

Oct-4 is associated with gastric cancer progression and prognosis

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Aim: To investigate the clinical significance of Oct-4 in the development and progression of gastric cancer.

Methods: Immunohistochemistry was used to analyze Oct-4 expression in 412 gastric cancer cases. Oct-4 protein levels were upregulated in gastric cancer tissues compared with adjacent noncancerous tissues.

Results: Positive expression of Oct-4 correlated with age, depth of invasion, Lauren classification, lymph node metastasis, distant metastasis, and TNM stage. In stages I, II, and III, the 5-year survival rate of patients with high expression of Oct-4 was significantly lower than that in patients with low expression of Oct-4. In stage IV, Oct-4 expression did not correlate with the 5-year survival rate. Furthermore, multivariate analysis suggested that the depth of invasion, lymph node metastasis, distant metastasis, TNM stage, and upregulation of Oct-4 were independent prognostic factors of gastric cancer.

Conclusion: Oct-4 protein is a useful marker in predicting tumor progression and prognosis.

Keywords: gastric carcinoma, invasion, metastasis, survival rate

Introduction

Gastric cancer is the second leading cause of cancer-related mortality worldwide. Despite recent advances in surgical techniques and chemotherapy, the overall 5-year survival rate in the People's Republic of China is still only 40%. Most gastric cancer is diagnosed as stage III or IV, and the rate of lymph node metastasis is high (50%–75%).¹ A recent study suggests that Oct-4 could be involved in the development of certain tumors. Oct-4 is essential for the identity of the pluripotent founder cell population in the mammalian embryo.^{2–4} Oct-4 was recently identified to be associated with tumorigenesis and malignant transformation of cancers. Oct-4 expression was significantly increased from normal epithelium (both ovarian epithelium and fallopian tube epithelium) to benign and borderline cystadenoma to carcinoma in the serous lesion subgroups.⁵ Oct-4 was expressed in hepatocellular carcinoma (HCC) cell lines and tumor specimens of HCC.⁶ Expression of Oct-4 in metaplastic pancreatic ducts was significantly stronger than in normal acini and pancreatic carcinoma.⁷ Oct-4 was significantly higher in sporadic colorectal cancer compared with adjacent normal colonic epithelium.⁸ It has been shown that Oct-4 may play a role in tumorigenesis. However, little is known regarding the efficacy of Oct-4 as a potential biomarker for cancer; thus, we investigated its expression in gastric cancer and its relationship with gastric cancer.

Materials and methods

Patients and tissue samples

Gastric cancer tissues were collected from the gastrectomy specimens of 412 patients (male:female 284:128; median age, 62 years; range, 30–85 years) treated at the

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Department of Gastrointestinal Surgery, Zhejiang Provincial People's Hospital and Juxian People's Hospital between January 2001 and January 2010. Tissues were formalin-fixed and paraffin-embedded. All patients were followed up for over 5 years. The follow-up deadline was December 2013. The survival time was measured from the date of surgery to the follow-up deadline, or date of death (usually the result of carcinoma recurrence or metastasis). Cancer was found in the cardia, body, and antrum in 84, 154, and 174 cases, respectively. According to the World Health Organization histological classification of gastric carcinoma formulated in 2002, there were 39 cases of distant metastasis. Eighty non-cancerous human gastric tissue samples were obtained from gastrectomy of adjacent gastric cancer margins (>5 cm). Chemotherapy (fluorouracil, cisplatin, and tetrahydrofolic acid) was given to patients with advanced-stage disease after surgery, but none of the patients received radiation treatment. This study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital.

Tissue microarrays

Blocks (412 cancer samples and 80 noncancer tissue samples) were prepared as previously described.^{9,10} Sections (4 mm) were cut from each tissue array block, deparaffinized, and dehydrated.

Immunohistochemistry

Immunohistochemical analysis was carried out to determine altered protein expression in the 80 noncancerous human gastric tissue controls and 412 human gastric cancer tissues. Rabbit anti-Oct-4 was provided by Santa Cruz (Mountain View, CA, USA). Cytoplasmic and nuclear Oct-4 was stained buffy brown. The degree of immunostaining was scored independently by two observers based on the proportion of positively stained tumor cells and the staining intensity.^{11,12} The staining index was calculated as the staining intensity

score to the proportion of positive tumor cells. The cut-off value for high and low expression levels was chosen based on a measure of heterogeneity using the log-rank test with respect to overall survival: a staining index score of ≥ 4 indicates high Oct-4 expression and a staining index score of ≤ 3 indicates low Oct-4 expression.

Statistical analysis

All statistical analyses were performed using SPSS 16.0 software (Chicago, IL, USA). Survival curves were estimated using the Kaplan–Meier method, and the log-rank test was used to compare differences between the curves. We assessed the prognostic value of protein expression levels by Cox proportional hazards regression model.

Results

The expression of Oct-4 in gastric cancer and noncancer mucosa

Oct-4 was detected in 26/80 (32.5%) nontumor mucosae, and all samples expressed the protein at low levels. Oct-4 was expressed in 310/412 (75.2%) gastric cancer cases, and high Oct-4 expression was detected in 252/412 (61.17%) tumors. Oct-4 was mainly localized in the cytoplasm, with some localization in the nuclei of cancer cells (Figure 1).

Expression of Oct-4 and clinicopathological features

The expression of Oct-4 was related to age, differentiation, invasion, Lauren classification, lymph node metastasis, distant metastasis, and TNM stage ($P < 0.05$) (Table 1). Oct-4 expression did not correlate with sex or histological type ($P > 0.05$) (Table 1). Possible prognostic factors for gastric cancer were determined by Cox regression analysis (Table 2). The study showed that depth of invasion ($P = 0.012$), lymph node metastasis ($P = 0.011$), distant metastasis ($P = 0.008$), TNM stage ($P = 0.009$), and the level of Oct-4 expression

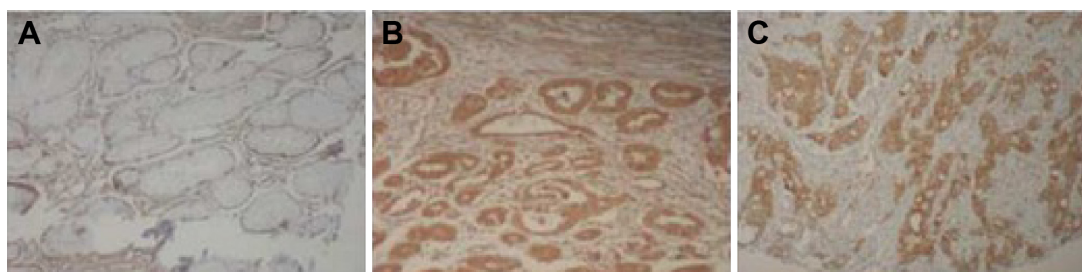


Figure 1 Immunohistochemical staining for Oct-4 in gastric cancer lesions and noncancerous tissues (SP $\times 100$).

Notes: (A) Oct-4 negative in noncancerous tissues; (B) Oct-4 was highly expressed in moderately differentiated adenocarcinoma; (C) Oct-4 was highly expressed in poorly differentiated adenocarcinoma.

Table 1 Relationship of Oct-4 expression with pathological parameters of tumor

Clinical parameters	Oct-4			
	Low, n (%)	High, n (%)	$t/\chi^2/r$	P-value
Age (years), mean \pm SD	56.91 \pm 10.99	61.22 \pm 12.66	3.43	0.001
Sex				
Male	104 (36.6)	180 (63.4)	0.944	0.331
Female	56 (43.8)	72 (56.2)		
Location				
Proximal	40 (47.6)	44 (52.4)	3.507	0.173
Middle	48 (31.2)	106 (68.8)		
Distal	72 (41.4)	102 (58.6)		
Size				
<5 cm	94 (47.5)	104 (52.5)	5.99	0.14
\geq 5 cm	66 (30.8)	148 (69.2)		
Lauren classification				
Intestinal	163 (73.1)	60 (26.9)	136.2	0.0001
Diffuse	37 (17.4)	176 (82.6)		
Histology				
Papillary adenocarcinoma	4 (40.0)	6 (60.0)	1.75	0.626
Tubular adenocarcinoma	106 (36.1)	188 (63.9)		
Mucinous adenocarcinoma	14 (46.7)	16 (53.3)		
Signet-ring cell carcinoma	36 (46.2)	42 (53.8)		
Histologic differentiation				
Well or moderately	64 (49.2)	66 (50.8)	4.32	0.038
Poorly or undifferentiated	96 (34.0)	186 (66.0)		
Invasion depth				
T1	12 (66.7)	6 (33.3)	8.36	0.039
T2	42 (48.8)	44 (51.2)		
T3	56 (41.2)	80 (58.8)		
T4	50 (29.1)	122 (70.9)		
TNM stages				
I	14 (77.8)	4 (22.2)	14.06	0.003
II	56 (51.9)	52 (48.1)		
III	78 (33.6)	154 (66.4)		
IV	12 (22.2)	42 (77.8)		
Lymph node metastasis				
No	102 (48.6)	108 (51.4)	8.54	0.003
Yes	58 (28.7)	144 (71.3)		
Distant metastasis				
No	150 (41.9)	208 (58.1)	5.39	0.02
Yes	10 (18.5)	44 (81.5)		

Note: Student's t-test was used.

Table 2 Multivariate analysis of the correlation between clinicopathological parameters and survival time in patients with gastric cancer

Covariates	Coefficient	Standard error	HR	95% CI for HR	P-value
Age	0.037	0.172	1.055	0.631–1.586	0.764
Tumor location	0.034	0.169	1.054	0.676–1.566	0.821
Tumor size	0.044	0.131	1.045	0.808–1.350	0.740
Differentiation	0.036	0.168	1.063	0.671–1.606	0.747
Lauren's classification	0.038	0.189	1.062	0.756–1.573	0.676
Depth of invasion	0.753	0.279	1.921	1.458–2.608	0.012
Lymph node metastasis	0.750	0.286	2.022	1.290–3.721	0.011
Distant metastasis	0.653	0.162	1.936	1.392–2.678	0.008
TNM stage	1.080	0.342	3.025	1.415–5.074	0.009
Oct-4 expression	0.924	0.271	2.567	1.475–2.563	0.009

Abbreviations: CI, confidence interval; HR, hazard ratio.

($P=0.009$) were all independent prognostic factors. However, the location of the tumor, tumor size, histological type, and differentiation had no prognostic value.

Oct-4 expression was correlated with patient prognosis

The 5-year survival rate for those with high Oct-4 expression (patients with stage I, II, or III disease) was significantly lower than that in patients with low Oct-4 expression. The cumulative 5-year survival rate was 92.9% in the low-expression group, but only 76.0% in the high-expression group ($P=0.001$, Figure 2) in stage I; the cumulative 5-year survival rate was 68.2% and 44.8% in the low- and high-expression group, respectively, ($P=0.0001$, Figure 3) in stage II; and in stage III, the cumulative 5-year survival rate was 39.3% and 21.6% in the low- and high-expression group, respectively, ($P=0.0001$, Figure 4). For stage IV, the expression of Oct-4 did not correlate with the 5-year survival rate (3.5% and 0% in the low- and high-expression group, respectively; $P=0.439$).

Discussion

Oct-4 was recently identified to be associated with tumorigenesis and malignant transformation of many cancers. Oct-4 induced dysplasia by inhibiting cellular differentiation.¹³ The expression of Oct-4 in mucosal tissues with severe gastritis, metaplastic/dysplastic transformation, and gastric cancer was significantly increased (2%, 6%, and 16%, respectively), and the results suggested that Oct-4 plays a role in the early stages of cancer development.¹⁴ Chen et al¹⁵ examined the

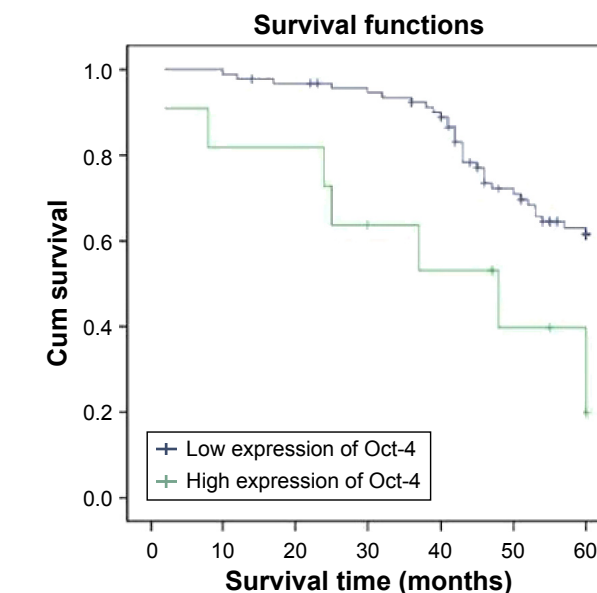


Figure 3 Kaplan–Meier curves with univariate analyses (log-rank) for patients with low Oct-4 expression versus those with high Oct-4 expression tumors in all gastric cancer in stage II.

Abbreviation: Cum, cumulative.

expression of Oct-4 in 62 gastric cancer cases using reverse transcription polymerase chain reaction and immunohistochemistry. The expression of Oct-4 was higher in gastric cancer tissues compared with the matching adjacent noncancerous tissues, and the expression of Oct-4 in gastric cancer was correlated with differentiation status, but not with age, sex, tumor size, TNM stage, depth of invasion, or lymph node metastasis.¹⁵ Little is known regarding the effect of Oct-4 as a potential biomarker of gastric cancer.

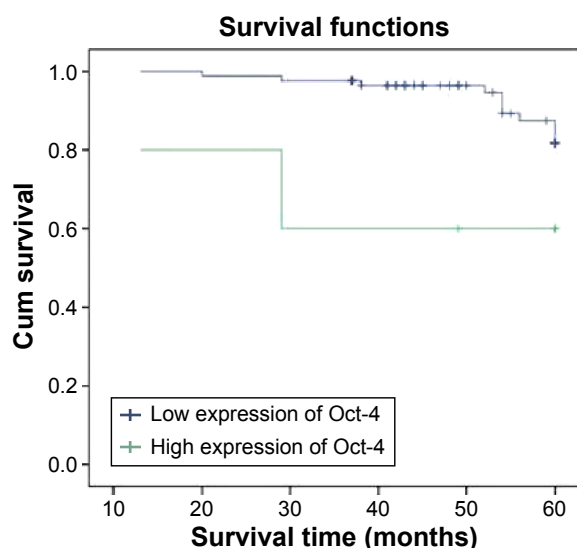


Figure 2 Kaplan–Meier curves with univariate analyses (log-rank) for patients with low Oct-4 expression versus those with high Oct-4 expression tumors in all gastric cancer in stage I.

Abbreviation: Cum, cumulative.

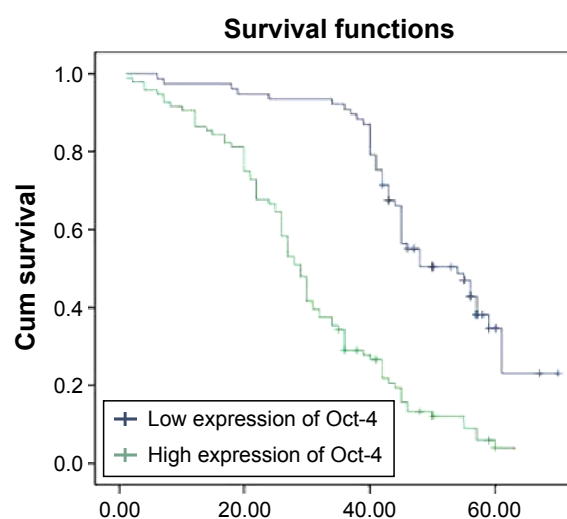


Figure 4 Kaplan–Meier curves with univariate analyses (log-rank) for patients with low Oct-4 expression versus those with high Oct-4 expression tumors in all gastric cancer in stage III.

Abbreviation: Cum, cumulative.

The expression of Oct-4 by immunostaining is a useful tool in assessing its clinical applications as a marker of neoplasia.^{16,17} Therefore, in this study, we detected the expression of Oct-4 in gastric cancer using immunohistochemistry and assessed its relationship with clinicopathological parameters. The study identified that Oct-4 was highly expressed in gastric cancer tissues compared with normal gastric tissues, and this was related significantly with prognosis. High expression of Oct-4 in gastric cancer cases was associated with age, tumor size, tumor location, depth of invasion, lymph node and distant metastasis, and TNM stage. The literature shows that the expression of Oct-4 was significantly associated with large tumor size and vascular invasion, and was an independent predictor of postoperative recurrence and poor prognosis of HCC.¹⁸ Expression of Oct-4 was significantly associated with poor differentiation and recurrence of HCC, and was an independent predictive factor in patients with HCC.¹⁹ Since the most important prognostic factor for gastric cancer is lymph node and distant metastasis,^{20,21} we analyzed the relationship between expression of Oct-4 and prognosis of patients with gastric cancer (staged according to TNM criteria). We found that the expression of Oct-4 was associated with poor prognosis in stage I–III, but was not associated with poor prognosis in stage IV.

The present study suggests that expression of Oct-4 in gastric cancer tissues may play an important role in the progression and metastases of gastric cancer, has a potential role in tumorigenesis of gastric cancer, and is a new tumor marker with potential value in the diagnosis and treatment of gastric cancer. Oct-4 might be a target of the β -catenin/TCF-mediated downstream gene in Wnt-activated cells, and uncovering Oct-4 downstream targets could further elucidate its part in tumorigenesis and could be a new target to combat cancer.²²

Acknowledgment

This work was supported by Zhejiang Provincial Medical Science Research Fund (grant 201337120).

Author contributions

Jiang WL and Zhang PF were equal contributors; Jiang WL and Zhang PF performed the majority of experiments and provided vital reagents and analytical tools and were also involved in writing the manuscript. Li GF and Wang XS provided the collection of all the human material in addition to providing financial support for this work. Dong JH and Wang YY designed the study and edited the manuscript.

All authors contributed toward data analysis, drafting, and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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