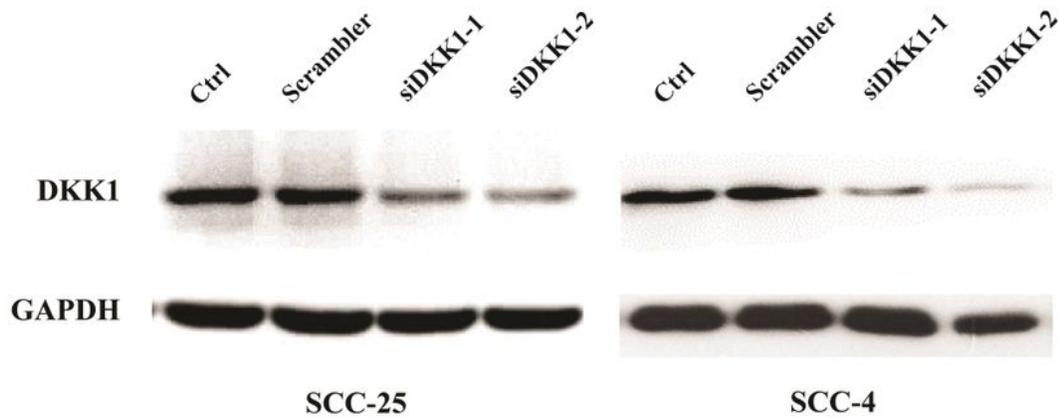
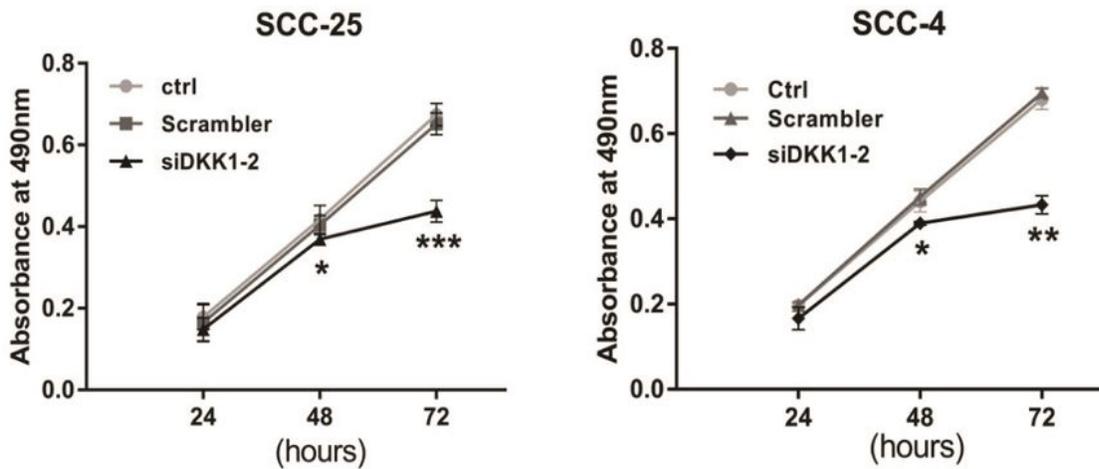


Figure 5

Expression of DKK1 in SCC-25 and SCC-4 of oral squamous cell lines (A) Quantitative PCR analysis of DKK1 genes expression in SCC-25 and SCC-4 of oral squamous cell lines and HOK of normal oral epithelial cell lines. The data show the fold changes of the expression for DKK1 in SCC-25 and SCC-4 of oral squamous cell lines compared to HOK of normal oral epithelial cell lines (* $P < 0.05$) ($n=8-10$ for each group). Values are mean \pm S.E.M. and expression of genes is corrected for the housekeeping gene GAPDH. (B) Western blot analysis for DKK1 in SCC-25 and SCC4 of oral squamous cell lines compared with HOK of normal oral epithelial cell lines (* $P < 0.05$) ($n=8-10$ for each group) Western blots of the target proteins were cropped as indicated, and full blot images are presented in Supplemental Fig.1. (C) Quantification of Western-blot analysis. Protein content is expressed relative to the control and represents three independent experiments with triplicate observations in each experiment. Volume is the sum of all pixel intensities within a band. All data are normalized to GAPDH and are expressed as mean \pm S.E.M..

A**B****C****Figure 6**

Silencing of DKK1 reduces OSCC cells growth (A) qRT-PCR and Western blot assays analysis of DKK1 expression by silencing of DKK1 using si-DKK1 in SCC-25 and SCC-4 cells. Western blots of the target proteins were cropped as indicated, and full blot images are presented in Supplemental Fig.2. (B) The proliferation capacity of OSCC cells was measured by MTT assay at 24, 48 and 72 h post-transfection

with si-DKK1 or its control. The data corresponds to the results of 3 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.0001.

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