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Esophagus

JMJD3 expression is an independent prognosticator in patients with esophageal squamous cell carcinoma ☆

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ABSTRACT

Background: The Jumonji-domain containing 3 has diverse roles in multiple cancers. Here, we investigated its prognostic significance in esophageal squamous cell carcinoma.
Methods: By using immunohistochemistry, the Jumonji-domain containing 3 expression was examined in 109 surgically resected esophageal squamous cell carcinomas and correlated with treatment outcome. The functional role of Jumonji-domain containing 3 in esophageal squamous cell carcinoma cells was determined by Jumonji-domain containing 3–mediated small interfering ribonucleic acid.
Results: Univariate analysis showed that Jumonji-domain containing 3 overexpression was associated with inferior overall survival ($P = .004$) and disease-free survival ($P = .002$). In a multivariate comparison, Jumonji-domain containing 3 overexpression remained independently associated with worse overall survival ($P = .017$, hazard ratio = 1.898) and disease-free survival ($P = .011$, hazard ratio = 1.901). The 5-year overall and disease-free survival rates were 66% and 58% in patients with a low expression of Jumonji-domain containing 3 and 34% and 27% in patients with overexpression of Jumonji-domain containing 3. Silencing Jumonji-domain containing 3 in esophageal squamous cell carcinoma cells inhibited cell growth rate and bromodeoxyuridine incorporation ability. In contrast, a gain of function of Jumonji-domain containing 3 promoted esophageal squamous cell carcinoma cell proliferation. Furthermore, Jumonji-domain containing 3 expression contributes to Ras/MEK pathway.
Conclusion: Jumonji-domain containing 3 overexpression was independently associated with poor prognosis in patients with esophageal squamous cell carcinoma. In vitro, Jumonji-domain containing 3 expression regulated esophageal squamous cell carcinoma cell growth. These results may further elucidate the role of Jumonji-domain containing 3 in esophageal squamous cell carcinoma and provide a potential new therapeutic approach for patients with esophageal squamous cell carcinoma.

Introduction

Despite improvements in surgical techniques and combination chemotherapy and radiation therapy, the prognosis of the patients with esophageal squamous cell carcinoma (ESCC) still remains poor.[1–3] Even after radical surgery and chemoradiotherapy, patients continue to develop recurrences.[1,3] Therefore, identification of prognostic biomarkers for ESCC could improve risk-adapted

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treatment strategies and may provide a useful insight that help the development of novel targeted therapeutic strategies.

Jumonji-domain containing 3 (JMJD3) belongs to the Jumonji C-domain–containing family of genes and regulates transcription by demethylating di- and tri-methyl-lysine 27 on histone H3.[4,5] Whether JMJD3 acts as an oncoprotein or tumor suppressor is still controversial. The tumor suppressive role of JMJD3 was noted in colorectal cancer,[6,7] pancreatic cancer,[8] and non-small cell lung cancer.[9] Studies have shown that JMJD3 activated p16/INK4a expression in human diploid fibroblasts[10] and mouse embryo fibroblasts.[11] Conversely, the oncogenic role of JMJD3 was reported in breast cancer,[12] prostate cancer,[13] hepatocellular carcinoma,[14] and renal cell carcinoma.[15] In hormone-dependent breast cancer cells, JMJD3 can activate the antiapoptotic gene BCL2.[16] Several studies also revealed that JMJD3 can induce epithelial-mesenchymal transition (EMT) in mammary epithelial cells,[12] hepatocellular carcinoma cells,[14] and renal cell carcinoma.[17] However, to the best of our knowledge, the clinical importance of JMJD3 in ESCC remains unclear.

The aim of this study is to evaluate the significance of JMJD3 in ESCC.

Methods

Patient population

Patients with ESCC who underwent surgical resection at Kaohsiung Chang Gung Memorial Hospital, Taiwan, Republic of China, from 1997 to 2012 were retrospectively reviewed. This retrospective study was approved by the Chang Gung Medical Foundation Institutional Review Board. Patients with synchronous cancers in other organs and patients receiving preoperative chemoradiotherapy, preoperative chemotherapy, or preoperative radiotherapy were excluded. During this period, 109 patients were identified. Patients undergoing surgery had a radical esophagectomy with cervical esophagogastric anastomosis (McKeown procedure) or an Ivor Lewis esophagectomy with intrathoracic anastomosis, reconstruction of the digestive tract with gastric tube, and pylorus drainage procedures. Two-field lymph node dissection was performed in all patients. The pathologic tumor, node, metastasis (TNM) stage was determined according to the 7th American Joint Committee on Cancer (AJCC) staging system.[18] After operation, patients were followed at 3-month intervals during years 1–2, 6-month intervals during years 3–5, and annually thereafter. Locoregional recurrence is defined as that occurring in the mediastinum or upper abdomen at the site of the previous esophageal resection and nodal clearance and that occurring in the cervical area where no lymphadenectomy had been performed.[19] Distant recurrence is defined as hematogenous if it developed within a solid organ or as transcoelomic if it is within the peritoneal cavity. If recurrence was found at more than 1 site on initial evaluation or was subsequently detected at another site within 30 days, it was defined as occurring synchronously in both areas. Overall survival (OS) was calculated from the time of surgery to death as a result of all causes. Disease-free survival (DFS) was computed from the time of surgery to the recurrence or death from any cause without evidence of recurrence.

Immunohistochemistry

Immunohistochemistry staining was performed using an immunoperoxidase technique. Staining was performed on slides (4 mm) of formalin-fixed, paraffin-embedded tissue sections with primary antibodies against JMJD3 (1:100, ab38113; Abcam). Briefly, after deparaffinization and rehydration, the retrieval of the antigen was performed by treating the slides in 10 mmol/L citrate buffer (pH 6.0) in a hot water bath (95°C) for 20 minutes. Endogenous peroxidase activity was blocked for 15 minutes in 0.3% hydrogen peroxide. After blocking with 1% goat serum for 1 hour at room temperature, the sections were incubated with primary antibodies for a minimum of 18 hours at 4°C. Immunodetection was performed using the LSAB2 kit (Dako, Carpinteria, CA, USA) followed by 3-3'-diaminobenzidine for color development and hematoxylin for counterstaining. Incubation without the primary antibody was used as a negative control, and hepatocellular carcinoma[14] was used as a positive control. The staining assessment was independently carried out by 2 pathologists (S.L.W. and W.T.H) without any information about clinicopathologic features or prognosis. We followed the method published elsewhere[15] to score the expression of JMJD3. Briefly, the proportions of JMJD3-expressing tumor cells were scored as follows: (0) no positive cells; (1) $<$5%; (2) 6%–25%; (3) 26%–50%; (4) 51%–75%; and (5) $>$75%. Staining intensity was graded according to the mean optical density: (0) no staining; (1) weak staining; (2) moderate staining; and (3) strong staining. The staining index was calculated as the product of the staining intensity score and the proportion of JMJD3-positive tumor cells. The criterion for positive staining was a specimen with a staining index $\geq$6.

Cell lines, transfection, and drug

The human esophageal squamous cell carcinoma cell lines TE8 were obtained from the American Type Culture Collection ([ATCC] Manassas, VA, USA) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) Invitrogen-Gibco, Carlsbad, CA, supplemented with 10% fetal bovine serum at 37°C and 5% CO_2. For plasmid transfection, the TE8 cells were prepared to 80% confluence in 6-well plates by using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. At 48 hours after transfection, cells were treated with G418 200 μg/mL for 2 weeks. Then cells were harvested for subsequent experiments. For short interfering ribonucleic acid (siRNA) transfection experiments, 2 synthetic JMJD3 targeting siRNAs and 1 sicontrol were applied. Cells were transfected in JMJD3-mediated siRNA in serum-free DMEM, using Plus/Lipofetamin Transfection Reagent (Invitrogen) according to the manufacturer's instructions. The 5 and 10 μM AZD6244 were used to inhibit the MEK pathway in TE8 cell.

Western blotting assay

TE8 cells transfected with negative control or JMJD3-siRNA were harvested and homogenized in radioimmunoprecipitation assay (RIPA) lysis buffer containing a protease inhibitor cocktail on ice for 30 minutes. The homogenate was centrifuged at 4°C at 15,000 rpm for 30 minutes, and lysates were collected. Protein concentration was determined using the bicinchoninic acid assay kit. The cell lysates were subjected to the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes. Then the membranes were probed with primary antibodies. The primary antibodies (anti-JMJD3, anti-Flag, anti-cyclin D1, and anti-β-actin) and the cell signaling technology horseradish peroxidase (HRP)–conjugated secondary antibodies were obtained from Sigma (St. Louis, MO, USA).

3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) bromodeoxyuridine (BrdU) incorporation assays

For the MTT assay, cells were seeded at a density of 1×10^4 cells per well in 96-well plates and maintained in DMEM supplemented with 10% FBS. After overnight incubation, the culture

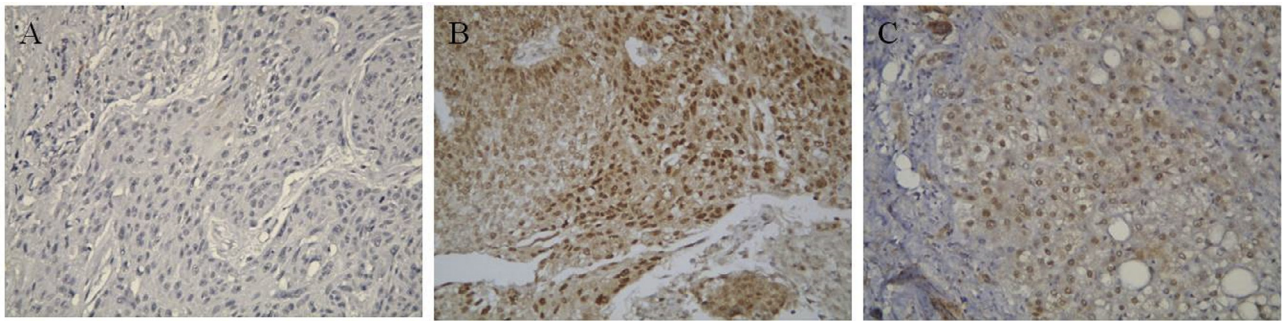

Fig 1. Immunohistochemical staining of JMJD3 in esophageal squamous cell carcinoma. (*A*) Low JMJD3 expression. (*B*) JMJD3 overexpression. (*C*) JMJD3 immunoreactivity was present in hepatocellular carcinoma used as a positive control.

medium was removed and the cells were washed with phosphate-buffered solution (PBS). The fresh completed DMEM medium was added in the presence or absence of the MEK inhibitor and cultured for 72 hours. The culture medium was then removed, and the cells were washed with phosphate-buffered solution. Cells were then incubated with 0.5 mg/mL MTT, in culture medium without FBS, for 4 hours at 37 °C in a 5% CO_2 atmosphere. The medium was removed and 100-μL dimethyl sulfoxide buffer was added and incubated in the dark for 10 minutes. Absorbance was measured on a microplate reader at 540 nm. For the BrdU incorporation assay, cells were seeded at a density of 5×10^3 cells/well in 96-well culture plates in the presence or absence of a MEK inhibitor for 48 hours. BrdU incorporation analysis was performed using the cell proliferation enzyme-linked immunoassay, BrdU kit (Roche, Burgess Hill, UK) according to the manufacturer's instructions.

Statistical analysis

For patient data, statistical analysis was performed using the SPSS v17 software package (IBM, Armonk, NY, USA). The χ^2 test and Fisher exact test were used to compare data between the two groups. For survival analysis, the Kaplan-Meier method was used for univariate analysis, and the difference between survival curves was tested by a log-rank test. In a stepwise forward fashion, significant parameters at univariate level were entered into the Cox regression model to analyze their relative prognostic importance. For cell line experiments, one-way ANOVA was used for the statistical analysis. For all analyses, two-sided tests of significance were used, with $P < .05$ considered significant.

Results

Patient characteristics

The clinicopathologic parameters of the 109 patients with ESCC are presented in Table 1. The median age for the study group of 109 patients, 104 men and 5 women, was 55 years (range, 28–80 years). The 7th AJCC stages of 109 esophageal squamous cell carcinomas were stage IA in 5 patients, stage IB in 34 patients, stage IIA in 18 patients, stage IIB in 27 patients, stage IIIA in 11 patients, stage IIIB in 3 patients, stage IIIC in 9 patients, and stage IV in 2 patients. The tumor locations were upper in 19 patients, middle in 37 patients, and lower in 53 patients. The histologic gradings were grade 1 in 10 patients, grade 2 in 72 patients, and grade 3 in 27 patients. Of these 109 patients, resection margins were positive for residual tumor in 15 patients. At the time of analysis, the median periods of follow-up were 72.4 months (range, 60.8–112 months) for the 47 survivors and 28.7 months (range, 3.5–112 months) for all 109 patients. The 5-year overall and disease-free survival rates of these 109 patients were 49% and 41%.

Table 1
Characteristics of 109 patients with esophageal squamous cell carcinoma receiving esophagectomy.

Age (years)	
Median	55
Mean	55.9
Range	29–80
Sex	
Male	104 (95%)
Female	5 (5%)
Primary tumor location	
Upper	19 (17%)
Middle	37 (34%)
Lower	53 (49%)
T classification	
T1	43 (39%)
T2	29 (27%)
T3	27 (25%)
T4	10 (9%)
N classification	
N0	73 (67%)
N1	25 (23%)
N2	9 (8%)
N3	2 (2%)
Seventh AJCC Stage	
IA	5 (5%)
IB	34 (31%)
IIA	18 (17%)
IIB	27 (25%)
IIIA	11 (10%)
IIIB	3 (3%)
IIIC	9 (8%)
IV	2 (2%)
Histologic grading	
Grade 1	10 (9%)
Grade 2	72 (66%)
Grade 3	27 (25%)
Surgical margin	
Negative	94 (86%)
Positive	15 (14%)
JMJD3 expression	
Low expression	50 (46%)
Overexpression	59 (54%)

AJCC, American Joint Committee on Cancer.

Correlation between clinicopathologic parameters and JMJD3 expression

Among the 109 patients, JMJD3 overexpression was identified in 59 (54%) patients (Fig 1). The correlations between clinicopathologic parameters and immunohistochemical expression of JMJD3 are summarized in Table 2. JMJD3 overexpression was significantly associated with advanced T classification and seventh AJCC stage.

Survival analyses

Correlations of clinicopathologic parameters and JMJD3 with overall survival, disease-free survival, locoregional recurrence-free

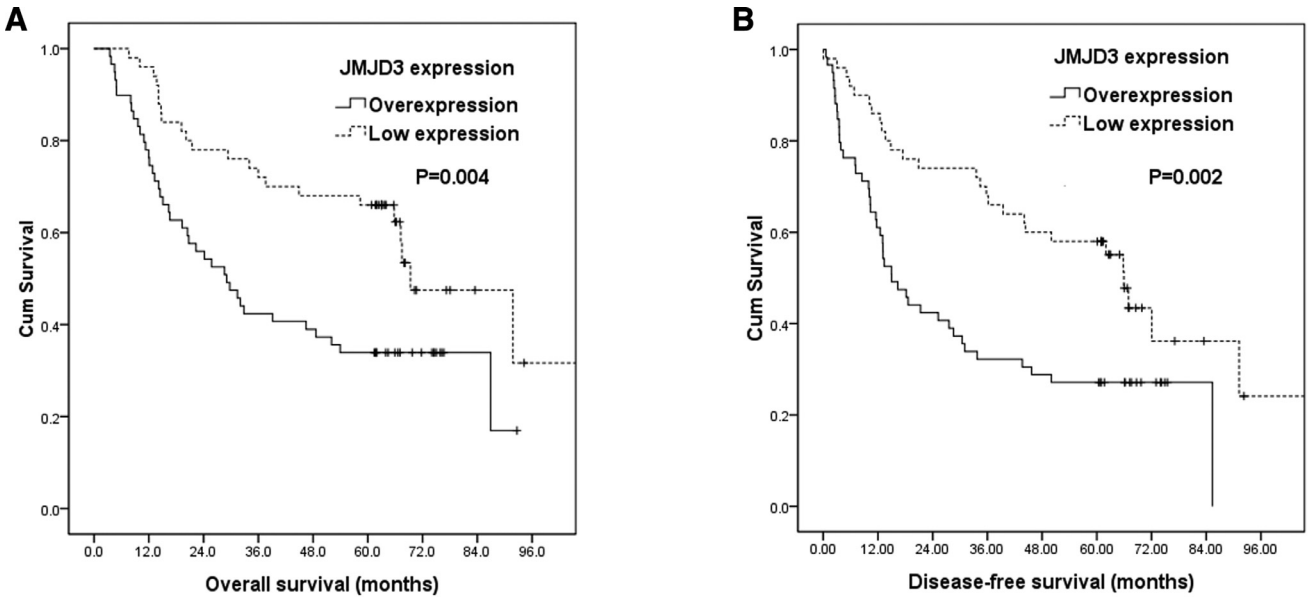

Fig 2. Kaplan-Meier curves according to JMJD3 status. (*A*) Overall survival according to JMJD3 status. (*B*) Disease-free survival according to JMJD3 status.

Table 2
Associations between JMJD3 expression and clinicopathologic parameters in 109 patients with esophageal squamous cell carcinoma receiving esophagectomy.

Parameters		JMJD3 expression		
		Low	Over	*P* value
Age (years)	<55	26	28	.64
	≧55	24	31	
Primary tumor location	U + M	28	28	.37
	L	22	31	
T classification	T1 + T2	42	30	< .001*
	T3 + T4	8	29	
N classification	N0	38	35	.065
	N1 + 2 + 3	12	24	
Seventh AJCC Stage	I + II	45	39	.003*
	III + IV	5	20	
	I	26	13	.001*
	II + III + IV	24	46	
Histologic grading	Grade 1 + 2	40	42	.29
	Grade 3	10	17	
Surgical margin	Negative	46	48	.11
	Positive	4	11	

* Statistically significant. *AJCC*, American Joint Committee on Cancer.

survival, and distant recurrence-free survival are presented in Table 3. Univariate analyses demonstrated that seventh AJCC stage III + IV ($P < .001$), T classification, T3/4 ($P = .011$), N classification, N1 + 2 + 3 ($P < .001$), positive surgical margin ($P = .014$), and JMJD3 overexpression ($P = .004$, Fig 2, *A*) were associated with inferior overall survival. In addition, seventh AJCC stage III + IV ($P < .001$), T classification, T3/4 ($P = .021$), N classification, N1 + 2 + 3 ($P < .001$), and JMJD3 overexpression ($P = .002$, Fig 2, *B*) were associated with worse disease-free survival. The seventh AJCC stage III + IV ($P = .007$), N classification, N1 + 2 + 3 ($P < .001$), positive surgical margin ($P = .019$), and JMJD3 overexpression ($P = .035$) were associated with worse locoregional recurrence-free survival. The seventh AJCC stage III + IV ($P < .001$), T classification ($P = .018$), N classification, N1 + 2 + 3 ($P < .001$), positive surgical margin ($P = .044$), and JMJD3 overexpression ($P = .001$) were associated with worse distant recurrence-free survival.

In multivariate comparison (Table 4), JMJD3 overexpression ($P = .017$, hazard ratio [HR] = 1.898, 95% confidence interval [CI]: 1.121∼3.215) remained independently associated with inferior overall survival, together with N classification, N1 + 2 + 3 ($P < .001$, HR = 3.552, 95% CI: 2.102∼6.005). For disease-free survival, JMJD3 overexpression ($P = .011$, HR = 1.901, 95% CI: 1.161∼3.115) and N classification, N1 + 2 +3 ($P < .001$, HR = 2.942, 95% CI: 1.803∼4.799) represented an independent adverse prognosticator. The 5-year overall and disease-free survival rates were 66% and 58% in patients with a low expression of JMJD3 and 34% and 27% in patients with an overexpression of JMJD3.

JMJD3 expression regulates the cell growth rate of ESCC cell line TE8

To explore the biologic function of JMJD3 in ESCC, we transfected TE8 cells with small siRNAs that target JMJD3 gene to knockdown the endogenous expression of JMJD3. The knockdown efficiency was revealed to be effective because approximately 90% of the JMJD3 expression was knockdown by Western blotting analysis (Fig 3, *A*). To determine the ability of JMJD3 silencing in inhibiting cell proliferation, MTT and BrdU assays were performed, and the results show significant decreases of cell growth and the BrdU incorporation rate after the JMJD3 expression was inhibited (Figs 3, *B, C*). Conversely, the cell proliferation was increased in the gain of function of the JMJD3 in TE8 cells by MTT and BrdU assays (Figs 3, *D–F*).

JMJD3 expression is modulated in MEK pathway in ESCC cell line TE8

Understanding the mechanisms by which JMJD3 promotes cell growth is important for developing novel methods for cancer treatment. Other studies have shown that oncogenic Ras activation caused a loss in the levels of H3K27me3 in human fibroblast cells, indicating that JMJD3 may be involved in the Ras/MEK signaling pathway.[11] To this end, we used the MEK inhibitor to examine the JMJD3 expression in TE8 cells. The protein levels of cyclin D1, a downstream target of MEK and JMJD3, were reduced in TE8 cells treated with MEK inhibitor in a dose-dependent manner (Fig 4, *A*). Using the same panel, we also found that cell growth abilities in MEK inhibitor–treated groups were decreased compared with the control group (Fig 4, *B*). Taken together, these results suggested that the JMJD3 expression participates in the Ras/MEK pathway in part, which regulates cell growth in the ESCC cell.

Table 3
Results of univariate log-rank analysis of prognostic factors for overall survival and disease-free survival in 109 patients with esophageal squamous cell carcinoma receiving esophagectomy.

Factors	Number of patients	Overall survival (OS)		Disease-free survival (DFS)		Locoregional recurrence- free survival (LRFS)		Distant recurrence-free survival (DRFS)	
		5-yr OS (%)	*P* value	5-yr DFS (%)	*P* value	5-yr LRFS (%)	*P* value	5-yr DRFS (%)	P value
Age (years)									
<55	54	56	.41	50	.19	57	.20	62%	.97
≧55	55	42		33		40		58%	
Location									
U + M	56	55	.15	45	.39	51	.49	68%	.18
L	53	42		38		47		52%	
T classification									
T1 + 2	72	57	.011*	47	.021*	54	.07	67%	.018*
T3 + 4	37	32		30		38		47%	
N classification									
N0	73	63	< .001*	52	< .001*	57	< .001*	73%	<.001*
N1 + 2 + 3	36	19		19		29		30%	
7th AJCC stage									
I + II	84	58	< .001*	49	< .001*	54	.007*	69%	<.001*
III + IV	25	16		16		29		27%	
I	39	64	.005*	56	.002*	58	.029*	73%	.01*
II + III + IV	70	40		33		43		52%	
Histologic grading									
Grade 1 + 2	82	50	.46	42	.61	48	.96	63%	.12
Grade 3	27	44		41		51		50%	
Surgical margin									
Negative	94	52	.014*	44	.11	52	.019*	63%	.044*
Positive	15	27		27		27		38%	
JMJD3 expression									
Low expression	50	66	.004*	58	.002*	61	.035*	77%	.001*
Overexpression	59	34		27		37		44%	

* Statistically significant. *AJCC*, American Joint Committee on Cancer.

Table 4
Results of multivariate Cox regression analysis for overall survival and disease-free survival in 109 patients with esophageal squamous cell carcinoma.

Factors	Overall survival		Disease-free survival	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
JMJD3 overexpression	1.898 (1.121∼3.215)	.017	1.901 (1.161∼3.115)	.011*
N1 + 2 + 3	3.552 (2.102∼6.005)	< .001*	2.942 (1.803∼4.799)	< .001*

* Statistically significant. *HR*, hazard ratio; *95% CI*, 95% confidence interval.

Discussion

The function of JMJD3 in human cancers is controversial. Several studies have indicated that JMJD3 plays an oncogenic role in a variety of cancers, such as prostate, breast, renal, and hepatocellular carcinoma.[13–15,20,21] Conversely, the property of tumor suppressor in other types of cancers, including myelodysplastic syndrome, lung adenocarcinoma, colorectal, gliomas, and pancreatic, have been reported.[8,10,22] However, to the best of our knowledge, the role of JMJD3 in ESCC remains unclear. Therefore, we conducted the present study to evaluate the significance of JMJD3 in ESCC.

Clinically, Li et al[17] reported that the JMJD3 overexpression was significantly correlated with higher T classification, higher N classification, advanced AJCC stage, and inferior overall survival in patients with clear cell renal cell carcinoma. However, Tokunaga et al[7] showed that low JMJD3 expression predicted a poor prognosis in surgically resected colorectal cancer patients. In the present study, we found that the JMJD3 overexpression in ESCC was significantly correlated with a higher T classification and an advanced AJCC stage. Furthermore, in our survival analysis, JMJD3 overexpression was significantly associated with worse overall and disease-free survival in patients with ESCC receiving esophagectomy, and it remained a poor independent prognosticator in multivariate analysis. Our clinical findings suggested that the JMJD3 expression may have an oncogenic property in ESCC. Despite significant progress having been made in surgical technique and postoperative care, there is still a large proportion of patients with ESCC who develop recurrences after esophagectomy. Therefore, identification of patients at high risk for recurrence who may benefit from postoperative adjuvant therapy is important. In the current study, JMJD3 overexpression was highly representative of biologic aggressiveness and independently associated with poor overall survival and disease-free survival. The 5-year overall and disease-free survival rates were 66% and 58%, respectively, in patients with a low JMJD3 expression, and 34% and 27%, respectively, in patients with JMJD3 overexpression, implying that JMJD3 status might be used to select some patients who have high risk of recurrence.

In vitro, our study showed that blockage endogenous JMJD3 expression restrained cell growth and BrdU incorporation ability, which further support our clinical findings in patients with ESCC. Other studies have shown that JMJD3 activates BCL2 in hormone-dependent breast cancer cells to raise cell growth.[16] In Madin Darby Canine Kidney (MDCK) cells, gain of function of JMJD3 promotes EMT expression by inducing Snail2. In mammary epithelial cells, JMJD3 participates in TGF-β-induced EMT through increas-

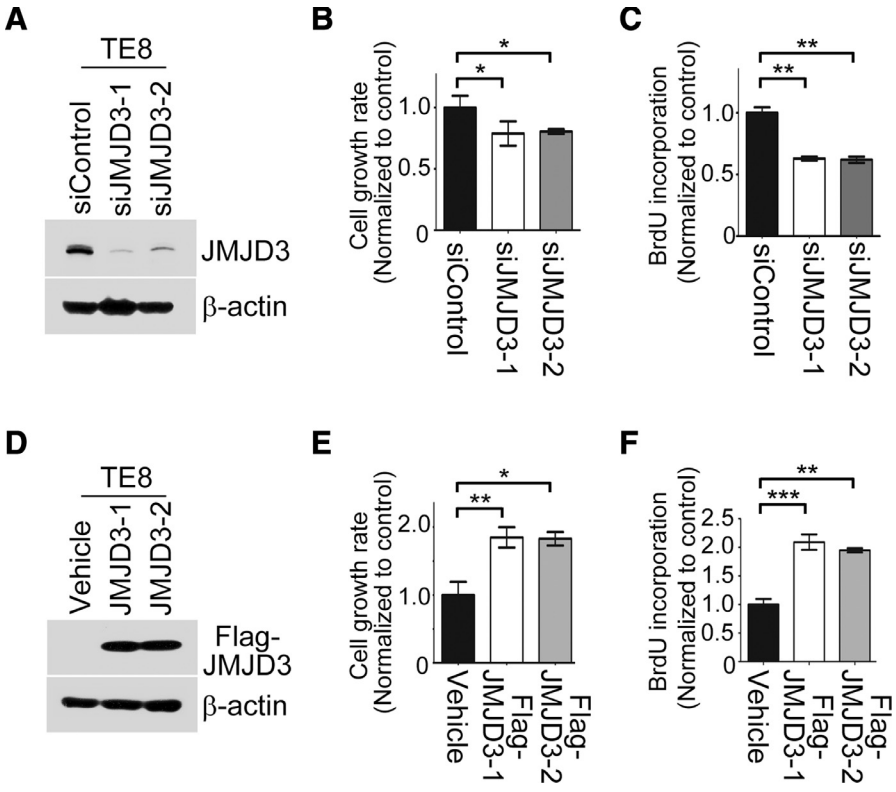

Fig 3. JMJD3 expression modulates TE8 cell growth. (*A, D*) The protein expression levels of JMJD3 were demonstrated in TE8 cells transfected with siControl and siJMJD3 or vehicle and JMJD3 transfectants by Western blotting. (*B, C*) The cell growth abilities of siControl and siJMJD3 in TE8 cells were measured by MTT and BrdU assays. (*E, F*) The cell growth abilities of vehicle and JMJD3 transfectants in TE8 cells were measured by MTT and BrdU assays. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

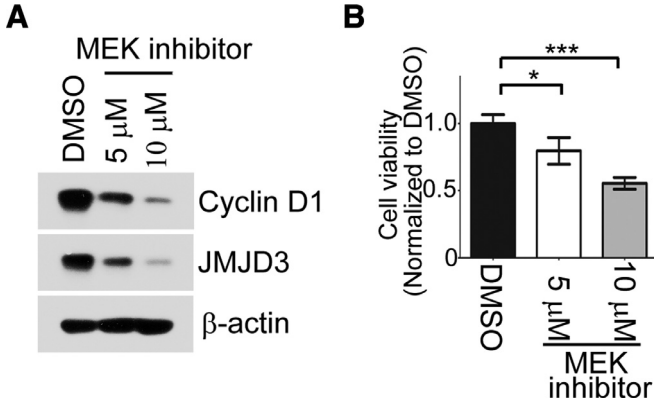

Fig 4. JMJD3 expression is governed in MEK signaling in TE8 cells. (*A*) The protein expression levels of JMJD3 and cyclin D1 were examined in TE8 cells stimulated with an MEK inhibitor, AZD6244 (5 μM and 10 μM), in a dose-dependent manner by Western blotting. (*B*) Using the same panel A, the cell growth ability was determined by the MTT assay at 72 hours. $^{*}P < .05$; $^{***}P < .001$.

ing expressions of *SNAI1 and TWIST*, leading to breast cancer invasion.[12] In clear cell renal cell carcinoma, Li et al[17] also reported that JMJD3 could induce EMT through the activation of Slug. JMJD3 is able to transcribe and associate with RUNX2 to enhance proliferation of chondrocytes.[23] An elevated level of the JMJD3 expression promotes p16 upregulation to inhibit the reprogramming of mouse embryo fibroblast (MEF) to become induced pluripotent stem (iPS) cells and prevents Schwann cell proliferation.[24,25] The results of other studies have suggested that JMJD3 plays a crucial component in the activation of the *INK4a/ARF* locus by oncogenic Ras. Ras/MEK signaling reduces the extent of H3K27me3 by upregulating JMJD3 and downregulating EZH2 in fibroblast cells,[11] suggesting that JMJD3 might contribute to the Ras/MEK signaling to regulate cell progression. In the present data, we showed that the JMJD3 protein expression was decreased and cells stimulated with the MEK inhibitor in dose-dependent manner, indicating that JMJD3, in part, plays an important role in the regulation of the Ras/MEK pathway for cancer cell development in ESCC cells.

On the other hand, several studies have revealed that JMJD3 serves as a crucial regulator of tumor suppressor genes. Many reports suggest that cytosolic—but not nuclear—JMJD3 is able to interact with p53 and regulates p53 distribution in cancer cells.[26] Recently, JMJD3 was reported to induce growth-suppressive cluster miR99a/let7c/125b-2 in prostate cancer and modulating p15/INK4B expression in colorectal cancer.[7,27] Taken together, these inconsistent findings suggest that the effect of JMJD3 may be tumor type-specific in a variety of cancer cells. However, the complicated mechanisms of oncogenic role of JMJD3 regulating in ESCC progression should be further investigated in the future.

The present study has important limitations. First, our study was a retrospective analysis. Second, our observations were based on a relatively small number of patients. Third, we could not confirm the effect of JMJD3 using JMJD3 overexpressed cells. Further research is needed to confirm our findings.

In conclusion, the JMJD3 overexpression was independently associated with poor prognosis in patients with ESCC. In ESCC cell lines, dysregulation of JMJD3 in ESCC influenced the cell growth. These results may further elucidate the role of JMJD3 in ESCC and provide a potential new therapeutic approach for patients with ESCC.

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