ORIGINAL ARTICLES

Department of Otolaryngology[1], The Fourth Medical Center of PLA General Hospital; Department of Otolaryngology[2], Wuhan Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China

SIRT4 expression in laryngeal squamous cell carcinoma

Wuhanhui Wan[1,#], Yanni Li[1,#,*], Chengyong Zhou[2], Baochun Sun[2], Shuzhi Yang[2], Chengyong Zhou[1]

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**Correspondence author: Prof. Yanni Li, Department of Otorhinolaryngology, Wuhan Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, 473 Hanzheng Street, Qiaokou District, Wuhan, Hubei Province, P.R. China 430032*
jumplyn@163.com
[#]These authors contributed equally o this work.

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SIRT4 has been reported to be abnormally expressed in many malignant tumor tissues, but data in laryngeal squamous cell carcinoma (LSCC) is lacking. In the present study, we detected the expression of SIRT4 in 168 pairs of LSCC tissues and adjacent normal tissues using RT-qPCR, immunoblotting and immunohistochemical staining, and analyzed its clinical implication. We found that SIRT4 expression was low in LSCC tissues, and was significantly related to histological grade, T classification, clinical stage, lymph node metastasis and recurrence of LSCC patients. *In vitro*, knockdown of SIRT4 promoted the proliferation and migration of LSCC cells, while overexpression of SIRT4 inhibits the proliferation and migration of LSCC cells. Moreover, the expression of SIRT4 protein was an independent factor affecting the disease-free survival (DFS) (HR=0.562, 95%CI=0.129-0.834) and overall survival rates (OS) (HR=0.628, 95%CI=0.267-0.935) of LSCC patients. The 5-years DFS and OS in LSCC patients with low SIRT4 expression were significantly lower than that in LSCC patients with high SIRT4 expression. In conclusion, SIRT4 was lowly expressed in LSCC patients, which might be related to more aggressive tumor behaviour and a poor prognosis.

1. Introduction

Laryngeal squamous cell carcinoma (LSCC) is the most common pathological classification of laryngeal carcinoma, with a high incidence, accounting for 1 to 5% of systemic tumors, and is the most common upper respiratory tract malignant tumor (Chen et al. 2016; Schorn and Miles 2014). Generally, LSCC is considered to be related to alcoholism, long-term inhalation of harmful substances, and papillomavirus infection, so LSCC patients are mostly middle-aged and elderly men (Farshadpour et al. 2011; Lee et al. 2005). In China, surgery or surgery combined with radiotherapy and chemotherapy is a common treatment option for patients with LSCC, but it is less effective for patients with advanced LSCC (Liu et al. 2013; Marioni et al. 2006). In fact, there are currently no effective treatment options for patients with advanced LSCC, their prognosis is poor, and mortality is high (Forastiere et al. 2015). Therefore, early diagnosis and treatment is very important for patients with LSCC, because it can improve the survival rate of patients after surgery (Allegra et al. 2013; García-Fernández et al. 2011). Additionally, it is possible to preserve the pronunciation function of the larynx as long as possible and reduce postoperative complications.

SIRT4 is a member of the Sirtuin (SIRT) protein family, which is mainly located in the mitochondria, and its substrate is also mainly related to enzymes related to mitochondrial metabolism, such as glutamate dehydrogenase (GDH) (Komlos et al. 2013), adenine glucuronide transporters (ANT) -2 and ANT-3 (Ahuja et al. 2007), malonyl-CoA decarboxylase (MCD) (Laurent et al. 2013) and mitochondrial trifunctional protein α (MTPα) (Guo et al. 2016). Interestingly, SIRT4 has also been found to be abnormally expressed in many malignant tumor tissues like other members of the SIRT protein family, including colorectal cancer (Miyo et al. 2015), breast cancer (Huang et al. 2017), pancreatic cancer (Hu et al. 2019), non-small cell lung cancer (Fu et al. 2016) and so on. SIRT4 can promote DNA damage repair by inhibiting mitochondrial GDH (Jeong et al. 2013). However, the expression of SIRT4 in LSCC patients and its clinical implication is still unclear. Here, we report the expression of SIRT4 in LSCC tissues and normal tissues adjacent to tumor tissues, and analyzed the correlation between SIRT4 and clinicopathological parameters and prognostic significance for LSCC patients.

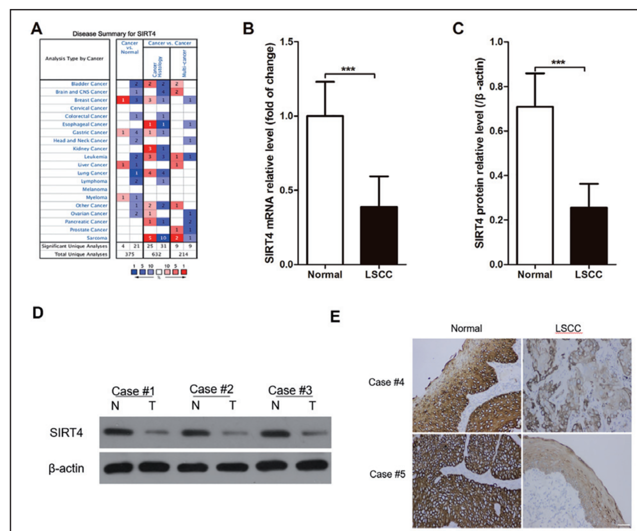

Fig. 1: SIRT4 was lowly expressed in LSCC tissues. A, Disease summary for SIRT4 in Oncomine database (www.oncomine.org). The parameters were set as follows: P<0.05, fold change (all), gene rank (top 10%) and data type (all); B-C, Statistical analysis of SIRT4 mRNA (B) and protein (C) expression in LSCC tissues and paired tissues adjacent to cancer. Data was expressed as (mean ± SD), and P value was calculated by paired t test, *** meant P <0.001; D-E, Representative immunoblotting (D) and immunohistochemical staining (E) for SIRT4 protein in primary LSCC tissues and adjacent noncarcinoma epithelial tissues.

2. Investigations and results

2.1. *SIRT4 expression in LSCC specimens*

According to the data from the Oncomine database (Fig. 1A), SIRT4 is abnormally expressed in many tumor tissues, such as in bladder cancer, breast cancer, and colorectal cancer, but data is lacking for LSCC tissues. Therefore, we first detected the expression of SIRT4 mRNA in LSCC tissues and paired tissues adjacent to LSCC tissues, and we found that the expression of SIRT4 mRNA in 168 LSCC tissues were significantly lower than those in 168 paired tissues adjacent to cancer (Fig. 1B). Next, we detected the expression of SIRT4 protein using immunoblotting and immunohistochemical staining, and the data of immunoblotting show that the expression of SIRT4 protein in 168 LSCC tissues were significantly lower than those in 168 paired tissues adjacent to cancer (Fig. 1C and 1D). Similarly, the data of immunohistochemical staining suggested that SIRT4 localized in the cytoplasm was more highly expressed in normal tissues adjacent to cancer in LSCC patients (Fig. 1E).

Table 1: Relationship between SIRT4 protein expression and clinicopathological parameters of patients with LSCC

Parameters	Number	SIRT4 expression		χ2	P
		Low	High		
Age					
<60	80	45	35	0.139	0.710
≥60	88	52	36		
Gender					
Male	153	89	64	0.131	0.717
Female	15	8	7		
Alcohol consumption					
Yes	89	58	31	4.749	0.093
No	67	32	35		
Unknown	12	7	5		
Smoking history					
Yes	123	72	51	1.420	0.490
No	37	22	15		
Unknown	8	3	5		
Histological grade					
G1	102	50	52	8.088	0.004
G2+G3	66	47	19		
Primary tumor grade					
Glottic	108	65	43	0.742	0.389
Others	60	32	28		
T classification					
T1+T2	92	41	51	14.462	<0.001
T3+T4	76	56	20		
Clinical stage					
I+II	81	40	41	4.475	0.034
III+IV	87	57	30		
Lymph node metastasis					
Yes	62	43	19	5.434	0.020
No	106	54	52		
Recurrence					
Yes	86	68	18	32.857	<0.001
No	82	29	53		

2.2. *Correlation between SIRT4 and clinicopathological parameters of LSCC patients*

To further analyze the significance of SIRT4 expression in LSCC patients, we collected the clinicopathological parameters of LSCC patients, including age, gender, alcohol consumption, smoking history, histological grade, primary tumor grade, T classification, clinical stage, lymph node metastasis and recurrence, and then we assigned 168 patients with LSCC to two groups based on SIRT4 protein expression level determined immunohistochemical staining. At last, we analyzed the correlation between SIRT4 protein expression and clinicopathological parameters of the patients. As shown in Table 1, there was no significant difference between SIRT4 protein expression and age (P=0.710), gender (P=0.717), alcohol consumption (P=0.093), smoking history (P=0.490) and primary tumor grade (P=0.389), but significant differences between SIRT4 protein expression and histological grade (P=0.004), T classification (P<0.001), clinical stage (P=0.034), lymph node metastasis (P=0.020) and recurrence (P<0.001) of LSCC.

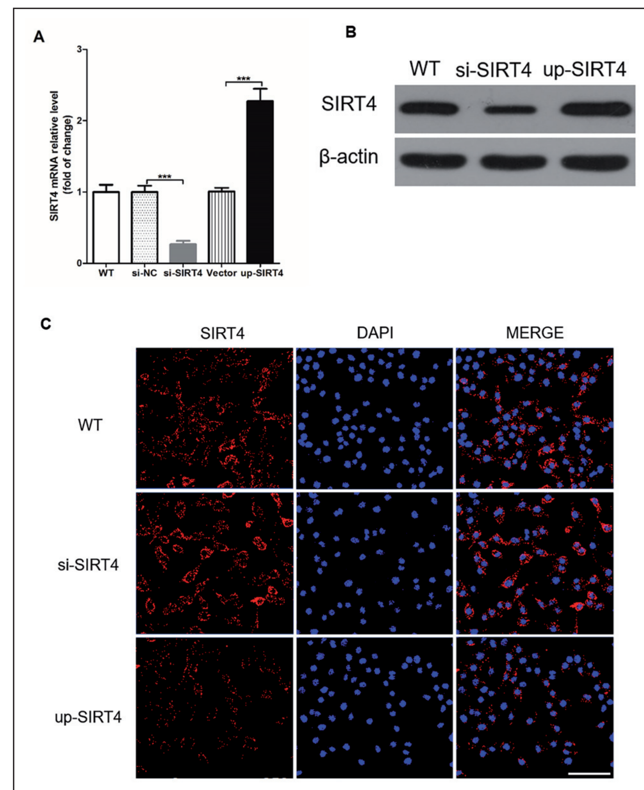

Fig. 2: Establishment of SIRT4 knockdown and overexpressed LSCC cells. A, RT-qPCR analysis of the indicated SIRT4 mRNA levels in different AMC-HN-8 cells; B-C, Representative immunoblotting (B) and immunofluorescence staining (C) for SIRT4 protein in different AMC-HN-8 cells.

2.3. *Effect of SIRT4 expression on proliferation and metastasis of LSCC cells in vitro*

To study the function of SIRT4 in LSCC cells, we transferred the small interfering RNA to knock down the expression of SIRT4, and transferred the overexpression plasmid to overexpress the expression of SIRT4 in AMC-HN-8 cells. Fortunately, the data of RT-qPCR suggested that (Fig. 2A) the negative control small interfering RNA (si-NC) and the empty plasmid (vector) did not affect the expression of SIRT4 mRNA in AMC-HN-8 cells, and SIRT4 small interfering RNA (si-SIRT4) successfully knocked down the expression of SIRT4, SIRT4 overexpression plasmid (up-SIRT4) successfully upregulated the expression of SIRT4 in AMC-HN-8 cells. Furthermore, the data of immunoblotting (Fig. 2B) and cellular immunofluorescence staining (Fig. 2C) all confirmed that we successfully established SIRT4 knockdown and overexpression AMC-HN-8 cells.

Next, we used these AMC-HN-8 cells to study the effect of SIRT4 expression on proliferation and metastasis of LSCC cells *in vitro*. As shown in Fig. 3, the number of LSCC cell-forming cell clones knocking down SIRT4 expression was significantly lower than that of wild-type (WT) LSCC cells, while the number of LSCC

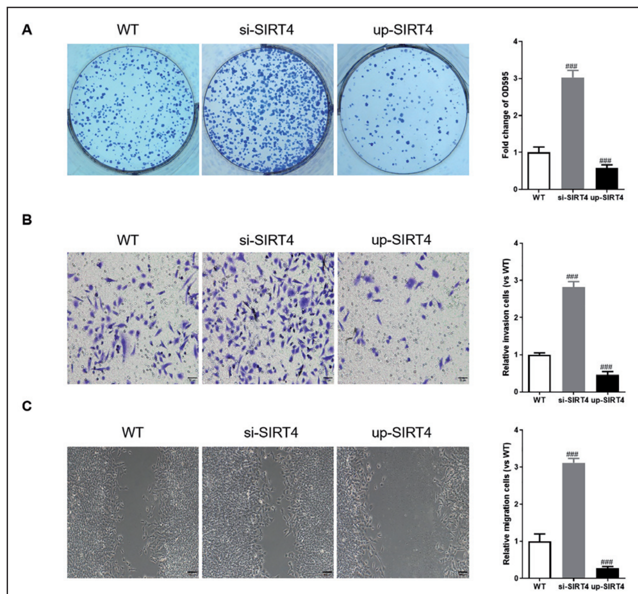

Fig. 3: SIRT4 regulated the proliferation and metastasis of LSCC cells. A, Representative cell clones of AMC-HN-8 after staining with crystal violet (left), and statistical analysis of the number of cell clones expressed by OD595 (right); B, Representative invading AMC-HN-8 cells after staining with crystal violet (left), and statistical analysis of the number of invading AMC-HN-8 cells (right); C, Representative migrating AMC-HN-8 cells after staining with crystal violet (left), and statistical analysis of the number of migrating AMC-HN-8 cells (right). Data was expressed as (mean ± SD), and P value was calculated by post-hoc comparisons, ### meant P<0.001 vs WT group.

cell-forming cell clones overexpressing SIRT4 was significantly higher than that of wild-type LSCC cells (Fig. 3A). In addition, knocking down SIRT4 not only significantly increased the number of invading LSCC cells (Fig. 3B), but also significantly increased the number of migrating LSCC cells (Fig. 3C). However, upregulating SIRT4 expression not only significantly reduced the number of invading LSCC cells (Fig. 3B), but also significantly reduced the number of migrating LSCC cells (Fig. 3C).

2.4. Prognostic significance of SIRT4 protein in the survival of LSCC patients

As an influencing factor, SIRT4 protein expression level was considered during Cox regression analysis of disease-free survival and overall survival in patients with LSCC. As shown in Table 2, the expression of SIRT4 protein was an independent factor affecting the disease-free survival (DFS) (HR=0.562, 95%CI=0.129-0.834) and overall survival (OS) (HR=0.628, 95%CI=0.267-0.935) of LSCC patients.

Next, we assigned 168 patients with LSCC to two groups based on SIRT4 protein expression level using immunohistochemical staining, namely low SIRT4 expression (n=97) and high SIRT4 expression (n=71). And then Kaplan-Meier survival analysis was used to analyze the effect of DFS and OS in LSCC patients based on SIRT4 protein expression. We found that the 5-years DFS and OS in LSCC patients with low SIRT4 expression were significantly lower than that in LSCC patients with high SIRT4 expression (Fig. 4).

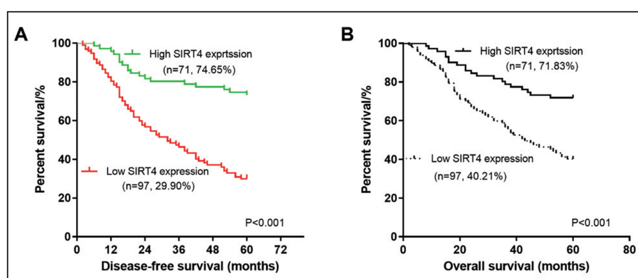

Fig. 4: Kaplan-Meier survival analysis of (A) DFS and (B) OS in LSCC patients, according to the SIRT4 protein expression. The log-rank test was used to calculate the p value.

Table 2: Cox regression analysis of influencing factors on the disease-free survival and overall survival of LSCC patients

	Disease-free survival			Overall survival		
	P	HR	95%CI	P	HR	95%CI
Univariate						
Age	0.523	0.560	0.280-0.987	0.385	0.395	0.119-0.682
Gender	0.329	0.383	0.325-0.967	0.657	0.958	0.864-1.659
Alcohol consumption	0.421	0.235	0.127-1.032	0.297	1.035	0.561-2.358
Smoking history	0.328	1.123	0.917-2.354	0.451	3.254	2.056-5.627
Histological grade	0.015	0.462	0.231-0.825	0.010	0.925	0.652-1.658
Primary tumor grade	0.056	1.235	0.954-1.658	0.072	1.562	0.564-2.035
T classification	0.038	2.341	1.250-3.264	<0.001	1.824	1.231-2.652
Lymph node metastasis	<0.001	0.892	0.234-2.385	0.002	1.035	0.523-1.967
SIRT4 levels	0.0152	2.567	1.235-3.024	<0.001	3.021	2.522-5.647
Multivariate						
Histological grade	0.008	2.635	1.523-5.624	0.002	3.015	1.625-6.238
T classification	0.058	1.235	0.689-2.152	0.128	1.568	1.023-3.124
Lymph node metastasis	<0.001	2.058	1.244–3.405	<0.001	2.138	1.652-3.056
SIRT4 levels	0.025	0.562	0.129-0.834	0.019	0.628	0.267-0.935

3. Discussion

So far, except for mitochondria, SIRT4 has not been found anywhere else and has been found to be closely related to mitochondrial energy metabolism. As a NAD^+ dependent ADP-ribose transferase, SIRT4 responds to pressure changes in the availability of cellular nutrients by controlling mitochondrial energy (Haigis et al. 2006). Importantly, DNA damage elicits a cellular signaling response that initiates cell cycle arrest and DNA repair. However, if cell growth is uncontrolled after DNA damage, damaged cells continue to proliferate and can cause cancer (Friedberg 2003; Hoeijmakers 2009). When SIRT4 works normally, SIRT4 senses energy metabolism after DNA damage, blocks cell cycle and inhibits tumor formation by blocking glutamine metabolism (Jeong et al. 2013). In this study, we found that the expression of SIRT4 in LSCC tissues was significantly lower than that in normal tissues, the expression of SIRT4 was lowly expressed LSCC tissues, and was significantly related to histological grade, T classification, clinical stage, lymph node metastasis and recurrence of LSCC patients.

Previous research found that SIRT4 is a potential target for fighting tumors (Huang and Zhu 2018; Jeong et al. 2014). It was found that SIRT4 was lowly expressed in malignant tumor tissues as in our study, such as Miyo et al. (2015) found that SIRT4 was lowly expressed in human colorectal cancer tissues, and inhibited the proliferation, migration and invasion of colorectal cancer cells through inhibition of glutamine metabolism (Miyo et al. 2015). Similarly, SIRT4, which was lowly expressed in gastric cancer tissues, was also found to inhibit the proliferation, invasion and migration of gastric cancer cells (Sun et al. 2018). In animal research, SIRT4-knockout mice developed lung cancer spontaneously in 15 months (Jeong et al. 2013) and loss of SIRT4 accelerated development of Myc-induced B-cell lymphoma in SIRT4-knockout mice (Jeong et al. 2014); In addition, SIRT4-knockout rats had more aneuploidy, showing increase in genomic instability (Choi and Mostoslavsky 2014).

To study the function of SIRT4 on LSCC cells, we established a LSCC cell line that knocked down or overexpressed SIRT4, and the data from *in vitro* tests suggested that SIRT4 knockdown promoted cell proliferation, invasion and migration of LSCC cells, while overexpression of SIRT4 inhibited them. Although we have

not conducted further research on the functional mechanism of SIRT4 to inhibit the proliferation and migration of LSCC cells, previous research provides us with some references. Under normal physiological conditions, SIRT4 stabilizes cAMP responsive element binding 2 by sensing the mammalian target of rapamycin complex 1, regulating glutamate dehydrogenase activity and glutamine metabolism (Csibi et al. 2013; Menon and Manning 2008). Therefore, loss of SIRT4 would increase glutamate dehydrogenase activity and glutamine metabolism (Menon and Manning 2008). Glutamine is a metabolite necessary for cell proliferation and promoting the transition of cells from G1 phase to S phase, while rapid proliferation is one of the characteristics of tumor cell metabolism (Colombo et al. 2011). Therefore, the low expression of SIRT4 in tumor cells promotes glutamine into the TCA cycle, increases cell energy supply, accelerates the cell cycle, and enhances cell proliferation, invasion, and migration capabilities.
Our current data, together with previous reports (Akoğlu et al. 2005; Elsheikh et al. 2006), suggested that cell proliferation and metastasis was inversely correlated with the prognosis for patients with LSCC. In this study, we found that the expression of SIRT4 protein was an independent factor affecting the DFS and OS of LSCC patients, and the 5-years DFS and OS in LSCC patients with low SIRT4 expression were significantly lower than that in LSCC patients with high SIRT4 expression. In conclusion, our data suggested that SIRT4 was lowly expressed in LSCC patients, and was related to development and prognosis of LSCC patients. However, due to the lack of specific molecular mechanism research, our data can only indicate that SIRT4 is a potential diagnostic indicator of LSCC, but whether it can be used for treatment requires further research.

4. Experimental

4.1. Specimens and ethics statement

There were 168 pairs LSCC tissues and normal tissue adjacent to tumor tissues in the present study, these tissues were collected from January 2011 to December 2014 at the Department of Otolaryngology in The Fourth Hospital of Wuhan, Wuhan, Hubei, China. The LSCC donors who donated the above tissues for the present study had to follow these inclusion criteria: Signed informed consent; no history of treatment before obtaining tissue: including chemotherapy, radiotherapy, and targeted therapy; no other malignant tumors; all clinical data in Table 1 are complete; 5-year follow-up completed. In addition, the present study was reviewed and supervised by the Ethics Committee of The Fourth Hospital of Wuhan.

4.2. RT-qPCR analysis

The total RNA from tissues and cells was extracted by RNAiso Plus (TARAKA, Japan), and reverse transcribed into cDNA using a cDNA kit (TARAKA, Japan). At last, 20 µL of qPCR system was prepared and analyzed as described in the instructions of GoTaq qPCR Master Mix (promega, USA). The relative expression of mRNA was calculated by $2^{-\Delta\Delta Ct}$ method, and β-actin was used as a loading control. Primers was showed as follow: SIRT4-forword: 5'-GCTTTGCGTTGACTTTCAGGT-3', SIRT4-reverse: 5'-CCAATGGAGGCTTTCGAGCA-3'; β-actin-forward: 5'-GGCTGTATTCCCCTC-CATCG-3', β-actin-reverse: 5'-CCAGTTGGTAACAATGCCATGT-3'.

4.3. Immunoblotting

We extracted the total protein from cells and tissues using a Total Protein Extraction Kit (Applygen, China), and detected the concentration of total protein using a BCA kit (Beyotime Biotechnology, China). A total of 40 µg protein was analyzed using a 10% SDS-PAGE. Next, the protein was transferred into a PVDF membrane (Thermo Fisher Scientific, USA). After blocking with 5% BSA for 2 h at room temperature, the SIRT4 antibody (ABCAM, UK) was added to incubate over night at 4 °C. After washing with PBS for 3 times, the secondary antibody which was conjugated with HRP was added to incubate for 1 h at room temperature. At last, Imag J 3.0 (IBM, USA) was used to analyze the gray value of protein band and β-actin was loaded as control.

4.4. Immunohistochemical staining

We detected the expression of SIRT4 protein in tissues using immunohistochemical staining as previously described. Briefly, the slices are first blocked by 5% BSA for 1 h at room temperature, and then the antibody target to SIRT4 (ABCAM, UK) was added to incubate over night at 4 °C, and the second antibody was incubated at room temperature for 1 h. No color was negative (0), faint yellow or yellow was weak positive (1), yellow was positive (2), brown-yellow or tan was strongly positive (3). At the same time, count the proportion of positive cells in each field of view. Immunohistochemical score of protein = color score × positive cell ratio. In this study, an immunohistochemical score of protein > 1.5 was considered to be high expression.

4.5. SIRT4 knockdown and overexpression

AMC-HN-8 cell was purchased from Shanghai Honsun Biological Technology Co., Ltd, China. We cultured AMC-HN-8 cells in DMEM medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gbico, USA) at 37 °C with 5% CO_2. To knock down SIRT4, we used small interfering RNA (siRNA), 50 nmol/l of si-SIRT4 (forward: 5'- AUUCUAGAGAAAAAGAAGCCU-3', reverse: 5'- GCUUCU-UUUUCUCUAGAAUGA-3'.) was directly transfected into 2.5 x 10^6 AMC-HN-8 cell using Lipofectamine 2000 (Thermofisher, USA) according to the manufacturer's protocols, and si-NC (forward: 5'- UGAUGGAACGCUGUAACCUAAG-3', reverse: 5'- GCUUCUUUUUCUCUAGAAUGA-3'.) was used as negative control. We performed experiments 72 h after transfection. To overexpress SIRT4, we first cloned the SIRT4 gene sequence (NCBI Reference Sequence: NC_000012.12) into pLenti-C-Myc-DDK-IRES-Puro Tagged Cloning Vector (ORIGEN, USA), and then used Lentiviral Packaging Kits (ORIGEN, USA) to prepare lentiviruses, and infected the cells to establish a SIRT4 overexpressing cell line.

4.6. Cellular immunofluorescence

We seeded cells in the lab-tak chambered coverglass (Thermo Fisher Scientific, USA). Twelve hours later, we removed the cell culture medium and washed cells with PBS, and fixed with 4% formaldehyde for 15 min at room temperature. After being blocked with 5% of BSA in 0.3 Triton X-100 for 1 h at room temperature, SIRT4 antibody (ABCAM, UK) was added to incubate the cell overnight at 4 °C. After incubating with secondary antibody, all slides were counterstained the nucleus with 5 µg/mL DAPI for 5 min at room temperature. Slides were analyzed under a Leica TCS SP5 microscope (Leica microsystem) with the LAS AF Lite 4.0 image browser software.

4.7. Cell clone test

Cells (2 x 10^3) were seeded in the 6-well cell culture dish (Thermo Fisher Scientific, USA) to culture at 37 °C with 5% CO_2, cell culture medium was changed every 4 days. When we could see the cell clone, we removed the cell culture medium and washed cells with PBS, and fixed with 4% formaldehyde for 15 min. The supernatant was drawn, stained with 0.25% crystal violet for 25 min, and slowly rinsed with sterile water. Plates were placed in a sterile clean bench for drying, and pictures were taken after drying. The relative proliferation was determined by measuring the absorbance at 595 nm.

4.8. Transwell assay

Cells (4 x 10^5) were seeded in the upper chamber of a 24-well transwell plate (Corning, USA), and then 1 mL cell culture medium was added to the lower chamber, which contained 20% FBS (Gibco, USA). Cells were cultured for 24 h at 37 °C with 5% CO_2. And we removed the cell culture medium of lower chamber and washed cells with PBS, and fixed with 4% formaldehyde for 15 min. The supernatant was drawn, stained with 0.25% crystal violet for 25 min, and slowly rinsed with sterile water. Plates were placed in sterile clean bench for drying, and taken pictures after drying.

4.9. Cell scratch test

Cells (4 x 10^5) were seeded in the 24-well cell culture dish (Thermo Fisher Scientific, USA). Twelve hours later, we made a scratch with tips, and then washed away non-adherent cells with PBS. Next, we added 1.0 mL cell culture medium into the dish for 72 h at 37 °C with 5% CO_2. At last, we took pictures of the cells and counted the number of migrated cells.

4.10. Statistical analysis

We used graphpad prism 8 to analyze the data in this study. Paired t test and chi-square test were used to compare the difference between two groups, and we used one-way ANOVA with duncan test as *post hoc* test to compare the difference between multiple groups. Cox regression analysis was used to analyze the independent prognostic indicators for LSCC patients, and long rank test was used to compare the difference of 5-year DFS and OS based on SIRT4 expression in LSCC patients.

Conflicts of interest: None declared.

References

Ahuja N, Schwer B, Carobbio S, Waltregny D, North BJ, Castronovo V, Maechler P, Verdin E (2007) Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. J Biol Chem 282: 33583–33592.
Akoğlu E, Dutipek M, Bekiş R, Değirmenci B, Ada E, Güneri A (2005) Assessment of cervical lymph node metastasis with different imaging methods in patients with head and neck squamous cell carcinoma. J Otolaryngology 34: 384–394.
Allegra E, Caltabiano R, Amorosi A, Vasquez E, Garozzo A, Puzzo L (2013) Expression of BMI1 and p16 in laryngeal squamous cell carcinoma. Head Neck 35: 847–851.
Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J (2016) Cancer statistics in China, 2015. Ca Cancer J Clin 66: 115–132.
Choi JE, Mostoslavsky R (2014) Sirtuins, metabolism, and DNA repair. Current Opin Genet Devel 26: 24–32.
Colombo SL, Palacios-Callender M, Frakich N, Carcamo S, Kovacs I, Tudzarova S, Moncada S (2011) Molecular basis for the differential use of glucose and glutamine in cell proliferation as revealed by synchronized HeLa cells. Proc Natl Acad Sci USA 108: 21069–21074.

Csibi A, Fendt S-M, Li C, Poulogiannis G, Choo AY, Chapski DJ, Jeong SM, Dempsey JM, Parkhitko A, Morrison T (2013) The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. Cell 153: 840–854.

Elsheikh MN, Rinaldo A, Hamakawa H, Mahfouz ME, Rodrigo JP, Brennan J, Devaney KO, Grandis JR, Ferlito A (2006) Importance of molecular analysis in detecting cervical lymph node metastasis in head and neck squamous cell carcinoma. Head & Neck 28: 842–849.

Farshadpour F, Kranenborg H, Calkoen EVB, Hordijk GJ, Koole R, Slootweg PJ, Terhaard CH (2011) Survival analysis of head and neck squamous cell carcinoma: influence of smoking and drinking. Head Neck 33: 817–823.

Forastiere AA, Weber RS, Trotti A (2015) Organ preservation for advanced larynx cancer: issues and outcomes. J Clin Oncol 33: 3262–3268.

Friedberg EC (2003) DNA damage and repair. Oxid Damage Repair 421: 436–440.

Fu L, Dong Q, He J, Wang X, Xing J, Wang E, Qiu X, Li Q (2016) SIRT4 inhibits malignancy progression of NSCLCs, through mitochondrial dynamics mediated by the ERK-Drp1 pathway. Oncogene 36: 2724–2736.

García-Fernández E, Diego JID, Collantes-Bellido E, Mendiola M, Hardisson D (2011) Aurora B kinase expression in laryngeal squamous cell carcinoma and its prognostic implications. Histopathology 58: 368–376.

Guo L, Zhou SR, Wei XB, Liu Y, Chang XX, Liu Y, Ge X, Dou X, Huang HY, Qian SW (2016) Acetylation of mitochondrial trifunctional protein α-subunit enhances its stability to promote fatty acid oxidation and is decreased in NAFLD. Mol Cell Biol 36: 2553–2567.

Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G (2006) SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic β cells. Cell 126: 941–954.

Hoeijmakers JHJ (2009) DNA damage, aging, and cancer. New Engl J Med 361: 1475–1485.

Hu Q, Qin Y, Ji S, Xu W, Liu W, Sun Q, Zhang Z, Liu M, Ni Q, Yu X (2019) UHRF1 promotes aerobic glycolysis and proliferation via suppression of SIRT4 in pancreatic cancer. Cancer Lett 452: 226–236.

Huang G, Lin Y, Zhu G (2017) SIRT4 is upregulated in breast cancer and promotes the proliferation, migration and invasion of breast cancer cells. Int J Clin Exp Pathol 10: 11849–11856.

Huang G, Zhu G (2018) Sirtuin-4 (SIRT4), a therapeutic target with oncogenic and tumor-suppressive activity in cancer. Onco Targets Ther 11: 3395–3400.

Jeong SM, Lee A, Lee J, Haigis MC (2014) SIRT4 protein suppresses tumor formation in genetic models of myc-induced B cell lymphoma. J Biol Chem 289: 4135–4144.

Jeong SM, Xiao C, Finley LW, Lahusen T, Souza AL, Pierce K, Li Y-H, Wang X, Laurent G, German NJ (2013) SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glutamine metabolism. Cancer Cell 23: 450–463.

Komlos D, Mann KD, Zhuo Y, Ricupero CL, Hart RP, Liu YC, Firestein BL (2013) Glutamate dehydrogenase 1 and SIRT4 regulate glial development. Glia 61: 394–408.

Laurent G, German NJ, Saha AK, Boer VCJD, Haigis MC (2013) SIRT4 coordinates the balance between lipid synthesis and catabolism by repressing malonyl CoA decarboxylase. Mol Cell 50: 686–698.

Lee KW, Kuo WR, Tsai SM, Wu DC, Wang WM, Fang FM, Chiang FY, Ho KY, Wang LF, Tai CF (2005) Different impact from betel quid, alcohol and cigarette: risk factors for pharyngeal and laryngeal cancer. Int J Cancer 117: 831–836.

Liu Y, Su Z, Li G, Yu C, Ren S (2013) Increased expression of metadherin protein predicts worse disease-free and overall survival in laryngeal squamous cell carcinoma. Int J Cancer 133: 671-679.

Marioni G, Marchese-Ragona R, Cartei G, Marchese F, Staffieri A (2006) Current opinion in diagnosis and treatment of laryngeal carcinoma. Cancer Treat Rev 32: 504–515.

Menon S, Manning BD (2008) Common corruption of the mTOR signaling network in human tumors. Oncogene 27 Suppl 2: S43–51.

Miyo M, Yamamoto H, Konno M, Colvin H, Nishida N, Koseki J, Kawamoto K, Ogawa H, Hamabe A, Uemura M (2015) Tumour-suppressive function of SIRT4 in human colorectal cancer. Brit J Cancer 113: 492–499.

Schorn VJ, Miles BA (2014) Laryngeal squamous cell carcinoma. In: Lin FY, Patel ZM: ENT board prep: High yield review for the otolaryngology in-service and board exams. pp. 227–233.

Sun H, Huang D, Liu G, Jian F, Zhu J, Zhang L (2018) SIRT4 acts as a tumor suppressor in gastric cancer by inhibiting cell proliferation, migration, and invasion. Onco Targets Ther 11: 3959–3968.