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Decreased adiponectin level is associated with aggressive phenotype of tongue squamous cell carcinoma

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Circulating adiponectin levels are inversely associated with risk of various obesity-related cancers. However, the effect of adiponectin on carcinogenesis and progression of tongue squamous cell carcinoma (TSCC) remains unknown. We measured serum adiponectin levels in 59 patients with TSCC and 50 healthy controls. Expression of adiponectin and its receptors in paired tumor and paracancerous specimens were determined by immunohistochemical staining (n = 37) and western blot (n = 30), respectively. Serum adiponectin level was lower in patients than in controls (5.0 ± 2.4 *vs* 8.4 ± 3.5 μg/mL, P < 0.01), and was inversely associated with histological grade and lymph node metastasis but not tumor size. Local adiponectin levels in tumor tissue gradually decreased as tumor-node-metastasis stage increased, while the expression of adiponectin receptors was unchanged. In addition, serum adiponectin levels in the TSCC patients without metabolic and cardiovascular diseases, or without smoking and drinking habits, were still lower than in controls. Furthermore, adiponectin inhibited the migration, but not proliferation, of SCC15 cells *in vitro*. These results indicate that a decreased adiponectin level is associated with risk of TSCC. Hypoadiponectinemia might be used as a biomarker to predict an aggressive phenotype of TSCC. (*Cancer Sci*, doi: 10.1111/cas.12077, 2013)

Tongue squamous cell carcinoma (TSCC) is one of the most common cancers in the oral cavity and is characterized by rapid growth, diffuse invasion, high propensity for cervical nodal metastasis and high recurrence.[1] Lymph node metastasis affects the probability of regional control and is the strongest prognostic factor for survival with TSCC.[2] Despite refinement of surgical techniques and chemotherapy or radiotherapy, 5-year survival is still unsatisfactory.[3] Considerable epidemiological evidence indicates that smoking, alcohol intake, chronic mechanical stimulation and betel quid chewing are associated with the incidence of TSCC,[4,5] but the molecular mechanisms responsible for TSCC remain unclear. Furthermore, TSCC has become increasingly prevalent among young and middle-aged populations.[5] Therefore, it is necessary to develop new strategies to improve the diagnosis and therapy for TSCC.

Adiponectin is an adipokine produced predominantly by adipocytes that circulates abundantly in plasma.[6] Adiponectin acts through two different membrane-bound adiponectin receptors, AdipoR1 and AdipoR2, and functions as an anti-diabetic, anti-atherogenic, anti-inflammatory and anti-angiogenic hormone.[7,8] Circulating adiponectin levels are lower in patients with obesity, type 2 diabetes and coronary artery disease.[8,9] Hypoadiponectinemia may be a biomarker for metabolic and cardiovascular diseases. Recent epidemiological studies indicate that hypoadiponectinemia is associated with risk of various cancers, including breast, endometrial, and colorectal cancers.[10–12] In addition, adiponectin has anti-proliferative and pro-apoptotic effects on breast cancer cells.[13] These results suggest that adiponectin plays a direct and/or indirect role in carcinogenesis and progression of obesity-related cancers. However, serum adiponectin level is unchanged in lung cancer and even increased in pancreatic and hepatocellular carcinoma.[14–16] Therefore, the exact roles of adiponectin in cancer development require further investigation.

Despite numerous reports indicating the important roles of adiponectin in various obesity-related cancers, a potential association between adiponectin and nonobesity-related cancers, such as TSCC, has not been explored. Specifically, the expression of adiponectin receptors in tongue tissue remains unknown. We hypothesized that changes in adiponectin and its receptors may be linked to carcinogenesis and progression of TSCC. Here, we investigated serum and local adiponectin levels as well as the expression of AdipoRs in TSCC, and evaluated their association with clinicopathologic features of TSCC. Moreover, we examined the effects of adiponectin on proliferation and migration of SCC15, a TSCC cell line.

Materials and Methods

Patients and samples. We included 59 TSCC patients from the Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology from January 2010 to November 2011. None of the patients had received preoperative radiation or chemotherapy. A self-administered questionnaire was used to assess lifestyle characteristics and disease histories. The extent of smoking was evaluated by quartiles of the smoking index, calculated by multiplying cigarettes per day and number of smoking years, as previously described.[17] Surgically-removed tongue tissue was routinely processed and divided into tumor and adjacent non-malignant epithelium (paracancer), collected at sites at least 2 cm away from the tumor mass.[18] In addition, 50 age-matched and gender-matched healthy controls (during routine health screening) were recruited as volunteers. The study was approved by the Ethics Committee of the Peking University Health Science Center, and all participants signed an informed consent before blood and tissue collection.

Measurement of blood biochemical variables. Fasting blood glucose, total cholesterol and triglyceride levels were measured

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using a model 7180 clinical autoanalyzer (Hitachi, Tokyo, Japan). Adiponectin in serum and culture medium was assessed by enzyme-linked immunosorbent assay using a commercially available kit (Adipobiotech, Santa Clara, CA, USA). Serum insulin level was measured using an I^{125}-radioimmunoassay kit (BNIBT, Beijing, China).

Histopathology. The histological grade and clinical stage of tumors were determined based on the criteria of the World Health Organization classification and the TNM system.[19,20] For immunohistochemistry, consecutive 4-μm thick sections were incubated with primary antibodies against adiponectin (1:100, ab22554; Abcam, Cambridge, UK), AdipoR1 (aa 357–375, H-001-44), AdipoR2 (aa 374–386, H-001-23) (both 1:200; Phoenix Pharmaceuticals, Belmont, CA, USA) or CK AE1/AE3 (ZM0069; Zhongshan Laboratories, Beijing, China) at 4°C overnight, then with HRP-conjugated secondary antibodies. Immunoreactions were visualized with 3-3′ diaminobenzidine tetrahydrochloride. Immunohistochemical staining was evaluated using a semi-quantitative immunoreactivity score, as previously reported.[21] Image analysis was performed using ImageJ 1.44 software (NIH, Bethesda, MD, USA) and the LEICA 550IW system, with five randomly chosen fields from each section, as previously described.[22]

RT-PCR. Total RNA of SCC15 cells was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA was generated from 5 μg of total RNA with M-MLV reverse-transcriptase (Promega, Madison, WI, USA). The sense and antisense primers used were 5′-CATGACCAGGAAACCACGACT-3′, 5′-TGAATGCTGAG CGGTAT-3′ for adiponectin, 5′-ACGTTGGAGAGTCATCCC GTAT-3′, 5′-TCTTGAAGCAAGCCCGAAAG-3′ for AdipoR1, and 5′-AGCCTCTATATCACCGGAGCTG-3′, 5′-GCTGATG AGAGTGAAACCAGATGT-3′ for AdipoR2. The PCR products were electrophorosed on a 1.5% agarose gel and stained with ethidium bromide.

Western blot analysis. Protein concentrations in extracts of tissue or cells were measured, as previously described.[23] Equal amounts of proteins (40 μg) were separated on 12% SDS-PAGE and transferred to polyvinylidene difluoride membrane. The membrane was blocked with 5% non-fat milk, and probed with primary antibodies against adiponectin, AdipoR1 or AdipoR2 (all 1:1000) at 4°C overnight, then probed with HRP-conjugated secondary antibodies. Immunoreactive bands were visualized by enhanced chemiluminescence (Pierce, Rockford, IL, USA).

Cell culture and proliferation assay. SCC15 cell line was purchased from American Type Culture Collection (Manassas, VA, USA) and cultured according to standard protocols. The effect of adiponectin on cell proliferation was evaluated by a colorimetric assay using the CellTiter 96 AQ_{ueous} One Solution (MTS) reagent (Promega). Briefly, SCC15 cells were grown on 96-well plates (8×10^3 cells at 100 μL/well) and cultured with or without 5% FBS in the presence or absence of globular adiponectin (gAd, 0.5–8 μg/mL; Peprotech, Rocky Hill, NJ, USA) for 48 h, before undergoing MTS assay. All experiments were performed at least three times in triplicate.

Migration assays. Migration of SCC15 cells was measured by transwell and wound-healing assays. For the transwell assay, cells (3×10^5) were added to the upper chamber of a 24-well cell culture chamber (8-μm pore size; Corning, New York, NY, USA) in 100 μL serum-free medium with or without 1–4 μg/mL gAd. After 24 h, cells were fixed with 4% paraformaldehyde, stained with 0.2% crystal violet (Amresco, Solon, OH, USA) and photographed. For wound-healing assay, SCC15 cells were grown to confluence in six-well plates and the bottom of monolayer cells was scraped off using a sterile p200 pipette tip. Cells were then treated with gAd (1–4 μg /mL) in the presence of 1% FBS and allowed to migrate to the denuded area for 24 h. For each well, four randomly selected regions were photographed (Olympus CKX41, Tokyo, Japan) at 0 and after 24 h of incubation, and the relative speed of migration was measured by the mean linear movement speed of wound edges over 24 h.

Statistical analysis. Characteristics of patients and controls are presented as percentage (%) or mean ± SD. For comparison of two groups, categorical variables were analyzed by chi-square-test and continuous variables by *t*-test. Comparison of more than two groups involved one-way ANOVA followed by Fisher's least significant difference test. Pearson's correlation coefficient was used to examine correlation among variables. Univariate and multivariate logistic regression models were used to assess the association of adiponectin with risk of TSCC. The odds ratio (OR) and 95% confidence interval (95% CI) were estimated as described previously.[24] In these models, case control was used as the outcome variable, while adiponectin levels (in increments of 1 SD of the hormone among controls) along with classical epidemiological risk factors of TSCC and potential confounders were used as predictor variables. Risk factors considered in the study were age, cigarette smoking and alcohol intake; potential confounders were insulin, fasting blood glucose and body mass index (BMI). $P < 0.05$ was considered significant. All analyses were conducted using SPSS v11.5 (SPSS, Chicago, IL, USA).

Results

Clinical characteristics of subject. There was no significant difference in age, gender, BMI, total cholesterol, triglyceride and insulin levels between controls and TSCC patients (Table 1). The patients were more likely to smoke heavily, to drink, and to have a history of diabetes mellitus, hypertension and heart disease. Fasting blood glucose was higher in patients than in controls.

Serum adiponectin level reduces in tongue squamous cell carcinoma patients. Serum adiponectin level was lower in patients than in controls (5.0 ± 2.4 *vs* 8.4 ± 3.5 μg/mL, $P < 0.01$, Fig. 1). In addition, TSCC patients with no history of metabolic and cardiovascular diseases, or without smoking and drinking habits also had lower adiponectin levels than the controls. Serum adiponectin level was inversely correlated with weight, BMI, fasting blood glucose or insulin level in the controls, but not in the TSCC patients (Table 2).

Decreased serum adiponectin levels are associated with increased risk of tongue squamous cell carcinoma. To further establish association between serum adiponectin levels and TSCC risk, we performed univariate analysis, which revealed that low serum adiponectin ($P < 0.001$), fasting blood glucose ($P = 0.007$), heavy smoking (smoking index ≥ 900, $P = 0.017$) and alcohol intake ($P < 0.001$) were positively associated with TSCC (Table 3). After adjustment for these factors, multivariate analysis revealed that decreased serum adiponectin was still associated with increased risk of TSCC ($P < 0.001$). Furthermore, the same association exists in patients without metabolic and cardiovascular diseases ($P = 0.007$). In addition, in both entire and subgroups, reduced BMI and alcohol intake were significantly associated with increased risk of TSCC.

Serum adiponectin levels are inversely associated with histological grade and lymph node metastasis. Histopathologic characteristics were assessed by H&E staining, while epithelial tumor cells were identified using cytokeratin antibody CK AE1/AE3 immunostaining (Fig. 2). Serum adiponectin levels were lower in patients with moderately-differentiated and poorly-differentiated than well-differentiated TSCC (Fig. 3A), but were not correlated with tumor size (Fig. 3B). Moreover, adiponectin levels were lower in patients with lymph node

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Table 1. Clinical characteristics of tongue squamous cell carcinoma patients and controls

Characteristics	Controls (n = 50)	Cases (n = 59)	P-value
Age (years), mean ± SD	52.7 ± 11.6	57.2 ± 12.3	0.054
Sex			
Male	35 (70.0)	34 (57.6)	0.232
Female	15 (30.0)	25 (42.4)	
Body mass index (kg/m^2)			
<25	22 (44.0)	35 (59.3)	0.214
≥25, <30	27 (54.0)	22 (37.3)	
≥30	1 (2.0)	2 (3.4)	
Smoking index			
0	30 (60.0)	27 (45.8)	0.008†
1–344	8 (16.0)	4 (6.8)	
345–519	6 (12.0)	8 (13.6)	
520–899	3 (6.0)	6 (10.1)	
≥900	3 (6.0)	14 (23.7)	
Alcohol intake			
Yes	5 (10.0)	25 (42.4)	<0.001
No	45 (90.0)	34 (57.6)	
Diabetes mellitus			
Yes	0 (0.0)	8 (13.6)	0.007
No	50 (100.0)	51 (86.4)	
Hypertension			
Yes	0 (0.0)	35 (59.3)	<0.001
No	50 (100.0)	24 (40.7)	
Heart disease			
Yes	0 (0.0)	9 (15.3)	0.004
No	50 (100.0)	50 (84.7)	
Fasting blood glucose (mM), mean ± SD	4.9 ± 0.7	5.5 ± 1.2	0.003
Total cholesterol level (mM), mean ± SD	4.7 ± 1.0	4.8 ± 1.0	0.542
Triglycerides level (mM), mean ± SD	1.4 ± 1.1	1.6 ± 0.7	0.397
Insulin level (μU/mL), mean ± SD	11.6 ± 5.8	13.9 ± 6.4	0.053

Data represent number (%) unless indicated. †P-value from chi-square-test for trend. Body mass index: calculated as weight in kilograms divided by the square of height in meters. Diabetes mellitus: self-reported history of diabetes mellitus or fasting glucose level >7.0 mM. Hypertention: self-reported history of hypertension or blood pressure ≥140 mmHg for systolic or 90 mmHg for diastolic blood pressure. Heart disease: self-reported history of coronary heart disease or pulmonary hypertension.

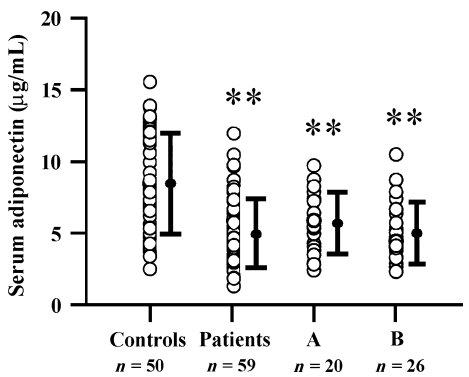

Fig. 1. Serum adiponectin levels in patients with tongue squamous cell carcinoma (TSCC) and controls. Serum adiponectin levels of controls, TSCC patients, patients without metabolic and cardiovascular diseases (A) or without smoking and drinking habits (B) were measured by ELISA. Open circles represent each subject and vertical lines indicate mean ± SD. **P < 0.01 *vs* control.

Table 2. Correlation of serum adiponectin with anthropometric and biochemical factors in cases and controls†

Characteristics	Serum adiponectin level			
	Controls (n = 50)		Cases (n = 59)	
	r	P-value	R	P-value
Age	−0.185	0.199	−0.035	0.793
Height	0.078	0.590	0.005	0.968
Weight	−0.403	0.004	−0.079	0.550
Body mass index	−0.538	<0.001	−0.079	0.553
Fasting blood glucose	−0.332	0.019	0.082	0.539
Insulin level	−0.391	0.005	0.092	0.488
Triglycerides level	−0.192	0.181	−0.198	0.133
Total cholesterol level	−0.117	0.417	−0.066	0.620

†Correlation determined by Pearson correlation.

metastasis of N1 and N2 stage than N0 stage (Fig. 3C). Taken together, reduced serum adiponectin levels were observed in TSCC patients with more severe histological grade or with lymph node metastasis.

Adiponectin and AdipoRs are expressed in tongue tissue. Immunohistochemical staining showed that expression of adiponectin was intense in epithelial cells of the stratum basale, but only marginal in the stratum spinosum, the most upper layers of the stratum superficial and the hyper-orthokeratotic surface (Fig. 4A). AdipoR1 and AdipoR2 staining appeared with similar distribution in the stratum basal and spinosum, and was occasionally detected in the stratum superficial or hyper-orthokeratotic surface (Fig. 4B,C). In well-differentiated tumor samples, the three proteins were co-localizated mostly in basolateral cells (Fig. 4D–F). In moderately-differentiated tumors, the proteins were expressed in almost all cells except for the keratin pearl (Fig. 4G–I), while in poorly-differentiated tumors, the proteins were widespread in all cancer cells (Fig. 4J–L). Their expression did not differ by histological grade of TSCC (data not shown). The expression of adiponectin and its receptors in human fat, muscle or rat liver served as positive controls (Fig. 4M–O).

Local adiponectin level decreases with increasing clinical stage of tongue squamous cell carcinoma. As Figure 5(A,B) show, the expressions of adiponectin were higher in tumors at stages I and II than in paracancer tissue, but gradually decreased with increasing clinical stage. Western blot analysis confirmed the immunohistochemistry results (Fig. 5C). AdipoR1 and AdipoR2 expression did not differ by clinical stage (data not shown).

Adiponectin inhibits migration of SCC15 cells. The mRNA and protein expression of adiponectin, AdipoR1 and AdipoR2 in SCC15 cells are shown in Figure 6(A,B). The level of secreted adiponectin in the culture medium was higher after incubation of SCC15 cells for 8 h (Fig. 6C). MTS results showed that gAd (0.5–8 μg/mL) had no effect on proliferation of SCC15 after incubation for 48 h with or without 5% FBS (Fig. 7A,B). Transwell migration assay showed that incubation with gAd (1–4 μg/mL) for 24 h markedly inhibited migration of SCC15 cells (Fig. 7C,E), which was further confirmed by wound healing assay (Fig. 7D,F).

Discussion

Our study presents three major novel findings. First, we demonstrated that serum adiponectin level was reduced in TSCC, and inversely associated with histological grade and lymph node metastasis of TSCC. Second, we identified the tongue as a new origin and target of adiponectin by characterizing the

Table 3. Univariate and multivariate logistic regression analysis of the association of serum adiponectin level and risk of tongue squamous cell carcinoma

Variables	Category or increment	Univariate model			Multivariate model		
		OR	95% CI	*P*-value	OR	95% CI	*P*-value
All the cases (n = 59) and controls (n = 50)							
Adiponectin level	1 SD among controls	0.34	0.21–0.55	<0.001	0.25	0.14–0.46	<0.001
Insulin level	1 SD among controls	1.45	0.99–2.14	0.059			
Fasting blood glucose level	1 SD among controls	1.69	1.15–2.47	0.007			
Age	10 years	1.37	0.99–1.90	0.057	1.52	1.00–2.31	0.049
Body mass index	2 kg/m^2	0.84	0.67–1.06	0.151	0.59	0.41–0.84	0.004
Smoking index	0	Baseline					
	1–344	0.56	0.15–2.06	0.378			
	345–539	1.48	0.46–4.82	0.514			
	540–899	2.22	0.51–9.76	0.290			
	≥900	5.19	1.34–20.02	0.017			
Alcohol intake	Yes versus no	6.62	2.30–19.07	<0.001	6.26	1.64–23.97	0.007
Cases (n = 20) and controls (n = 50) without diabetes mellitus, hypertension and heart disease							
Adiponectin level	1 SD among controls	0.55	0.31–0.97	0.039	0.38	0.19–0.77	0.007
Insulin level	1 SD among controls	1.13	0.69–1.87	0.626			
Fasting blood glucose level	1 SD among controls	1.16	0.71–1.91	0.551			
Age	10 yr	0.77	0.49–1.22	0.265			
Body mass index	2 kg/m^2	0.75	0.55–1.03	0.074	0.62	0.41–0.94	0.022
Smoking index	0	Baseline					
	1–345	0.42	0.05–3.79	0.437			
	345–539	2.78	0.68–11.28	0.153			
	540–899	1.11	0.10–12.04	0.931			
	≥900	4.44	0.84–23.66	0.080			
Alcohol intake	Yes versus no	6.00	1.66–21.71	0.006	5.60	1.27–24.65	0.023

CI, confidence interval; OR, odds ratio; SD, standard deviation.

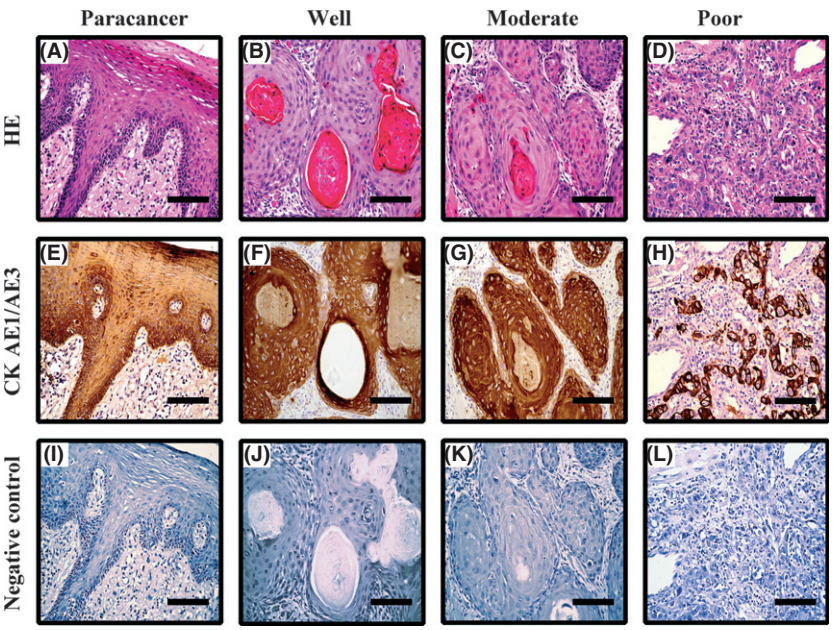

Fig. 2. Histopathology assessment of tongue squamous cell carcinoma (TSCC). Hematoxylin and eosin staining for paracancerous epithelium (A), well-differentiated (B), moderately-differentiated (C), and poorly-differentiated tumors (D). Immunohistochemical staining with anti-cytokeratin antibody CK AE1/AE3 to characterize epithelial tumor cells (E, F, G, H). Primary antibodies were omitted in negative controls (I, J, K, L). Bar = 100 μm.

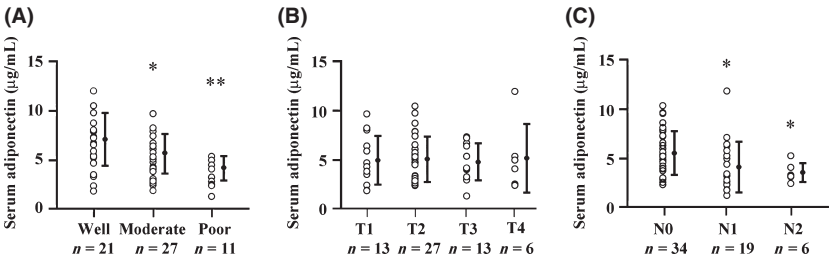

Fig. 3. Relationship between serum adiponectin level and clinicopathological features of tongue squamous cell carcinoma (TSCC). Serum adiponectin levels of TSCC with different histological grade (A), clinical T stage (B) and N stage (C). *P < 0.05; **P < 0.01 *vs* well-differentiated or N0.

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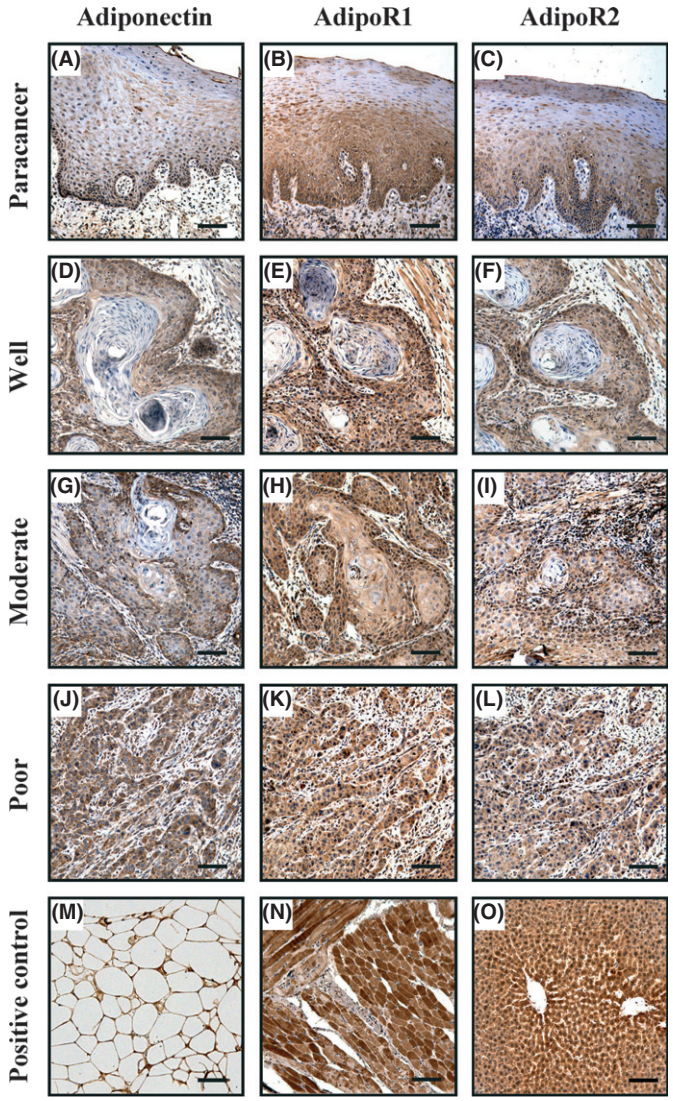

Fig. 4. Expression and distribution of adiponectin and its receptors in tongue tissue. Immunohistochemical staining of adiponectin (A, D, G, J), AdipoR1 (B, E, H, K), and AdipoR2 (C, F, I, L) in paracancer (A, B, C), well-differentiated (D, E, F), moderately-differentiated (G, H, I), and poorly-differentiated tumors (J, K, L). Tissues from human adipose (M), skeletal muscle (N) or rat liver (O) was used as positive controls, respectively. Nuclei were counterstained with hematoxylin (blue). Bar = 100 μm.

expression and distribution of adiponectin and its receptors in human tongue tissue and SCC15 cells. Moreover, the adiponectin levels in TSCC tissue were inversely associated with the clinical stages of TSCC, although adiponectin receptor levels remained unchanged. Third, adiponectin inhibited the migration of SCC15 cells *in vitro*. These results suggest that a decreased adiponectin level is associated with an aggressive phenotype of TSCC.

Although mainly secreted by adipose tissue, adiponectin levels are inversely correlated with BMI.[6] Recently, adiponectin has been identified as a novel risk marker in various obesity-related cancers, such as breast, endometrial, renal, colon and prostate cancer.[10–12,24,25] However, only a few studies have focused on the effect of adiponectin on nonobesity-related cancers. Serum adiponectin was found to be lower in esophagus squamous cell carcinoma, but unchanged in lung cancer.[14,26] In obese women, weight loss is associated with increased circulating adiponectin and reduced risk of breast cancer.[27] However, so far, obesity has not been associated with TSCC. In fact, most patients with TSCC lose weight due to difficulty chewing, swallowing and appetite loss, especially at later stages.[28] Here, we found that serum adiponectin level was only inversely correlated with weight, BMI, fasting blood glucose and insulin level in healthy controls, as reported previously,[24,29] but not in TSCC patients. These results are consistent with reports on gastric and esophageal cancer, and the impairment of this correlation might be due to the cachexic status of cancer patients.[26,30] Furthermore, serum adiponectin level was significantly lower in TSCC patients and inversely associated with the risk and severity of TSCC, suggesting that adiponectin has a potential role in the carcinogenesis and progression of this nonobesity-related cancer.

Serum adiponectin level is regulated by numerous physiological and pathological factors. Hypoadiponectinemia is closely associated with obesity, type 2 diabetes, dyslipidemia and cardiovascular disease.[8,9] Medications including thiazolidinediones, angiotensin-converting enzyme inhibitors and angiotensin type-1 receptor blockers have been shown to increase circulating adiponectin levels.[31–33] To account for possible interferences of existing diseases and medication, we performed further analysis in a TSCC subgroup without metabolic and cardiovascular diseases. Serum adiponectin level in patients without the related diseases was still lower than in controls, and remained inversely associated with the risk of TSCC. In addition, hypoadiponectinemia was found in individuals with smoking and drinking habits, both as major risk factors of TSCC.[34,35] However, hypoadiponectinemia still existed in patients without these habits. Consistently, numerous studies have indicated that hypoadiponectinemia is correlated with cancer risk even after adjustment for the potential confounders including BMI, diabetes, hypertension, smoking, or drinking in endometrial,[36] colorectal[12,17] and renal cancers.[24] Taken together, these results indicate that hypoadiponectinemia is associated with TSCC, which might not be due to the effect of related diseases, habits or medication.

Circulating adiponectin exists as a full length protein (fAd) as well as a biological active globular C-terminal domain (gAd). gAd exists as a trimer, while fAd exists as a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and a high-molecular-weight (HMW) multimer.[37] Recent studies show that a decreased HMW adiponectin level is related to increased risk of breast and liver cancers,[38,39] while there is no clear association between HMW adiponectin level and the risk of colorectal cancer.[40] Conversely, LMW level is inversely associated with risk of liver and colorectal cancers.[40,41] In the present study, adiponectin detected by ELISA was total adiponectin including both fAd and gAd. Hence, the correlation of serum adiponectin subtypes with TSCC should be further explored.

Recently, growing evidence has revealed that adiponectin and its receptors are ubiquitously expressed in various cell types, including breast, endometrium and colon cancer cells.[42] We identified the expression and distribution of adiponectin and its receptors in tongue tissue and SCC15 cells. Moreover, adiponectin could be secreted by SCC15. These results suggest that the tongue is a new origin and target of adiponectin, and that adiponectin might affect the metabolism and function of tongue tissue as via endocrine as well as paracrine and/or autocrine pathways. However, the exact effect of adiponectin on the tongue remains to be explored.

We evaluated the correlation of serum and local adiponectin levels with histopathologic features of TSCC. Hypoadiponectinemia was associated with moderately-differentiated and

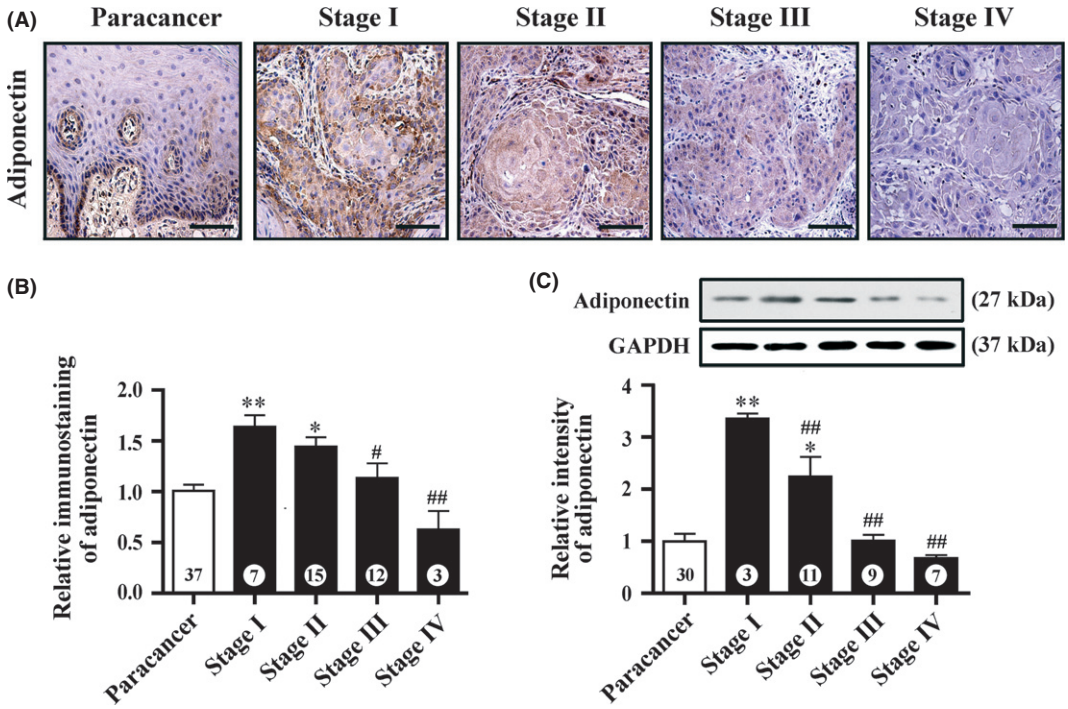

Fig. 5. Local adiponectin levels in different clinical stages of tongue squamous cell carcinoma (TSCC). (A) Representative immunohistochemical staining (A), relative immunohistochemical score (B), and representative western blot and quantitative analysis (C) of adiponectin in tongue tissue by TNM system. Bar = 100 μm. Data are mean ± SD from indicated sample numbers in the columns. $^{*}P < 0.05$; $^{**}P < 0.01$ *vs* paracancer, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ *vs* stage I.

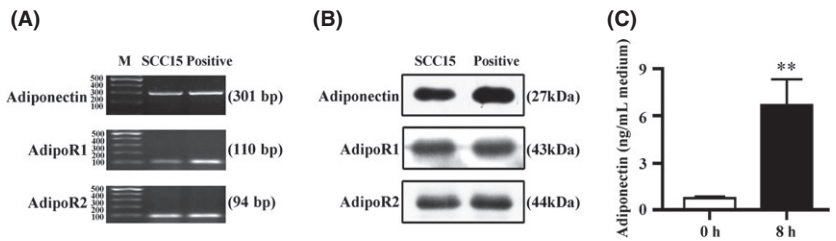

Fig. 6. The expression of adiponectin and its receptors in SCC15 cells. Expressions of adiponectin, AdipoR1, and AdipoR2 mRNA (A) and protein (B) in SCC15 cells were detected by RT-PCR and western blot. Human adipose, skeletal muscle or liver was used as positive control for adiponectin, AdipoR1 and AdipoR2, respectively. M: DNA marker. (C) Adiponectin levels in the medium were measured by ELISA after SCC15 cells were incubated for 8 h. Data are mean ± SD of three independent experiments. $^{**}P < 0.01$ *vs* 0 h.

poorly-differentiated TSCC and lymph node metastasis. Of note, although important in the TNM system, tumor size was not significantly associated with serum adiponectin level. It is likely that the rate of lymph node metastasis was not associated with increased tumor size, but rather more closely associated with the degree of differentiation in oral cancer, as described previously.[43] Compared with paracancer, TSCC tissues showed higher adiponectin levels at an early stage, when tumors were <4 cm and without lymph node metastasis. Studies of breast and colorectal cancers show similar results.[42,44] However, the reason for increased local adiponectin level remains unknown and might be an early response to the alteration of tumor microenvironment. More importantly, tissue levels of adiponectin gradually decreased with increasingly clinical stage, which was consistent with the inverse association of serum adiponectin level and TSCC grade and N stage. The expression of AdipoR1 and AdipoR2 in TSCC tissue was unchanged. These results suggest that hypoadiponectinemia is correlated with histopathologic features of TSCC, and might be a new biomarker of aggressive phenotype in TSCC.

Despite the inverse correlation between adiponectin and various cancers, the underlying mechanisms of adiponectin in potential cancer suppression are not fully elucidated. Both gAd and fAd exhibit potent anti-inflammatory and anti-atherosclerotic effects, and play important roles in regulating glucose and lipid metabolism.[45] gAd and fAd inhibit the proliferation of colorectal cancer cells.[46,47] However, gAd and fAd behave differently in inhibiting the proliferation of esophageal adenocarcinoma, breast and prostate cancer cells.[48–50] In the present study, gAd significantly inhibited the migration of SCC15 cells, but had no effect on their proliferation *in vitro*. This suggests that adiponectin, at least in globular form, might have a direct protective role in progression of TSCC.

In conclusion, we have provided the first evidence that serum adiponectin level is inversely associated with risk of TSCC in human. The decreased serum and local adiponectin levels were associated with clinicopathological characteristics of TSCC, notably histological grade, lymph node metastasis and clinical stage. Adiponectin could directly inhibit the migration of SCC15 cells. These findings may improve our understanding of the roles of adiponectin in the carcinogenesis

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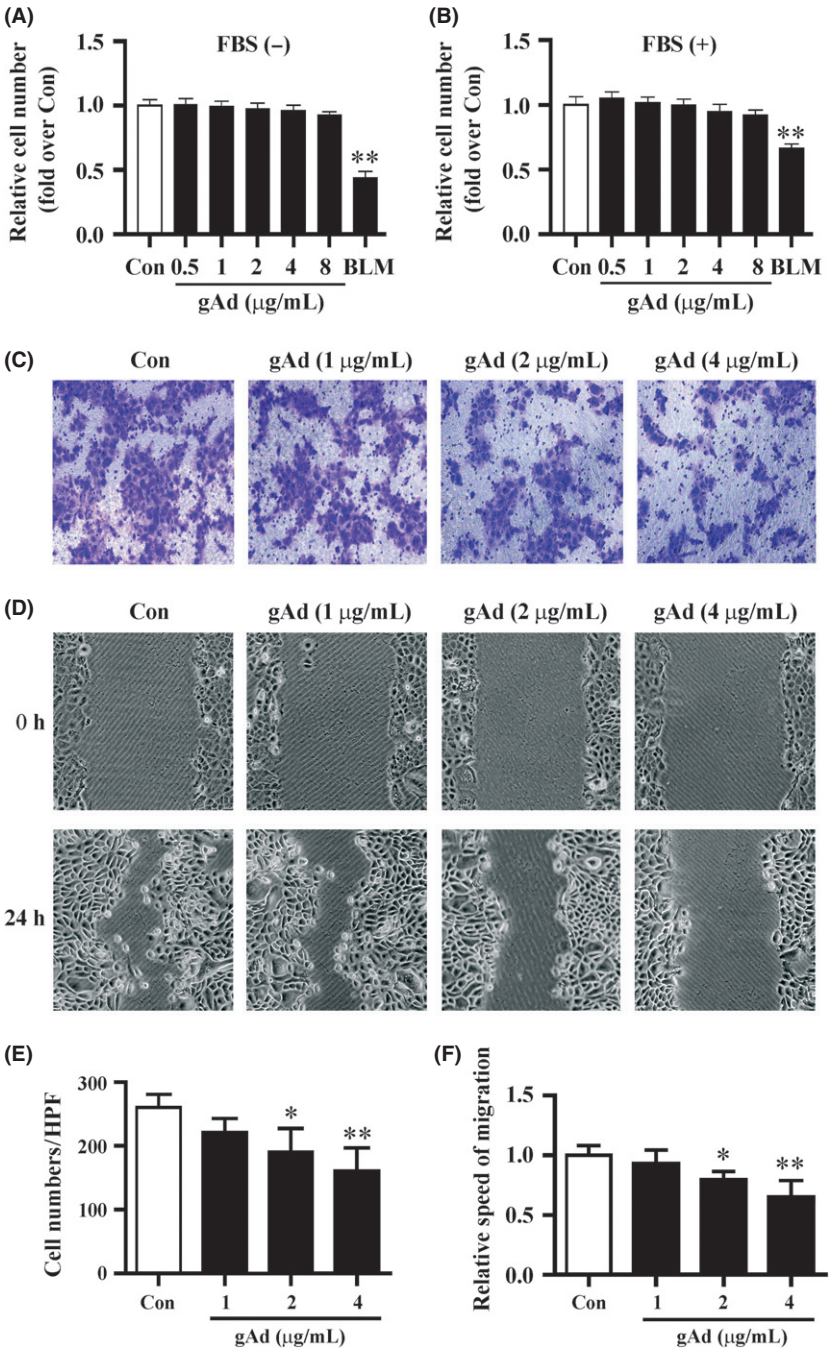

Fig. 7. Effects of adiponectin on proliferation and migration of SCC15 cells. SCC15 cells were cultured with gAd without (A) or with (B) 5% FBS for 48 h. Cell proliferation was measured from five independent experiments by MTS assay, using bleomycin (10 μg/mL) as positive control. (C) Representative images of cells migrating at 24 h. (D) Representative images of wound-healing assays at 0 and 24 h. (E) Migrated cells were quantified by averaging five randomly chosen high-power fields (HPF) of three independent experiments. (F) The relative speed of migration was measured by the mean linear movement speed of wound edges over 24 h. Data are mean ± SD of four separate fields of three independent experiments. Con, control, $*P < 0.05$; $**P < 0.01$ *vs* control.

and progression of TSCC and provide insights for novel diagnostic and therapeutic targets in TSCC.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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