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Original Article

Histone demethylase GASC1, a potential prognostic and predictive marker in esophageal squamous cell carcinoma

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Abstract: Gene amplified in squamous cell carcinoma 1 (GASC1) is a member of Jumonji C-domain containing histone demethylases that play an essential role in affecting chromatin architecture and gene expression. The purpose of this study was to determine the expression features and the clinical significance of GASC1 in esophageal squamous cell carcinoma (ESCC). GASC1 expression was detected on tissue microarrays of ESCC samples in 185 cases using immunohistochemical staining. Strong nuclear staining for GASC1 was observed in a subset of ESCC samples. The nuclear expression of GASC1 was significantly associated with lymph node metastasis (P=0.030) and tumor-node metastasis stages (P=0.013). Kaplan-Meier survival analysis showed a tendency that high expression of GASC1 in the nucleus was associated with poor survival of ESCC patients, with a 5-year survival rate of 26.5%, as compared to 43.7% for patients with GASC1-negative/low expression. Furthermore, multivariate analysis revealed that high expression of GASC1 likely acts as a predictive factor for overall survival of ESCC patients, despite the P-value failing to reach significance (P=0.059). The findings indicate that histone demethylase GASC1 may play an important role in promoting cancer metastasis, and shed new light on the importance of targeting GASC1 to suppress metastatic disease in various tumor types, including ESCC.

Keywords: Histone demethylase, GASC1, lymph node metastasis, immunohistochemistry, esophageal squamous cell carcinoma

Introduction

Esophageal cancer is the eighth most common cancer in the world and the fourth most common in China with a high mortality rate [1, 2]. Amongst all histological subtypes, esophageal squamous cell carcinoma (ESCC) is the predominant one in Asia [3]. The overall 5-year survival rate of ESCC is less than 10% [4]. Diagnosis of ESCC at its early stages still remains difficult, and advanced ESCC frequently displays local invasion and lymph node metastasis, which is one key reason for its poor prognosis and low survival rate [5]. Recently, the treatment of cancer using molecular targets has brought promising results and attracted more attention. Thus, characterization of genes involved in the development and progression of ESCC may improve patient outcomes by enabling development of better biomarkers for patient risk stratification and novel targeted therapies.

Gene amplified in squamous cell carcinoma 1 (GASC1, also known as KDM4C and JMJD2C) was originally cloned from an amplified region at 9p24 in ESCC lines [6]. Later studies showed that GASC1 amplification/overexpression occurs in various tumor types, including lymphoma, medulloblastoma, lung, prostate, and breast cancers [7-11]. The GASC1 protein, which contains an N-terminal Jumonji catalytic domain as well as C-terminal plant homeo

Table 1. Patient Characteristics

Clinical Parameters	No.
Specimens	185
Mean age	54.8
Age	
≤54	82
>54	103
Gender	
Male	122
Female	63
Tumor size	
≤3 cm	57
3-5 cm	96
>5 cm	32
Differentiation grade	
G1	46
G2	119
G3	20
G4	0
Invasive depth	
Tis	0
T1	3
T2	37
T3	143
T4	2
Lymph node metastasis	
N0	123
N1	60
N2	2
N3	
TNM classification	
0	0
IA	2
IB	10
IIA	48
IIB	68
IIIA	50
IIIB	1
IIIC	0
IV	6

Staging System: 7th Edition of the AJCC Cancer Staging Manual.

domain (PHD) and Tudor domains, is a member of a newly discovered family of histone demethylases that are fundamental regulators of chromatin architecture and gene expression [12-15]. Very recently, abundant evidence indicates that GASC1 serves as a transforming oncogene in various tumor types. For example, overexpression of GASC1 in non-transformed mammary epithelial cells results in a transformed phenotype exhibiting increased proliferation, anchorage-independent growth, growth factor-independent proliferation, disorganization of cellular architecture, and increased mammosphere formation [11]. Furthermore, GASC1 knockdown significantly slows cell growth of breast cancer *in vitro* and in xenograft models [11, 16].

To date, however, the link between GASC1 expression and the clinical progression of ESCC is still unknown. Additional studies are needed to understand the expression features of GASC1 in ESCC, as well as to establish its clinical significance. Thus, based on these previous studies about the function of GASC1, in our current work we analyzed the correlation between GASC1 expression in ESCC cases and histological and clinical parameters of tumors to evaluate whether GASC1 expression features are of any prognostic value; data which may provide important information to guide tumor treatments.

Materials and methods

Patients and samples

For the retrospective study, archival formalin-fixed, paraffin-embedded samples from 185 primary ESCC patients, precancerous lesions and the normal esophageal mucosa samples were obtained from the Department of Pathology of Shantou Central Hospital from 1987 to 1997. The samples embedded in paraffin wax blocks underwent tissue microarray (TMA) construction before immunohistochemical staining. Information on various histopathological characteristics, shown in **Table 1** (Patient Characteristics), was obtained from the records of the Clinical Pathology Department. The follow-up for patients after esophageal resection continued until their deaths, and only patients who died from ESCC were included in the tumor-related deaths. Patients suffering from severe postoperative complications, other tumors, or death from other causes, were excluded. Furthermore, none of the patients underwent radiotherapy or chemotherapy treatment.

All of the tumors were confirmed as ESCC by pathologists in the Clinical Pathology Department of the Hospital, and the cases were classified according to the seventh edition of the tumor-node-metastasis (TNM) classification of the Union for International Cancer Control (UICC). Evaluation of tumor differentiation was based on histological criteria of the guidelines according to the WHO Pathological Classification of Tumors. The study was approved by the Ethics committee of the Center Hospital of Shantou City and the local ethics committee; only patients with written informed consent were included in the study.

Tissue microarray (TMA) construction

Tissue microarray construction of esophageal carcinoma tissue has been described earlier [17]. Briefly, TMAs for immunohistochemistry were generated from samples selected from those specimens with more tissue available for persistent correlative studies. In advance, representative regions of each tissue were singled out of hematoxylin-and eosin-stained sections and marked on the individual paraffin blocks. At least two tissue cores were acquired from each specimen, measuring 1.8 mm in diameter and 1.0–3.0 mm in length, depending on the depth of tissue in the donor block. Each core was precisely arrayed into a new paraffin block. These microarrays were serially sectioned (4 μm) and stained with hematoxylin and eosin to ensure tissue sampling and completeness. The unstained sections were baked overnight at 56°C in preparation for immunohistochemistry staining.

Immunohistochemical analysis

Rabbit polyclonal GASC1 antibody (dilution 1:100 in PBS, Abcam) was performed in this study. After de-waxed in xylene and rehydrated in a series of graded alcohols, TMA sections (4 μm) were subjected to immunostaining in the SuperPicTure™ Polymer Detection Kit and the Liquid DAB Substrate Kit (Zymed/Invitrogen). Briefly, Antigen retrieval was performed by microwave oven heating (10 minutes) in 0.01 M sodium citrate buffer (pH 6.0). Slides were submerged in a Peroxidase Quenching Solution, containing 1 part of 30% hydrogen peroxide to 9 parts of absolute methanol, for 10 minutes and were then washed with PBS for 2 minutes, 3 times. Next, 0.1 ml of serum blocking solution was added to each section, followed by incubation for 10 minutes, and then draining the solution. At this time, 0.1 ml of primary antibody was applied to each section and incubated in a moist chamber for 30 minutes. After rinsing with PBS for 2 minutes, 3 times, 0.1 ml of HRP Polymer Conjugate was added to each section, and incubated for 10 minutes, followed by a rinse with PBS. Finally, 0.1 ml of DAB chromogen was applied to each section and incubated for 3–10 minutes. The samples were rinsed well with distilled water. Thereafter, slides were counterstained with Maye's hematoxylin, dehydrated, and mounted. The negative controls were prepared by substituting PBS for the primary antibodies.

The immunohistochemical staining results were assigned a maximum score taking into consideration both the intensity of staining and the proportion of tumor cells showing unequivocal positive reaction. Positive reactions were defined as those showing brown signals in the cell cytoplasm, nucleus, and membrane. The intensity of staining: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. Tumor cells area: 0, positive staining in less than 5% of tumor cells; 1, positive staining in 5–25% of tumor cells; 2, positive staining in 26–50% of tumor cells; 3, positive staining in 51–75% of tumor cells; 4, positive staining in greater than 75% of tumor cells. For statistical analyses, a composite staining index (SI) was defined as the product of intensity and area scores, producing values from 0 to 12. Scores from 0–4 were considered ''-'' (negative staining), scores of 5–12 were considered ''+'' (positive staining). Negative staining represented low expression of the GASC1 protein while positive staining was defined as high expression of the GASC1 protein.

Statistical analysis

Associations of GASC1 expression and other clinicopathological characteristics, including age, gender, tumor size, differentiation, invasive depth, lymph node metastasis, and TNM classification, were assessed with Kendall's tau-b test. Kaplan-Meier curves were constructed for overall survival (OS) analysis using a log-rank test. OS was defined as the time

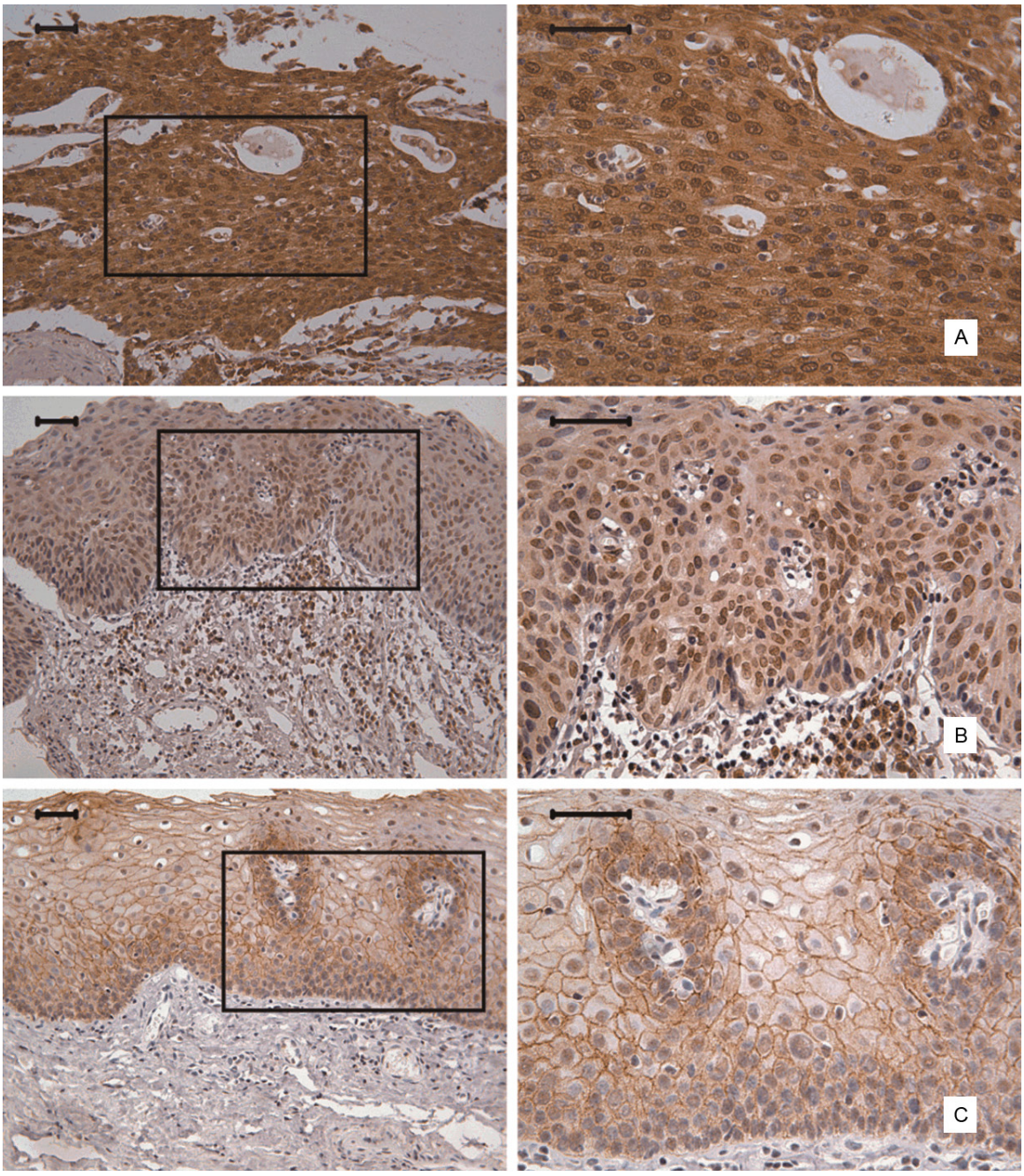

Figure 1. Results of immunohistochemical staining for GASC1. A: Strong immunoreactivity for GASC1 was observed in the nucleus of one repressive ESCC sample. B: Strong nuclear staining was also found in the cases of carcinoma *in situ*, while, C: weak or moderate GASC1 nuclear staining was observed in adjacent non-tumorigenic esophageal epithelium. A-C are all magnified versions (×400) of the corresponding embedded micrographs on the left (×200). The bar: 50 μm.

from the date of primary surgery to the date of death due to esophageal cancer. Data about survivors was censored at the last follow-up. The multivariate Cox proportional-hazards regression model was developed to correlate the clinical characteristics, survival, and the expression of GASC1. All analyses were performed with SPSS for Windows 13.0 software. Each value is two-tailed and the significance level is 0.05.

Table 2. Association between GASC1 expression and clinical data in ESCC for 185 patients

Clinical Parameters	Nucleus GASC1		*P*
	–	+	
Age (year)			
≤54	73	9	0.610
>54	95	8	
Gender			
Male	113	9	0.285
Female	55	8	
Tumor size			
≤3 cm	50	7	
3-5 cm	87	9	0.188
>5 cm	31	1	
Differentiation			
G1	41	5	
G2	110	9	0.994
G3	17	3	
Invasive depth			
T1	3	0	
T2	34	3	0.782
T3	129	14	
T4	2	0	
LN metastasis			
N0	116	7	0.030
N1+N2	52	10	
TNM classification			
IA+IB	11	1	
IIA+IIB	110	6	0.013
IIIA+IIIB+IIIC	43	8	
IV	4	2	

Statistical analysis: the Kendall's tall-b test; –: negative (score of (0-4)), +: positive (score of (5-12)). LN: lymph node.

Results

Correlation between GASC1 expression levels and clinicopathological parameters in ESCC patients

To determine the correlation between GASC1 expression levels and clinicopathological parameters in esophageal cancer, we performed immunohistochemical staining of a TMA containing 185 cases of primary esophageal cancer specimens. Clinicopathological characteristics of the patients can be found in **Table 1**. The median age of the population studied was 57.2 years (ranging from 34 to 73 years). Since GASC1 is a nuclear protein, we first focused on GASC1 nuclear expression in ESCC samples (**Figure 1A**). Immunohistochemical staining revealed a significant correlation between GASC1 nuclear expression levels and both lymph node metastasis (P=0.03) and tumor-node metastasis (TNM) (P=0.013) (**Table 2**). Cases with highly expressed nuclear GASC1 (scores between 5 and 12) were found in 8.3% (1 of 12) of TNM-I tumors, 5.2% (6 of 116) of TNM-II, 15.7% (8 of 51) of TNM-III, and 33.3% (2 of 6) of TNM-IV tumors. However, there were no significant correlations between GASC1 expression levels and other clinicopathological variables, including age, gender, tumor size, differentiation and invasive depth in patients with ESCC (**Table 2**).

We also performed GASC1 immunohistochemical staining in 22 adjacent non-tumor esophageal epithelium and 22 carcinoma in situ (CIS) samples. We found that weak or moderate GASC1 nuclear staining was observed in most normal squamous cell epithelia and hyperplasia lesions (**Figure 1C**), while strong nuclear staining was observed in the cases (7 of 22) of carcinoma in situ (CIS) (**Figure 1B**). Of interest, GASC1 protein staining was also observed in the membrane and/or cytoplasm in basal cells of the normal surface epithelia (**Figure 1C**). We found that GASC1 expression has a tendency to increase nuclear expression, while decreasing membrane/cytoplasm expression in the progression from normal epithelium to invasive carcinoma of the esophagus. Future investigations are required to validate this observation and more precisely address the possible roles of GASC1 expression for membranes and cytoplasm in normal epithelium.

Impact of GASC1 expression on the overall survival (OS) of ESCC patients

Having established the correlation between GASC1 expression and clinicopathological parameters, Kaplan-Meier survival analysis was next used to address the impact of GASC1 expression on the overall survival (OS) of ESCC patients. The survival information of the patients is shown in **Table 3**. High expression of GASC1 in the nucleus is likely associated with poor survival of ESCC patients (**Figure 2**). The Kaplan-Meier survival curve revealed a clear trend that patients with nuclear GASC1-positive tumors experienced accelerated death, with a

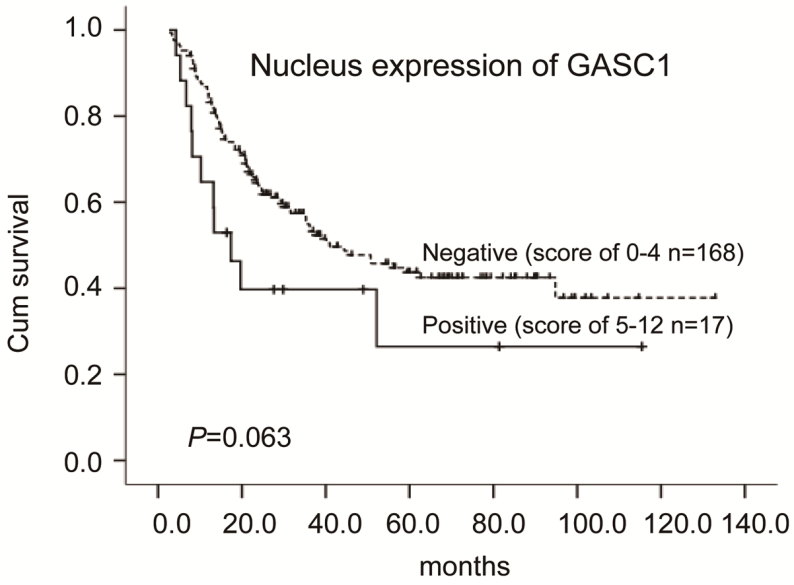

Figure 2. Overall survival of the patients with ESCC in relation to the expression level of GASC1 in the nucleus. Kaplan-Meier survival curve showing that patients with ESCC expressing high nuclear GASC1 have worse survival compared with patients with negative or low GASC1 expressing tumors.

5-year survival rate of 26.5%. That rate is compared to a 5-year survival rate of 43.7% for patients with GASC1-negative/low expression tumors (**Table 3**).

Univariate and multivariate analysis for the prognostic value of GASC1 expression

Finally, we included GASC1 expression in further prognostic value analysis. In univariate analysis, the following parameters were significantly associated with the overall five-year survival rate of the patients: tumor size (P=0.008), differentiation grade (P=0.007), invasive depth (P=0.000), lymph node metastasis (P=0.000), and TNM classification (P=0.000) (**Table 3**). Using the Cox proportional hazards model, we performed multivariate analysis to assess the independent predictive value of GASC1 nuclear expression for overall survival. The following prognostic variables: tumor size (P=0.026), invasive depth (P=0.002), and lymph node metastasis (P=0.016), can be used independently as prognostic indicators for ESCC patients (**Table 4**). Multivariate analysis revealed that high expression of GASC1 likely acts as a predictive factor for overall survival of ESCC patients, despite the P-value failing to reach significance (P=0.059), possibly due to limited patient number (**Table 4**). Thus, further studies are needed to test whether GASC1 acts independently as a prognostic marker in ESCCs.

Discussion

Using TMA, GASC1 was found to be highly overexpressed in a subset of ESCCs. More importantly, we have shown that GASC1 nuclear expression was significantly associated with TNM stages and metastasis, supporting the important role of this histone modifying factor in the progression of ESCC. This result was in keeping with previous studies demonstrating that GASC1 is overexpressed in a diverse array of human cancers, where it correlates with a poor prognosis for cancer patients [8-12, 16, 18-20]. The GASC1 gene was originally identified and cloned from the 9p24 amplicon of the ESCC line KYSE150 that was established from a poorly differentiated, aggressive ESCC in a 49-year old patient [6, 21]. In human breast cancer, GASC overexpression is more prevalent in the basal-type, which is particularly aggressive and accounts for a disproportionate number of breast cancer deaths [11]. Based on the MSKCC (Memorial Sloan-Kettering Cancer Center) and Oncomine databases, GASC1 expression increases with tumor stage and metastasis in prostate cancer [12, 22]. Furthermore, a large genetic epidemiological study revealed that variants in two SNPs of GASC1 are strongly associated with increased cancer risk of upper aerodigestive tract cancers, including oral cavity, pharynx, larynx, and esophagus [23].

In 2006, the GASC1 protein was discovered as a member of the Jumonji C domain-containing histone lysine demethylases [12-15]. Recent structural and biochemical studies indicated that GASC1, as well as its homologues KDM4A and B, catalyze the demethylation of H3K9me3/me2, and less efficiently (4-5 fold less than H3K9me3/me2), H3K36me3/me2 substrates; H3K9me3/me2 repressive methylation marks

Table 3. Survival information of the patient by clinical characteristics

Clinical Parameters	No.	Five Year Survival Rate (%)	*P*-Value
Age (year)			
≤54	82	43.5	0.538
>54	103	41.5	
Gender			
Male	122	41.4	0.918
Female	63	43.9	
Tumor size			
≤3 cm	57	49.9	0.008
3-5 cm	96	44.3	
>5 cm	32	22.0	
Differentiation grade			
G1	46	60.9	0.007
G2	119	38.8	
G3	20	19.3	
Invasive depth			
T1	3	66.7	0.000
T2	37	58.8	
T3	143	38.3	
T4	2	0.0	
Lymph node metastasis			
N0	123	50.5	0.000
N1+N2	62	25.6	
TNM classification			
IA+IB	12	59.5	0.000
IIA+IIB	116	51.9	
IIIA+IIIB+IIIC	51	18.8	
IV	6	16.7	
Nuclear expression of GASC1			
Low expression	168	43.7	0.063
High expression	17	26.5	

Statistical analysis: Kaplan-Meier curves (log-rank test); High expression: scores of 5-12. Low expression: scores of 0-4.

Table 4. Independent index of prognosis assessment by clinical characteristics

	Sig.	Exp(B)	95.0% CI for Exp(B)	
			Lower	Upper
Gender	0.624	1.118	0.716	1.745
Age	0.335	1.226	0.810	1.854
Tumor size	0.026	0.478	0.250	0.914
Differentiation grade	0.121	0.623	0.343	1.133
Invasive depth	0.002	0.021	0.002	0.248
Lymph node metastasis	0.016	1.794	1.115	2.886
Nucleus expression of GASC1	0.059	1.894	0.977	3.671

Statistical analysis: the multivariate Cox proportional-hazards regression.

are normally associated with gene silencing. Thus, GASC1 can function as a transcriptional activator by removing the euchromatic H3K9me3/me2 repressive marks [24]. Indeed, it has been shown that GASC1 regulates the expression of important cancer genes, such as MYC and MDM2, through demethylation of H3K9me3/me2 at corresponding promoter regions [20, 25, 26]. In addition, GASC1 interacts with hypoxia-inducible factor 1 (HIF1) and stimulates transcription of HIF-1 target genes, thereby promoting breast cancer growth and lung metastasis [16]. Furthermore, Loh *et al*. demonstrated that GASC1 enhanced the expression of Nanog, a cell-fate regulatory molecule known to be important for the self-renewal of embryonic stem cells [27]. Studies also demonstrated that overexpression of GASC1 enhances sphere formation, a characteristic property of stem/progenitor cells, in breast and colonic cancer cells by mediating expression of Wnt and Notch pathway genes [28].

A finding of particular interest from our current study is that GASC1 expression in the nucleus is significantly associated with metastatic progression of ESCC. Recent studies using loss- or gain-of-functions approaches indicate that histone demethylases, including GASC1, have critical roles in promoting cancer metastasis [29]. For example, with a functional *in vitro* small interfering RNA (siRNA) screening approach, Ding *et al*. identified several histone demethylases, including KDM4A and GASC1, that may be required for head and neck squamous cell carcinoma (SCC) metastasis [30]. Then, KDM4A was selected for a more detailed study, and it was demonstrated that KDM4A expression was significantly increased in human metastatic SCC in lymph nodes compared with that in primary human SCC. Furthermore, knockdown of KDM4A significantly inhibits the metastasis of SCC to cervical lymph nodes in a mouse model [30]. Another study by Luo *et al*. revealed that GASC1 knockdown inhibits breast tumor growth and

spontaneous metastasis to the lungs of mice following mammary fat pad injection of MDA-MB-435 cells [16]. Both studies suggest that histone demethylases KDM4A and/or GASC1 modulate histone methylation status and affect the expression of a set of key genes that are critical for cancer invasion and metastasis [16, 30].

In conclusion, this study has shown that GASC1 is over-expressed in a subset of primary ESCC samples and related to lymph node metastasis and TNM stages. We suggest that the GASC1 protein status might be a significant diagnostic biomarker for ESCC. Our study, together with others, indicates that histone demethylase GASC1 may play an important role in promoting cancer metastasis, and shed new light on the importance of targeting GASC1 to suppress metastatic disease in various tumor types, including ESCC.

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Disclosure of conflict of interest

No competing financial interests exist.

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References

[1] Kamangar F, Dores GM and Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 2006; 24: 2137-2150.

[2] Li L, Lu F and Zhang S. [Analysis of cancer modality and distribution in China from year 1990 through 1992--an epidemiologic study]. Zhonghua Zhong Liu Za Zhi 1996; 18: 403-407.

[3] Lehrbach DM, Nita ME and Cecconello I. Molecular aspects of esophageal squamous cell carcinoma carcinogenesis. Arq Gastroenterol 2003; 40: 256-261.

[4] Katlic MR, Wilkins EW Jr and Grillo HC. Three decades of treatment of esophageal squamous carcinoma at the Massachusetts General Hospital. J Thorac Cardiovasc Surg 1990; 99: 929-938.

[5] Enzinger PC and Mayer RJ. Esophageal cancer. N Engl J Med 2003; 349: 2241-2252.

[6] Yang ZQ, Imoto I, Fukuda Y, Pimkhaokham A, Shimada Y, Imamura M, Sugano S, Nakamura Y and Inazawa J. Identification of a novel gene, GASC1, within an amplicon at 9p23-24 frequently detected in esophageal cancer cell lines. Cancer Res 2000; 60: 4735-4739.

[7] Helias C, Struski S, Gervais C, Leymarie V, Mauvieux L, Herbrecht R and Lessard M. Polycythemia vera transforming to acute myeloid leukemia and complex abnormalities including 9p homogeneously staining region with amplification of MLLT3, JMJD2C, JAK2, and SMARCA2. Cancer Genet Cytogenet 2008; 180: 51-55.

[8] Northcott PA, Nakahara Y, Wu X, Feuk L, Ellison DW, Croul S, Mack S, Kongkham PN, Peacock J, Dubuc A, Ra YS, Zilberberg K, McLeod J, Scherer SW, Sunil Rao J, Eberhart CG, Grajkowska W, Gillespie Y, Lach B, Grundy R, Pollack IF, Hamilton RL, Van Meter T, Carlotti CG, Boop F, Bigner D, Gilbertson RJ, Rutka JT and Taylor MD. Multiple recurrent genetic events converge on control of histone lysine methylation in medulloblastoma. Nat Genet 2009; 41: 465-472.

[9] Italiano A, Attias R, Aurias A, Perot G, Burel-Vandenbos F, Otto J, Venissac N and Pedeutour F. Molecular cytogenetic characterization of a metastatic lung sarcomatoid carcinoma: 9p23 neocentromere and 9p23-p24 amplification including JAK2 and JMJD2C. Cancer Genet Cytogenet 2006; 167: 122-130.

[10] Suikki HE, Kujala PM, Tammela TL, van Weerden WM, Vessella RL and Visakorpi T. Genetic alterations and changes in expression of histone demethylases in prostate cancer. Prostate 2010; 70: 889-898.

[11] Liu G, Bollig-Fischer A, Kreike B, van de Vijver MJ, Abrams J, Ethier SP and Yang ZQ. Genomic amplification and oncogenic properties of the GASC1 histone demethylase gene in breast cancer. Oncogene 2009; 28: 4491-4500.

[12] Cloos PA, Christensen J, Agger K, Maiolica A, Rappsilber J, Antal T, Hansen KH and Helin K. The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. Nature 2006; 442: 307-311.
[13] Chen Z, Zang J, Whetstine J, Hong X, Davrazou F, Kutateladze TG, Simpson M, Mao Q, Pan CH, Dai S, Hagman J, Hansen K, Shi Y and Zhang G. Structural insights into histone demethylation by JMJD2 family members. Cell 2006; 125: 691-702.
[14] Whetstine JR, Nottke A, Lan F, Huarte M, Smolikov S, Chen Z, Spooner E, Li E, Zhang G, Colaiacovo M and Shi Y. Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. Cell 2006; 125: 467-481.
[15] Klose RJ, Kallin EM and Zhang Y. JmjC-domain-containing proteins and histone demethylation. Nat Rev Genet 2006; 7: 715-727.
[16] Luo W, Chang R, Zhong J, Pandey A and Semenza GL. Histone demethylase JMJD2C is a coactivator for hypoxia-inducible factor 1 that is required for breast cancer progression. Proc Natl Acad Sci U S A 2012; 109: E3367-3376.
[17] Zhang FR, Tao LH, Shen ZY, Lv Z, Xu LY and Li EM. Fascin expression in human embryonic, fetal, and normal adult tissue. J Histochem Cytochem 2008; 56: 193-199.
[18] Vinatzer U, Gollinger M, Mullauer L, Raderer M, Chott A and Streubel B. Mucosa-associated lymphoid tissue lymphoma: novel translocations including rearrangements of ODZ2, JMJD2C, and CNN3. Clin Cancer Res 2008; 14: 6426-6431.
[19] Wu J, Liu S, Liu G, Dombkowski A, Abrams J, Martin-Trevino R, Wicha MS, Ethier SP and Yang ZQ. Identification and functional analysis of 9p24 amplified genes in human breast cancer. Oncogene 2012; 31: 333-341.
[20] Rui L, Emre NC, Kruhlak MJ, Chung HJ, Steidl C, Slack G, Wright GW, Lenz G, Ngo VN, Shaffer AL, Xu W, Zhao H, Yang Y, Lamy L, Davis RE, Xiao W, Powell J, Maloney D, Thomas CJ, Moller P, Rosenwald A, Ott G, Muller-Hermelink HK, Savage K, Connors JM, Rimsza LM, Campo E, Jaffe ES, Delabie J, Smeland EB, Weisenburger DD, Chan WC, Gascoyne RD, Levens D and Staudt LM. Cooperative epigenetic modulation by cancer amplicon genes. Cancer Cell 2010; 18: 590-605.
[21] Shimada Y, Imamura M, Wagata T, Yamaguchi N and Tobe T. Characterization of 21 newly established esophageal cancer cell lines. Cancer 1992; 69: 277-284.
[22] Crea F, Sun L, Mai A, Chiang YT, Farrar WL, Danesi R and Helgason CD. The emerging role of histone lysine demethylases in prostate cancer. Mol Cancer 2012; 11: 52.
[23] Canova C, Hashibe M, Simonato L, Nelis M, Metspalu A, Lagiou P, Trichopoulos D, Ahrens W, Pigeot I, Merletti F, Richiardi L, Talamini R, Barzan L, Macfarlane GJ, Macfarlane TV, Holcatova I, Bencko V, Benhamou S, Bouchardy C, Kjaerheim K, Lowry R, Agudo A, Castellsague X, Conway DI, McKinney PA, Znaor A, McCartan BE, Healy CM, Marron M and Brennan P. Genetic associations of 115 polymorphisms with cancers of the upper aerodigestive tract across 10 European countries: the ARCAGE project. Cancer Res 2009; 69: 2956-2965.
[24] Hillringhaus L, Yue WW, Rose NR, Ng SS, Gileadi C, Loenarz C, Bello SH, Bray JE, Schofield CJ and Oppermann U. Structural and evolutionary basis for the dual substrate selectivity of human KDM4 histone demethylase family. J Biol Chem 2011; 286: 41616-41625.
[25] Wang J, Zhang M, Zhang Y, Kou Z, Han Z, Chen DY, Sun QY and Gao S. The histone demethylase JMJD2C is stage-specifically expressed in preimplantation mouse embryos and is required for embryonic development. Biol Reprod 2010; 82: 105-111.
[26] Ishimura A, Terashima M, Kimura H, Akagi K, Suzuki Y, Sugano S and Suzuki T. Jmjd2c histone demethylase enhances the expression of Mdm2 oncogene. Biochem Biophys Res Commun 2009; 389: 366-371.
[27] Loh YH, Zhang W, Chen X, George J and Ng HH. Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. Genes Dev 2007; 21: 2545-2557.
[28] Yamamoto S, Tateishi K, Kudo Y, Yamamoto K, Isagawa T, Nagae G, Nakatsuka T, Asaoka Y, Ijichi H, Hirata Y, Otsuka M, Ikenoue T, Aburatani H, Omata M and Koike K. Histone demethylase KDM4C regulates sphere formation by mediating the crosstalk between Wnt and Notch pathways in colonic cancer cells. Carcinogenesis 2013; 34: 2380-8.
[29] Cao J and Yan Q. Histone demethylases set the stage for cancer metastasis. Sci Signal 2013; 6: pe15, 1-2.
[30] Ding X, Pan H, Li J, Zhong Q, Chen X, Dry SM and Wang CY. Epigenetic activation of AP1 promotes squamous cell carcinoma metastasis. Sci Signal 2013; 6: ra28, 1-13, S0-15.