Activation of EGFR-Aurora A Induces Loss of Primary Cilia in Oral Squamous Cell Carcinoma

Fengying Yin
Zhejiang University School of Medicine Sir Run Run Shaw Hospital https://orcid.org/0000-0002-7418-1567

Qian Chen
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Yujie Shi
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Hao Xu
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Jie Huang
Sichuan University Huaxi College of Stomatology: Sichuan University West China College of Stomatology

Liang Zhong
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Maofeng Qing
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Jing Li
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Liang Xie
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Xin Zeng (zengxin22@163.com)
Sichuan University West China Hospital of Stomatology: Sichuan University West China College of Stomatology

Research

Keywords: Primary cilia, Oral leukoplakia, Oral squamous cell carcinoma, Epidermal growth factor receptor, Aurora A

Posted Date: November 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-108059/v1

Abstract

Background

Primary cilia are evolutionally conserved organelles involving multiple cell functions. Loss of primary cilia is frequently observed in various cancers, suggesting that the absence of them may promote tumorigenesis. However, little is known about the role of primary cilia in oral squamous cell carcinoma (OSCC).

Methods

Immunofluosense staining was applied to detect the incidence of primary cilia in normal, oral leukoplakia (OLK) and OSCC tissues. Differentially expressed ciliary genes of OSCC were screened from The Cancer Genome Atlas database and interested genes were identified by referring to Pubmed literature. Immunohistochemical analysis was used for validating the correlation between the cilia ratio and the protein expression levels, and their regulatory effect on primary cilia was further proved *in vitro* and *in vivo*.

Results

A significant decrease in the percentage of ciliated cells was found in OLK, especially in OSCC tissues. Multiple ciliary genes were abnormally expressed in OSCC and epidermal growth factor receptor (EGFR)-Aurora A signaling was chosen for further study. A parallel increase of EGFR-Aurora A activity was observed in OLK and OSCC tissues. Moreover, EGFR activation induced obvious cilia absorption by phosphorylating Aurora A, whereas inhibition of EGFR-Aurora A activity significantly restored cilia formation. Besides, Aurora A silencing significantly restored ciliary expression and decreased tumor growth *in vivo*.

Conclusions

The abnormal activation of EGFR-Aurora A signaling leads to the gradual loss of primary cilia in oral mucosa carcinogenesis. Primary cilia have the potential to be new biomarkers and therapeutic targets of oral mucosa carcinogenesis.

Background

Primary cilia, also known as immotile cilia, are hair-like cell structures that protrude from the cell surface and are almost ubiquitously expressed throughout the human body (1). It is comprised of nine outer microtubules and is anchored to the cell by the basal body (2). Primary cilia have been demonstrated to like “antennas” of cells due to the perception of its surrounding extracellular environment because multiple signal receptors are located on the cilia membrane, including hedgehog (Hh) signal receptors, platelet-derived growth factor (PDGF) receptor, Notch receptor, and Wnt signal receptors (3). Loss of primary cilia has been reported to have strong correlations with a variety of cancer types, such as breast,

renal, prostate, pancreatic, and ovarian cancer (4). Nevertheless, the frequency of primary cilia in the oral premalignant lesion and oral cancer has not been reported.

Oral squamous cell carcinoma (OSCC) accounts for the most common head and neck cancer (5), with a 5-year survival rate lower than 50% in patients(6) and is mainly developed via oral leukoplakia (OLK), the best-known oral potentially malignant disorders (OPMD)(7). Epidermal growth factor receptor (EGFR) signaling is a critical pathway that regulates multiple cellular functions in OSCC and leads to poor survival (8, 9). Recently, it has been shown that EGFR kinase suppresses the ciliogenesis of human retinal epithelia cells by stabilizing Aurora A (AURKA), a pivotal cilia disassembly kinase (10). However, the role of EGFR signaling in the primary ciliogenesis of cancer cells remains unclear.

In this study, we verified that the frequency of primary cilia was remarkably decreased in OLK and OSCC patient tissues with a corresponding elevation of the level of EGFR-Aurora A signaling. Further experiments showed that EGFR stimulated cilia absorption via regulation of Aurora A, which knockdown promoted the ciliary formation and decreased tumor growth in *vivo*. This study not only helped us to explore the biomarker of oral mucosa malignant transformation but also provided some new lights for the development of new therapeutic targets for oral cancer.

Methods

Immunofluorescencestaining

Immunofluorescence (IF) staining was performed as described previously (12). The antibodies used in this study are listed as follows: mouse anti-acetylated-α-tubulin (1:2000, T7451, Sigma-Aldrich), rabbit anti-γ-tubulin (1:500, T5192, Sigma-Aldrich), goat anti-mouse Alexa Fluor TRITC (1:500, T5393, Sigma-Aldrich), and goat anti-rabbit Alexa Fluor FITC (1:500, F0382, Sigma-Aldrich). The frozen sections were co-stained with acetylated-α-tubulin and γ-tubulin antibodies under the guidance of hematoxylin-eosin (HE) staining. Glass slides were then mounted in anti-fade mounting medium with DAPI (P0131, Beyotime). The images were photographed using a confocal microscope (Olympus F3000).

Bioinformatics and ciliary gene acquisition

The gene expression data of OSCC and its corresponding adjacent normal control tissues were downloaded from The Cancer Genome Atlas (TCGA) database and standardized through log2-transforming. All analyses were performed in R (R version 3.4.2) software. SYSCILIA, carried out by 18 partners from 7 countries, published the gold standard data set of ciliary proteins in 2013, which contains 303 proteins(13).In 2017, Reiter and Leroux identified a total of 428 genes associated with ciliary structures and/or functions(14). The above two data sets were combined to obtain the ciliary genes so far known.

Immunohistochemistry

Immunohistochemistry (IHC) was performed as described previously (15). The antibodies used in this study are listed as follows: rabbit anti-EGFR antibody (1:200, ab52894, Abcam), rabbit anti-p-EGFR (phospho Y1068) antibody (1:200, ab40815, Abcam), rabbit anti-Aurora A (1:200, 91590, CST) and rabbit anti-HDAC6 (1:1000, 7558, CST). Tissue slides were photographed by a high-resolution scanner (ScanScope GL, Aperio Technologies) and a scoring system was used to semi-quantify the protein expression levels of EGFR and phosphorylated EGFR as follows: staining intensity: 0-no detectable staining, 1-light yellow, 2-deep yellow, or 3-brown; staining proportion: 1 (<10%), 2 (10–30%), 3 (31–70%) or 4 (>71%); and final score: staining intensity multiplied by staining proportion. Tissues were considered positive if >1% of cells displayed immunoreactivity of Aurora A.

Western blot

The primary antibodies used for immunoblotting included rabbit anti-EGFR (1:5000, ab52894, Abcam), rabbit anti-p-EGFR (phospho Y1068) (1:1000, ab40815, Abcam), rabbit anti-HDAC6 (1:1000, 7558, CST), rabbit anti-Aurora A (1:1000, 91590, CST), rabbit anti-p-Aurora A (phospho T288) (1:1000, 3079, CST) and rabbit anti-GAPDH (1:2000, 5174, CST).Western blotting was performed as previously described (16). **RT-PCR**

Cell total RNA was isolated using Trizol (15596018, Thermo Fisher) and following reverse transcription reaction by PrimeScript^{TM}RT reagent kit (RR037A, Takara). Relative fluorescence quantitative PCR was performed on the ABI 7500 System (Thermo Fisher) with SYBR qPCR mix (4472908, Thermo Fisher). The primers used in this experiment are shown in Appendix Table 2.

Nude mouse tumor xenograft model

Fifteen female BALB/c nude mice, 4–5 weeks old, were obtained from Kekang (Changzhou, China). All the animals were kept in standard conditions, following the institutional guidelines. Human OSCC cells were injected into the subcutaneous tissues of the right upper flanks as 1 X 10^7 cells (0.1 mL). The tumor volume was calculated as (length X width^2) /2 every 3 days. All the animals were euthanized after tumor length reached 2 cm. The tumor was removed and half placed in 4% paraformaldehyde to prepare paraffin sections.

Statistical analysis

The correlation between cilia percentage and the expression level of protein was performed using the Spearman correlation test. The remained data were tested for significance using the Student's *t* test. Differences were considered significant when $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

Results

Reduced frequency of primary cilia in OLK and OSCC

To identify the role of primary cilia in the development of oral cancer, we first detect the frequency of primary cilia in OLK and OSCC patients. Tissues were made into continuous frozen sections. The HE slide was used as a reference to find the tissue types of interest on the adjacent serial section, thereby further guiding the IF co-staining of cilia using acetylated-α-tubulin (labeling the axoneme of primary cilia) and γ-tubulin (labeling the associated centrosomes) antibodies (Fig.1A). Then the percentage of primary cilia in epithelial was quantified and a total of 47,220 nuclei were counted with a range of 262-1264 nuclei per tissue. Significantly decreased frequency of primary cilia was observed in OLK tissues compared with normal epithelium (Fig.1A-B). While primary cilia were hardly detected in OSCC tissues (the median percentage was 1.11%) compared with normal or adjacent tissues. Additionally, there was no statistical difference in cilia length between normal, OLK, peri-tumor, and OSCC tissues (Fig.1B). These data suggested that primary cilia were gradually lost in the epithelium during the progression of oral mucosa carcinogenesis.

To further explore the differential expression of primary cilia, we assessed their expression on normal human oral keratinocyte cell line (HOK) and five OSCC cell lines (HSC-3, Cal27, SCC47, UM1, and SCC25). Although the specific mechanisms are not fully understood, serum starvation is widely by researchers to induce ciliary formation in cultured cells[11]. About 6.25% of cells were displayed primary cilia in HOK, while only 0.55% and 1.77% of cells in SCC47 and UM1 cells had cilia under conventional culture conditions (Fig.2A-B). After 24 hours of serum starvation induction, the percentage of primary cilia was both increased in SCC47 and UM1 cells. However, no primary cilia were detected in HSC-3, Cal27, and SCC25 cell lines neither in routine culture or serum starvation induction (Fig.2B). Because of the different culture systems, we did not compare the cilia ratio between HOK and OSCC cells directly. However, the results of cell culture still suggested that the formation of primary cilia was inhibited in OSCC cells, especially in HSC-3, Cal27, and SCC25 cells, which completely lost the ability to form primary cilia.

Multiple ciliary genes were abnormally expressed in OSCC

To explore the expression of ciliary genes in OSCC, bioinformatics analysis was conducted on the gene expression data of 32 pairs of OSCC and their corresponding adjacent normal tissues from TCGA database. A total of 1149 differentially expressed genes (DEGs) were obtained, among which 382 genes were upregulated and 767 genes were downregulated in OSCC tissues (Fig.3A). Then, Venn diagrams were used to calculate the intersection of the DEGs and the ciliary genes set, and 12 ciliary genes, including AURKA and Polo-like kinase 1 (PLK1) were screened in this study (Fig.3B-C). Aurora A is a critical conserved kinase inducing cilia disassembly, in which overactivity has been proved to result in cilia loss in multiple tumors (17). Therefore, the regulatory effect of Aurora A on primary cilia in oral cancer was verified preferentially in this study.

EGFR-Aurora A signaling suppresses primary ciliogenesis in OSCC

As EGFR kinase was recently shown to suppress ciliogenesis by stabilizing Aurora A(10), we tested the expression levels of EGFR-Aurora A signaling by IHC analysis in normal, OLK, peri-tumor, and OSCC tissues. The results showed that both EGFR and phosphorylated EGFR were notably overexpressed in

OLK tissues, especially in OSCC tissues compared to normal (Fig.4A-B). Correlation analysis further revealed a significant negative correlation between EGFR activity and cilia ratio (Fig.4C). Moreover, Aurora A was overexpressed in none of the normal or tumor-adjacent cases, 32% of OLK, and 78.9% of OSCC, as shown in Fig.4D and Table 1. The cilia ratio of Aurora A positive tissues was notably lower than negative tissues (Fig.4D).

Table 1. Positive rate of Aurora A in normal, peri-tumor, OLK, and OSCC groups.

	Total	Aurora A (IHC positive)
Normal	12	0
Peri-tumor	15	0
OLK	25	32%
OSCC	21	78.90%

To further determine whether the loss of primary cilia in oral cancer caused by high EGFR-Aurora A activity, we selected two OSCC cell lines under treating with hEGF or gefitinib (EGFR tyrosine kinase inhibitor) to activate or inhibit the EGFR kinase *in vitro* respectively. Immunoblot showed that hEGF successfully stimulated the phosphorylation of EGFR and Aurora A and gefitinib inversely blocked the activity of them (Fig.5A). The IF analysis showed that EGF stimulation induced the disassembly and disappearance of cilia under serum starvation, meanwhile, gefitinib significantly blocked the absorption of cilia caused by serum restimulation (Fig.5B-C). Next, EGFR and Aurora A were knocked down in OSCC cells. As expected, the cilia ratio increased after EGFR or Aurora A silencing in these two OSCC cell lines (Fig.5D-F and Appendix Fig.1). Thus EGFR-Aurora A signaling pathway was essential for the regulation of primary ciliogenesis in OSCC.

AURKA silencing induces ciliogenesis and reduces tumor growth

To determine whether AURKA silencing contributes to restoring cilia formation *in vivo*, AURKA silenced/unsilenced SCC47 cells were grafted to the flanks of nude mice by subcutaneous injection. Data from IF analysis showed that the cilia ratio increased significantly in two AURKA silenced groups compared to the NC controls (Fig.6A-B). Remarkably, the tumors derived from the silenced AURKA cells were smaller than the control cells and grew more slowly compared to the controls (Fig.6C). Together, these results suggested that Aurora A played an important role in the primary ciliogenesis of OSCC.

HDAC6 is not involved in the regulation of primary ciliogenesis in OSCC

Histone deacetylases 6 (HDAC6), a tubulin deacetylase, has been reported to be a key target of Aurora A and induce cilia disassembly by deacetylation of microtubules of the ciliary axoneme (18). So, we investigated whether HDAC6 is involved in the regulation of ciliogenesis in oral mucosa carcinogenesis. The IHC results showed that HDAC6 expression did not increase, but decreased gradually from normal to

OLK to OSCC tissues (Appendix Fig.2A-B). Then tubacin, an HDAC6 specific inhibitor, was used to treat OSCC cells *in vitro*. Accompany with the dose of tubacin increased, the expression of HDAC6 was gradually inhibited and meanwhile, acetylated α-tubulin, the main substrate of HDAC6-mediated cilia disassembly, was correspondingly increased, indicating that tubacin treatment substantially suppressed HDAC6 activity (Appendix Fig.2C). However, IF staining showed that the incidence of primary cilia in SCC47 or UM1 cells was not increased under the tubacin treatment (Appendix Fig.2D). Taken all together, these findings suggested that HDAC6 was not involved in the loss of primary cilia in oral mucosa carcinogenesis.

Discussion

Because of limited studies on the role of primary cilia in oral cancer, we initially characterized the incidence of primary cilia in the oral mucosa malignant transformation in detail and observed that the frequency of primary cilia was decreased in OLK and nearly absent in OSCC. This phenomenon, the gradual loss of primary cilia in carcinogenesis, is pretty similar to that in other cancer types, such as clear cell renal cell carcinoma (19), breast cancer (20), and pancreatic ductal adenocarcinoma (21). Besides, primary cilia have been shown to be associated with the malignant transformation of lung precancerous lesions. The lung premalignant process can progress into squamous cell lung cancer or regress into a relatively normal state, while only the lung premalignant lesions with reduced cilia rate lose the ability to regress (22).

EGFR is known to be overexpressed in OSCC compared to the normal epithelium, (23, 24) and our results were consistent with previous reports. However, the suppression of EGFR on primary cilia was first reported by Kousuke in 2018. Our results showed that EGFR-Aurora A activity was correlated with primary cilia incidence in OLK and OSCC patients. Meanwhile, primary ciliogenesis was blocked or promoted with the activation or inhibition of EGFR-Aurora A signaling *in vitro*. Moreover, silencing Aurora A could restore the ciliary expression of OSCC cells *in vivo*. Taken together, our work suggests that loss of primary cilia in OSCC cells may be accelerated by abnormal activation of EGFR-Aurora A signaling.

Aurora A, a serine/threonine kinase, is originally identified as a multifunctional protein during mitosis, which first proven function outside of mitosis is promoting cilia absorption (25). The overexpression of Aurora A in multiple cancers not only lead to centrosomal amplification and genomic instability (26, 27), but also induce the ciliary disassembly of tumor cells, resulting in cilia dysfunction (28). Additionally, restoration of primary cilia by Aurora A targeting has been reported as a tumor suppressor mechanism (17, 29). Furthermore, our *in vivo* experiments on a nude mouse tumor xenograft model showed a significant decrease in tumor growth associated with increased ciliary expression, suggesting that ciliary re-expression in tumor cells by targeting Aurora A may be a potential therapeutic approach for OSCC.

In this study, Aurora A has been identified as a downstream target of EGFR in the regulation of cilia formation in OSCC cells. While it remains unclear how Aurora A inhibits primary ciliogenesis in OSCC cells. HDAC6 is generally considered as a downstream regulator of Aurora A in the loss of primary cilia by

promoting destabilization of microtubules (18, 30). A previous study reported that the loss of primary cilia was dependent on HDAC6 in cholangiocarcinoma (31), renal cell carcinoma (17), and pheochromocytoma (32), while inversely, the remarkably suppression of ciliogenesis was found to be independent of HDAC6 in ovarian cancer and pancreatic ductal adenocarcinoma (28, 29). Similarly, we found no recovery of primary cilia in OSCC cells by inhibiting HDAC6 expression and its deacetylating activity, suggesting that the inhibitory effect of Aurora A on ciliogenesis may go through with distinct downstream target proteins in different tumors types.

As a complex multifunctional kinase, it is hard, but important, to distinguish the role of Aurora A in promoting tumor progression by regulating chromosomal separation and spindle assembly from inducing cilia disassembly and functional defects. Whether targeting Aurora A to restraint the tumor growth of OSCC by restoring cilia formation or by inhibiting chromosome segregation or by other mechanisms will continue to be the focus of this topic. In-depth research on such mechanisms will be helpful for the prevention and treatment of OSCC.

Conclusions

Our study demonstrates the gradual loss of primary cilia and a parallel increase of EGFR-Aurora A activity in the process of oral mucosa carcinogenesis. Besides, EGFR could induce cilia absorption through phosphorylating Aurora A in OSCC cell lines. Moreover, targeting Aurora A significantly restores ciliary expression and decreases tumor growth *in vivo*. Collectively, our study indicates that EGFR-Aurora A signaling is involved in the regulation of primary cilia and provides new sights for developing potential biomarkers and therapeutic targets of oral cancer.

Abbreviations

OSCC: oral squamous cell carcinoma; OLK: oral leukoplakia; EGFR: epidermal growth factor receptor; AURKA: Aurora A; TCGA: The Cancer Genome Atlas; Hh: hedgehog; PDGF: platelet-derived growth factor; OPMD: oral potentially malignant disorders; RT-PCR: reverse transcription-PCR; IF: Immunofluorescence; IHC: Immunohistochemistry; HOK: human oral keratinocyte cell; DEGs: differentially expressed genes; PLK1: Polo-like kinase 1; HDAC6: Histone deacetylases 6.

Declarations

Ethics approval and consent to participate

Approval and consent obtained for the use of human tissues were obtained from the ethics committee of the West China Hospital of Stomatology, Sichuan University (Date of approval: 2015-03-03, Approval number: WCHSIRB-2015-046).

Approval for all the mouse experiments was obtained from the Animal Care and Use Committee, State Key Laboratory of Oral Diseases, Sichuan University (Date of approval: 2015-02-05, Approval number:

WCCSIRB-D-2015-082).

Consent for publication

Not applicable.

Availability of data and materials

All the data generated or analysed in this study are included in this published article and its Additional files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China [grant number U19A2005, 81771081, 81730030 and 81520108009], the CAMS Innovation Fund for Medical Science [grant number 2019-I2M-5-004], and the Sichuan Science and Technology Program [grant number 2020YJ0286]. The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' contributions

YF, CQ, and SY performed experiments and analysis; XH performed the related bioinformatics analysis; HJ, QM, and ZL participated in the collection of samples. XL, LJ, and ZX coordinated the study and oversaw all experiments. YF and XL wrote the manuscript. All the authors approved the manuscript for publication.

References

1. Singla V, Reiter JF. The primary cilium as the cell's antenna: signaling at a sensory organelle. Science. 2006;313(5787):629-33.
2. Carvalho-Santos Z, Azimzadeh J, Pereira-Leal JB, Bettencourt-Dias M. Evolution: Tracing the origins of centrioles, cilia, and flagella. J Cell Biol. 2011;194(2):165-75.
3. Pala R, Alomari N, Nauli SM. Primary Cilium-Dependent Signaling Mechanisms. Int J Mol Sci. 2017;18(11).
4. Higgins M, Obaidi I, McMorrow T. Primary cilia and their role in cancer. Oncol Lett. 2019;17(3):3041-7.
5. Global Burden of Disease Cancer C, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncol. 2017;3(4):524-48.

6. Panzarella V, Pizzo G, Calvino F, Compilato D, Colella G, Campisi G. Diagnostic delay in oral squamous cell carcinoma: the role of cognitive and psychological variables. Int J Oral Sci. 2014;6(1):39-45.
7. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007;36(10):575-80.
8. Rajan A, Gibson-Corley KN, Choi AB, Ofori-Amanfo GK, Ten Eyck P, Espinosa-Cotton M, et al. Impact of Nuclear Interleukin-1 Alpha and EGFR Expression on Recurrence and Survival Outcomes in Oral Squamous Cell Carcinomas. J Oncol. 2019;2019:5859680.
9. Hsu PC, Jablons DM, Yang CT, You L. Epidermal Growth Factor Receptor (EGFR) Pathway, Yes-Associated Protein (YAP) and the Regulation of Programmed Death-Ligand 1 (PD-L1) in Non-Small Cell Lung Cancer (NSCLC). Int J Mol Sci. 2019;20(15).
10. Kasahara K, Aoki H, Kiyono T, Wang S, Kagiwada H, Yuge M, et al. EGF receptor kinase suppresses ciliogenesis through activation of USP8 deubiquitinase. Nat Commun. 2018;9(1):758.
11. Oh C, Kim HJ, Kim HM. Vitamin D maintains E-cadherin intercellular junctions by downregulating MMP-9 production in human gingival keratinocytes treated by TNF-alpha. J Periodontal Implant Sci. 2019;49(5):270-86.
12. Wong SW, Han D, Zhang H, Liu Y, Zhang X, Miao MZ, et al. Nine Novel PAX9 Mutations and a Distinct Tooth Agenesis Genotype-Phenotype. J Dent Res. 2018;97(2):155-62.
13. van Dam TJ, Wheway G, Slaats GG, Group SS, Huynen MA, Giles RH. The SYSCILIA gold standard (SCGSv1) of known ciliary components and its applications within a systems biology consortium. Cilia. 2013;2(1):7.
14. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. Nat Rev Mol Cell Biol. 2017;18(9):533-47.
15. Liu S, Liu D, Zeng X, Wang J, Liu J, Cheng J, et al. PA28gamma acts as a dual regulator of IL-6 and CCL2 and contributes to tumor angiogenesis in oral squamous cell carcinoma. Cancer Lett. 2018;428:192-200.
16. Li J, Guo Y, Feng X, Wang Z, Wang Y, Deng P, et al. Receptor for activated C kinase 1 (RACK1): a regulator for migration and invasion in oral squamous cell carcinoma cells. J Cancer Res Clin Oncol. 2012;138(4):563-71.
17. Dere R, Perkins AL, Bawa-Khalfe T, Jonasch D, Walker CL. beta-catenin links von Hippel-Lindau to aurora kinase A and loss of primary cilia in renal cell carcinoma. J Am Soc Nephrol. 2015;26(3):553-64.
18. Pugacheva EN, Jablonski SA, Hartman TR, Henske EP, Golemis EA. HEF1-dependent Aurora A activation induces disassembly of the primary cilium. Cell. 2007;129(7):1351-63.
19. Basten SG, Willekers S, Vermaat JS, Slaats GG, Voest EE, van Diest PJ, et al. Reduced cilia frequencies in human renal cell carcinomas versus neighboring parenchymal tissue. Cilia. 2013;2(1):2.

20. Menzl I, Lebeau L, Pandey R, Hassounah NB, Li FW, Nagle R, et al. Loss of primary cilia occurs early in breast cancer development. Cilia. 2014;3:7.
21. Seeley ES, Carriere C, Goetze T, Longnecker DS, Korc M. Pancreatic cancer and precursor pancreatic intraepithelial neoplasia lesions are devoid of primary cilia. Cancer Res. 2009;69(2):422-30.
22. Denisov EV, Schegoleva AA, Gerashchenko TS, Skryabin NA, Sleptcov AA, Yakushina VD, et al. Gene Expression Profiling Revealed 2 Types of Bronchial Basal Cell Hyperplasia and Squamous Metaplasia With Different Progression Potentials. Appl Immunohistochem Mol Morphol. 2019.
23. Kuttan NA, Bhakthan NM. Epidermal growth factor receptor (EGFR) in oral squamous cell carcinomas: overexpression, localization and therapeutic implications. Indian J Dent Res. 1997;8(1):9-18.
24. Panarese I, Aquino G, Ronchi A, Longo F, Montella M, Cozzolino I, et al. Oral and Oropharyngeal squamous cell carcinoma: prognostic and predictive parameters in the etiopathogenetic route. Expert Rev Anticancer Ther. 2019;19(2):105-19.
25. Pugacheva EN, Golemis EA. The focal adhesion scaffolding protein HEF1 regulates activation of the Aurora-A and Nek2 kinases at the centrosome. Nat Cell Biol. 2005;7(10):937-46.
26. Gritsko TM, Coppola D, Paciga JE, Yang L, Sun M, Shelley SA, et al. Activation and overexpression of centrosome kinase BTAK/Aurora-A in human ovarian cancer. Clin Cancer Res. 2003;9(4):1420-6.
27. Goepfert TM, Adigun YE, Zhong L, Gay J, Medina D, Brinkley WR. Centrosome amplification and overexpression of aurora A are early events in rat mammary carcinogenesis. Cancer Res. 2002;62(14):4115-22.
28. Kobayashi T, Nakazono K, Tokuda M, Mashima Y, Dynlacht BD, Itoh H. HDAC2 promotes loss of primary cilia in pancreatic ductal adenocarcinoma. EMBO Rep. 2017;18(2):334-43.
29. Egeberg DL, Lethan M, Manguso R, Schneider L, Awan A, Jorgensen TS, et al. Primary cilia and aberrant cell signaling in epithelial ovarian cancer. Cilia. 2012;1(1):15.
30. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, et al. HDAC6 is a microtubule-associated deacetylase. Nature. 2002;417(6887):455-8.
31. Gradilone SA, Radtke BN, Bogert PS, Huang BQ, Gajdos GB, LaRusso NF. HDAC6 inhibition restores ciliary expression and decreases tumor growth. Cancer Res. 2013;73(7):2259-70.
32. O'Toole SM, Watson DS, Novoselova TV, Romano LEL, King PJ, Bradshaw TY, et al. Oncometabolite induced primary cilia loss in pheochromocytoma. Endocr Relat Cancer. 2019;26(1):165-80.

Figures

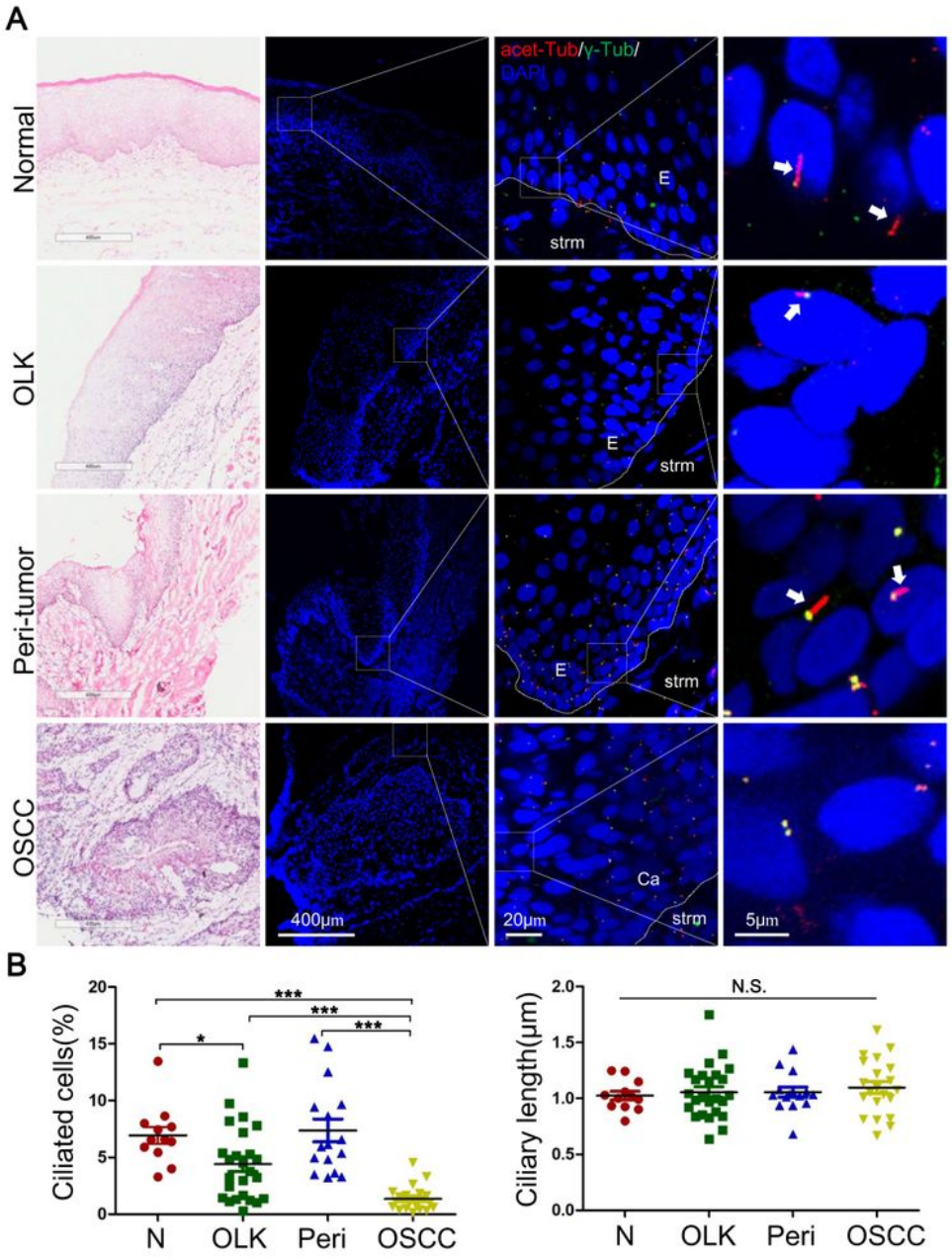

Figure 1

Primary cilia are gradually lost in OLK and OSCC tissues. (A) Representative images of the normal oral mucosa, OLK, adjacent to cancer (peri-tumor) and OSCC tissues. acet-Tub: acetylated-α-tubulin; γ-Tub: γ-tubulin. The white arrows indicate the primary cilia. Labeled structures are epithelium (E), stroma (Strm), and cancer (Ca). Percentages of ciliated cells and ciliary length are shown in (B). *P<0.05, ***P<0.001; N.S., not significant, two-tailed Student's t tests.

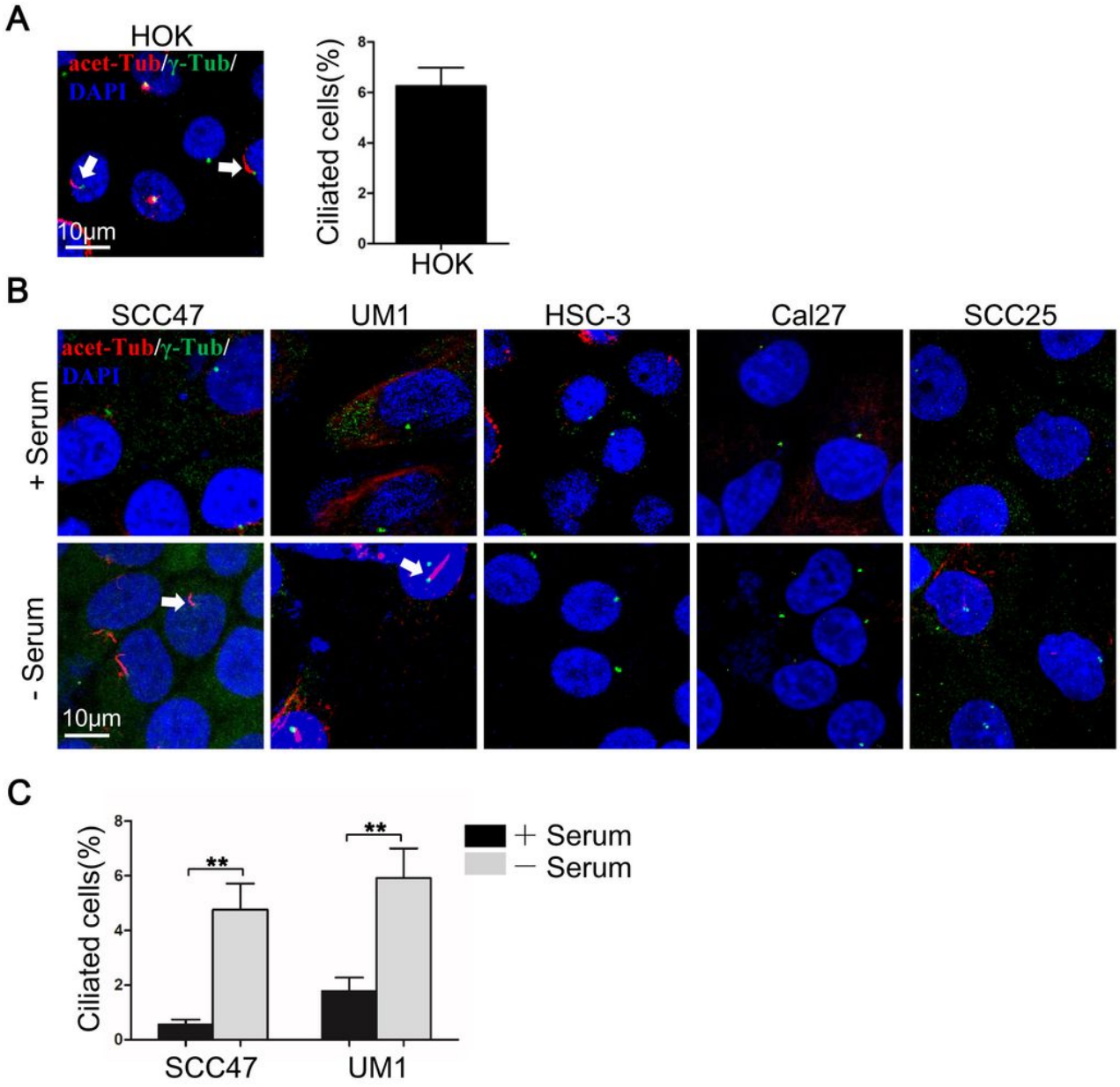

Figure 2

Primary Cilia formation is inhibited in OSCC cells in vitro. (A) Representative IF images and the percentages of primary cilia in HOK cells. The white arrows indicate the primary cilia. (B) Representative IF images of primary cilia in OSCC cell lines. “+Serum” means cultured with serum; “-serum” means cultured without serum. (C) Cilia ratio of SCC47 and UM1 cell lines with or without serum starvation. HSC-3, Cal27, and SCC25 cell lines are unable to form primary cilia neither in routine or serum starvation culture. **P < 0.01, two-tailed Student’s t test.

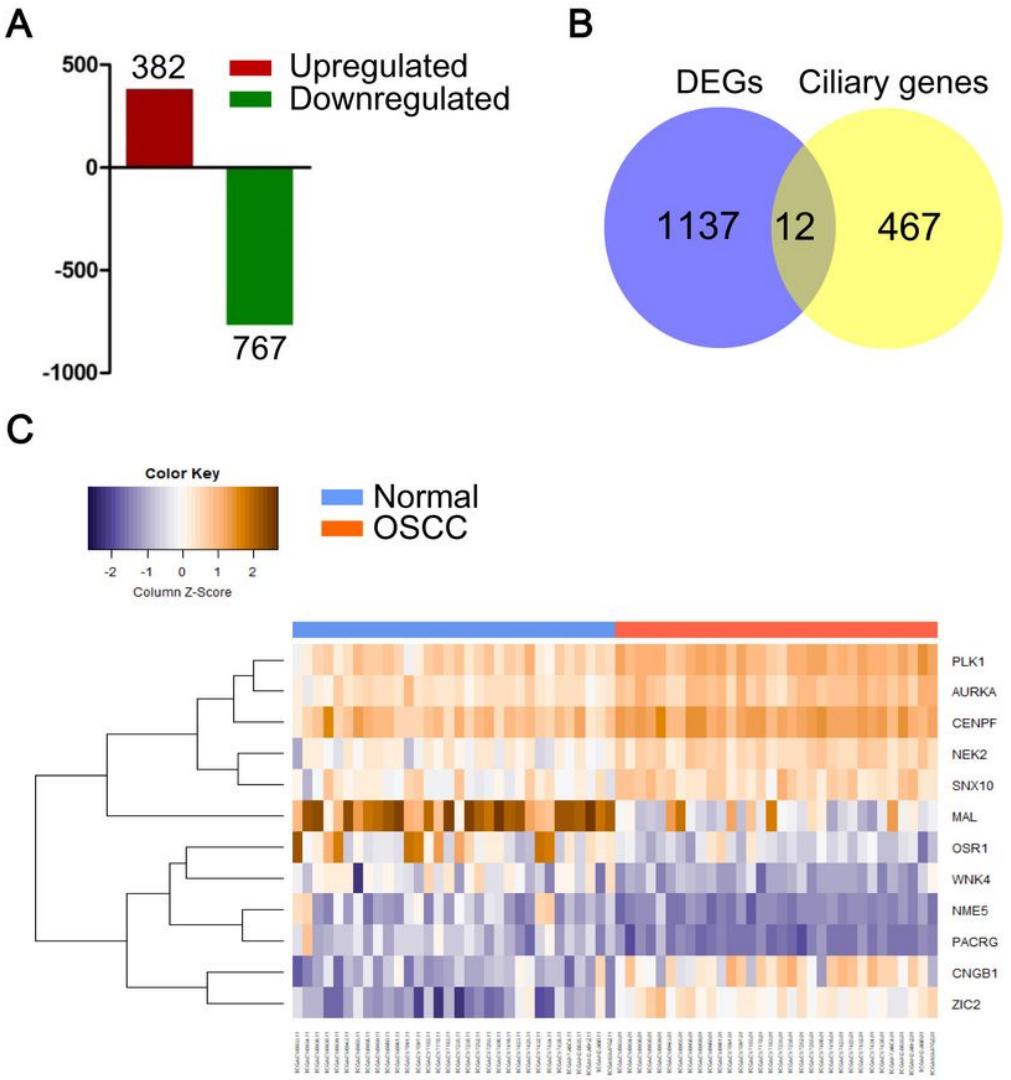

Figure 3

Differentially expressed ciliary genes in OSCC. (A) 382 upregulated genes and 767 downregulated genes were identified in OSCC. The intersection of the differentially expressed genes (DEGs) and ciliary genes calculated by Venn diagrams (B) and 12 DEGs shown by heatmap (C).

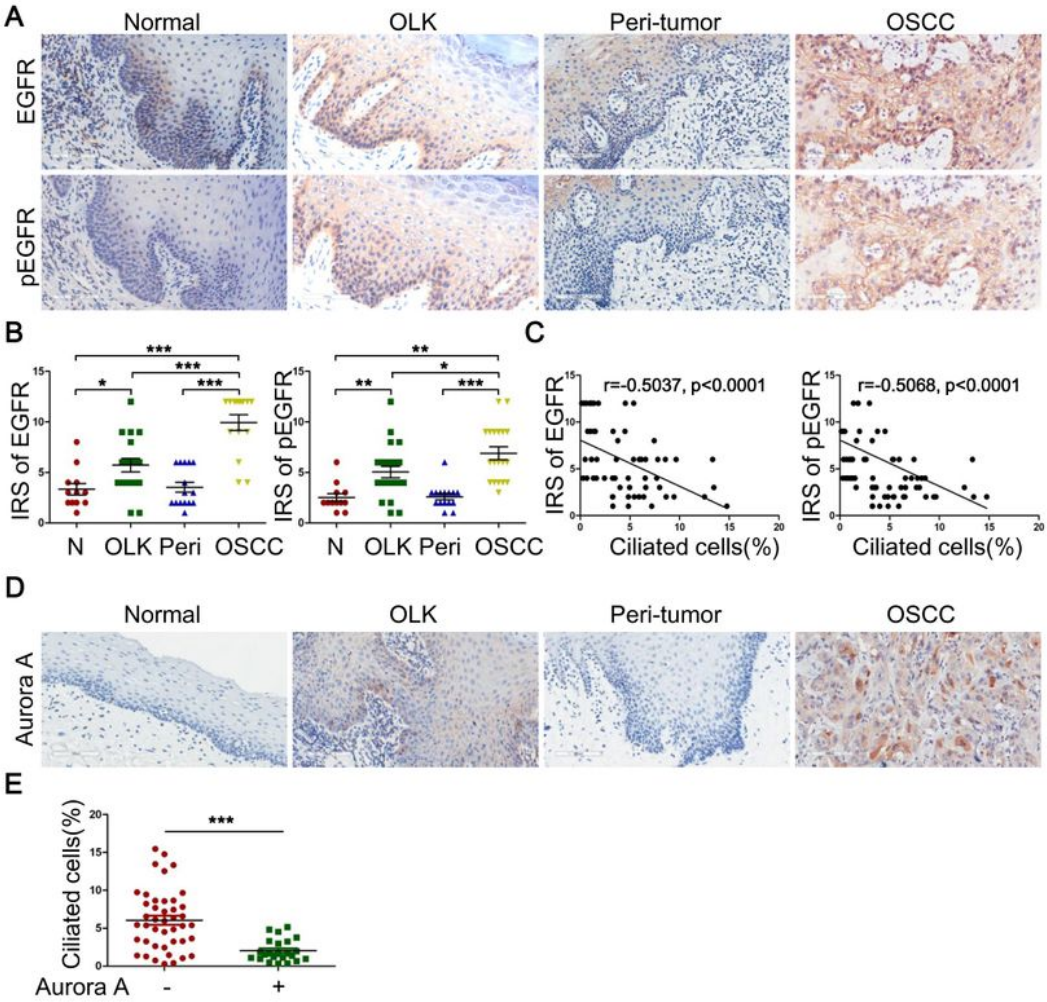

Figure 4

Primary cilia loss correlates with elevated EGFR-Aurora A activity. (A) Representative IHC images of EGFR and pEGFR (Y1068) in normal oral mucosa, OLK, Peri-tumor, and OSCC tissues. Bars=100μM. (B) Comparison of the immunoreactive scores (IRS) of EGFR (B, left) and pEGFR (Y1068) (B, right) between four groups. (C) Spearman correlation analysis was used to test the correlation between EGFR (C, left) and pEGFR (Y1068) (C, right) IRS and cilia percentages in normal, OLK, peri-tumor, and OSCC tissues. (D)

Representative IHC images of Aurora A in normal oral mucosa, OLK, Peri-tumor and OSCC tissues. (E) The percentage of cilia in Aurora A positive and negative tissues. *P<0.05; **P<0.01; ***P<0.001; two-tailed Student's t tests.

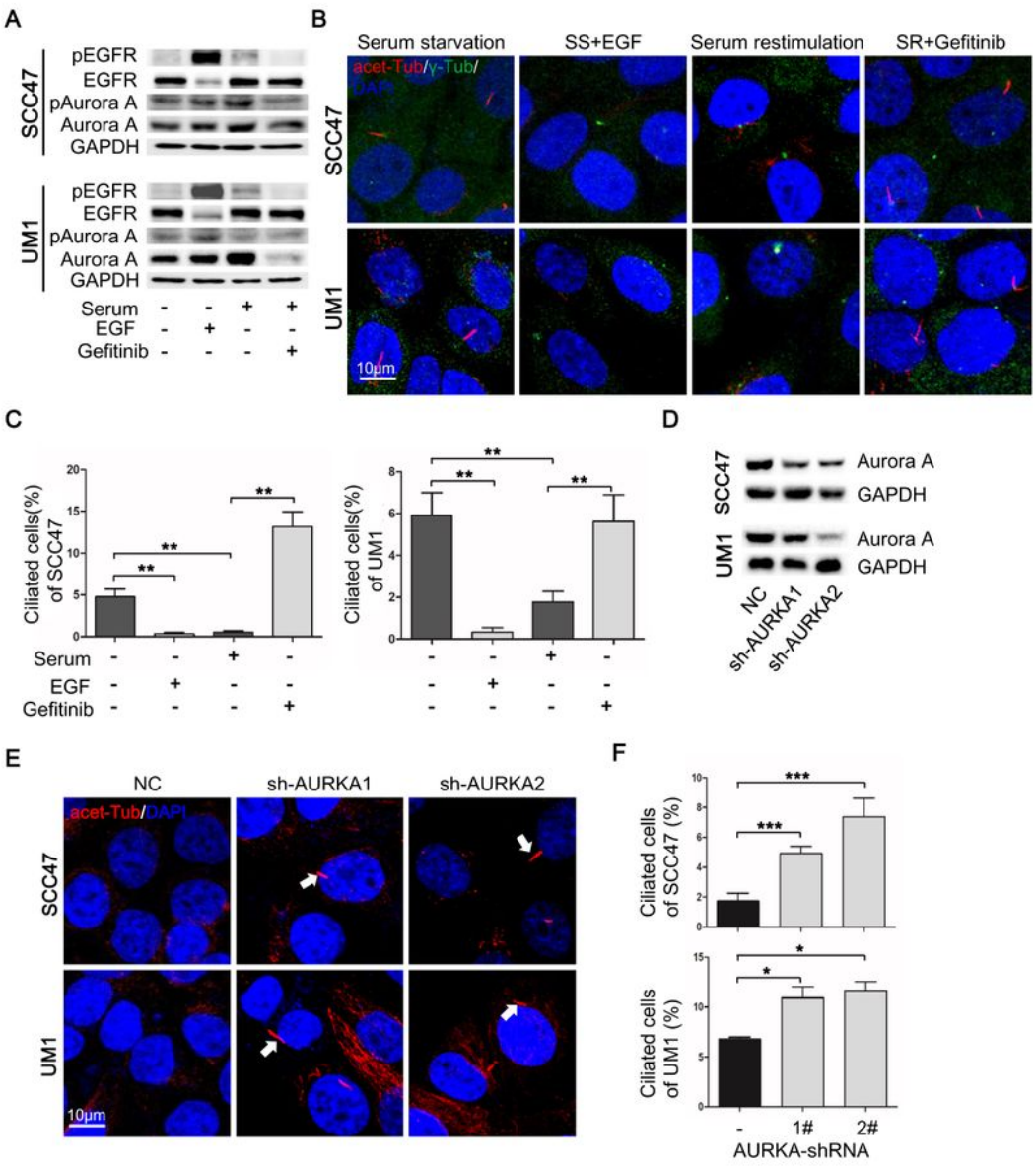

Figure 5

EGFR-Aurora A signaling suppresses ciliogenesis of OSCC. Serum starvation for 24 hours after SCC47 or UM1 cells confluence to induce cilia formation. Then cells were treated with EGF (10ng/ml), FBS (10%), or

10% FBS and gefitinib (10μM) for 24 hours. The cells were analyzed by immunoblotting with indicated antibodies (A), and IF staining with anti-acetylated-α-tubulin and anti-γ-tubulin (B) to valuate cilia incidence (C). SS means serum starvation; SR means serum restimulation. (D) SCC47 and UM1 cell lines of Aurora A silenced were constructed by shRNA. Then primary cilia were detected by IF staining (E). Percentages of ciliated cells are shown in (F). Six 400X visual fields were randomly selected and more than 500 nuclei were counted for each group. Mean ± SEM, n=6. *P<0.05; **P<0.01; ***P<0.001, two-tailed Student's t tests.

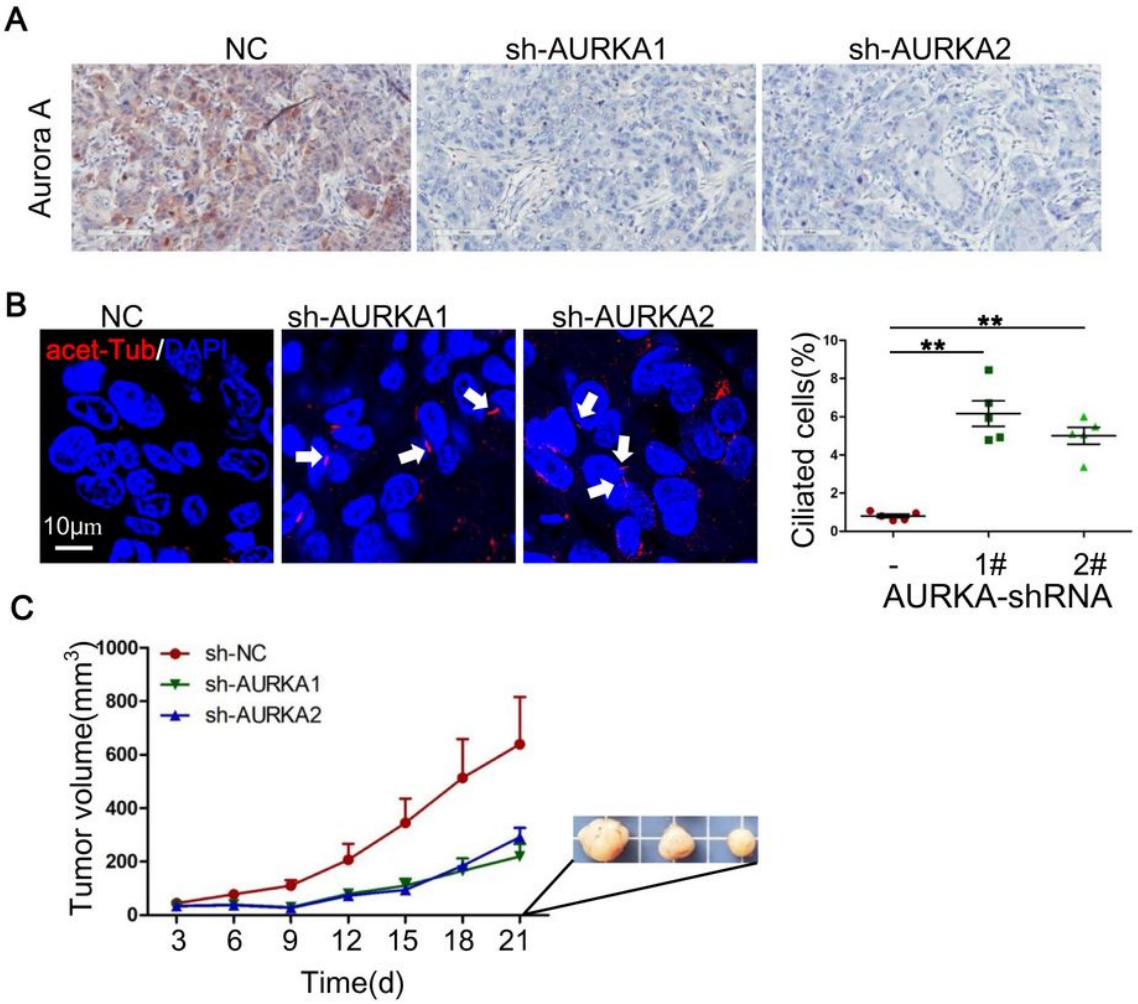

Figure 6

AURKA silencing restores the expression of primary cilia in an OSCC xenograft model. (A) Representative IHC images of Aurora A in the xenograft model of SCC47 cells. Bars=100μM. (B) Representative IF images and statistical analysis of cilia expression in the tumor cells. (C) The tumor volumes of two AURKA silencing groups were significantly smaller than the NC groups. **P<0.01, two-tailed Student's t tests.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Appendixmaterials.docx