ORIGINAL RESEARCH

The relationship between Lin28 and the chemotherapy response of gastric cancer

Rong Yue Teng[1,*]
Ji Chun Zhou[1,*]
Zi Nong Jiang[2]
Chao Yang Xu[3]
Zi Duo Li[1]
Qing Chuan Wang[1]
Chen Pu Xu[1]
Ju Feng Guo[1]
Jian Guo Shen[1]
Lin Bo Wang[1,4]

[1]Department of Surgical Oncology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, [2]Department of Pathology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, [3]Department of Breast and Thyroid Surgery, Shaoxing People's Hospital, The First Affiliated Hospital of Shaoxing Liberal Art College, Shaoxing, [4]Biomedical Research Center and Key Laboratory of Biotherapy of Zhejiang Province, Hangzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jian Guo Shen; Lin Bo Wang
Department of Surgical Oncology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou 310016, People's Republic of China
Tel +86 571 86002170;
+86 571 87236878
Email drshenjianguo@yahoo.com.cn;
wanglinbo@medmail.com.cn

Objective: The aim of the study reported here was to identify whether a stem cell biomarker, Lin28, may predict the pathologic tumor response to neoadjuvant chemotherapy for patients with locally advanced gastric cancer.

Methods: The study enrolled 47 patients with gastric cancer who underwent neoadjuvant chemotherapy followed by surgery between July 2004 and March 2012. Cancer tissue was biopsied by gastroscopy and Lin28 expression in the tissue was measured by immunohistochemistry. Statistical analyses were performed to identify the relationship between Lin28 expression and tumor regression grade.

Results: Of the 47 cases, pathologic nonresponse was observed in 29 (61.7%) and pathologic response in 18 (38.3%). Receiver-operating characteristic curve analysis showed that the histo-score of Lin28 expression with 0.325 as a cutoff value could differentiate between pathologic response and nonresponse. Multivariable analysis showed that Lin28 expression was an independent predictive factor for pathologic response to neoadjuvant chemotherapy ($P = 0.006$).

Conclusion: Lin28 expression was associated with pathologic tumor response in locally advanced gastric cancer patients undergoing neoadjuvant chemotherapy. This may suggest that Lin28 can serve as a predictive biomarker for neoadjuvant chemotherapy in patients with gastric cancer.

Keywords: neoadjuvant chemotherapy, pathologic tumor response, stem cell biomarker, immunohistochemistry

Introduction

Gastric cancer is now the fourth most common cancer worldwide, and the third most common cause of death from cancer.[1] Preoperative chemotherapy is currently wildly used in local advanced gastric cancer because it may improve patient survival. However, there may be risk in delaying surgery in unresponsive cases. Several studies[2,3] have found biomarkers in patients that can predict the chemotherapy response before chemotherapy, but there are still limitations of these for clinical use. Lin28 is a highly conserved RNA-binding protein[4] that has been shown to participate in inducing pluripotent stem cells and to the maintenance of stem cell-like cells in cancer.[5] Recent studies suggest that Lin28 functions as an oncogene, which may promote malignant transformation and tumor progression.[6] Moreover, several recent studies have demonstrated that Lin28 expression correlates with the survival of patients with malignant diseases.[7] Our previous study showed that Lin28 expression is associated with poor survival in gastric cancer patients[8] and other research has demonstrated that the expression of Lin28 is correlated with radioresistance in human cancer cells.[9]

However, as far as we are aware, there is no evidence to support the correlation of Lin28 expression and chemotherapy response in gastric cancer.

The study reported here aimed to find the relationship between Lin28 expression and chemotherapy response to neoadjuvant chemotherapy in patients with locally advanced gastric cancer, with the intention of determining whether Lin28 could be a marker that may predict the efficacy of neoadjuvant chemotherapy in gastric cancer patients.

Patients and methods

Tissue was collected from 47 patients with locally advanced gastric cancer by endoscopic biopsy from July 2004 to March 2012 at the Department of Surgical Oncology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China. Inclusion criteria and exclusion criteria, pre-treatment staging, and surgical procedure were consistent with our previously published study.[10] All patients received standard neoadjuvant chemotherapy or radio-chemotherapy followed by curative resection (R0 resection) and adjuvant chemotherapy.

Assessment of pathologic response

After surgery, all tumor specimens were examined by pathologists. Pathologic response was evaluated according to Becker's criteria, in which residual tumor cells were microscopically evaluated.[11] Briefly, tumor regression was classified into three grades: Grade 1, complete or subtotal regression (<10% residual tumor per tumor bed); Grade 2, partial tumor regression (10%–50% residual tumor per tumor bed); and Grade 3, minimal or no tumor regression (>50% residual tumor per tumor bed). All patients with Grade 1 or 2 regression were defined as having experienced "tumor regression," while the definition of Grade 3 present "pathologic nonresponse" was taken from by Liu et al.[12]

Immunohistochemical staining for detection of Lin28 expression

Immunohistochemical analysis to determine Lin28 expression was performed on formalin-fixed, paraffin-embedded specimens. The slides were de-paraffinized in xylene and rehydrated in gradient ethanol solutions. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 10 minutes. The slides were immersed in 10 mM citric buffer (pH 6.0) with heating for 15 minutes for antigen retrieval. Nonspecific binding was blocked by preincubation with 10% fetal calf serum in phosphate-buffered saline with 0.01% sodium azide, and the slides were incubated in a humid chamber for

1 hour with an antibody against Lin28 (rabbit polyclonal antibody, cat no 11724-1-AP, PTGLAB (Proteintech Group, Inc, Chicago, USA); 1:75). Following this, the slides were incubated with undiluted EnVision™ HRP complex (Dako Denmark A/S, Glostrup, Denmark) for 60 minutes, then visualized with diaminobenzidine (Dako Denmark A/S) and counterstained with hematoxylin.

For substitute negative controls, the primary antibody was replaced with phosphate-buffered saline. Positive control was normal testis tissue known to exhibit high expression of Lin28. Evaluation of immunoreactivity for Lin28 was conducted according to the intensity and distribution of cell cytoplasm staining by diaminobenzidine chromogen. Cell cytoplasm staining intensity was scored using a weighted-histoscore method.[13] The intensity of staining was graded as 3+ (dark brown; strongly positive), 2+ (brown; moderately positive), 1+ (light brown; weekly positive), and negative. The final histoscores were calculated as follows:

$$(1 \times \text{percentage of cells staining light brown}) + (2 \times \text{percentage of cells staining brown}) + (3 \times \text{percentage of cells staining dark brown}).$$

Scoring was undertaken by two independent observers who did not know the clinical outcomes. Discrepant scoring was reevaluated by the two observers and agreement was reached after appropriate discussion.

Statistical analysis

Chi-square or Fisher's exact tests were utilized to determine the significance of associations between the pathologic findings and categorical variables. For multivariable analysis, a logistic regression model was applied to identify correlations between pathologic response and clinicopathological variables. A receiver-operating characteristic curve was constructed to define the cutoff value for the various continuous variables that were correlated with predicting pathologic tumor response. SPSS (v 16.0; IBM Corporation, Armonk, NY, USA) was implemented for all statistical calculations. In all tests, a two-sided $P < 0.05$ was considered to indicate significance.

Results
Clinicopathologic variables and pathologic tumor response

Pathological nonresponse (tumor regression grade [TRG] 3) was observed in 61.7% (29/47) of patients, and pathological response (TRG 1–2) in 38.3% (18/47) of patients. The chemotherapy efficiency was 38.3%. On univariate analysis,

Table 1 Relationship between patient characteristics and chemo-response

Variable	Pathologic tumor response		P value
	No response	Response	
Age			
≤60	16 (34.0%)	10 (21.3%)	
>60	13 (27.7%)	8 (17.0%)	0.609
Sex			
Male	18 (38.3%)	14 (29.8%)	
Female	11 (23.4%)	4 (8.5%)	0.213
Tumor size			
≤4.25 cm	21 (44.7%)	6 (12.7%)	
>4.25 cm	8 (17.1%)	12 (25.5%)	0.010
Tumor differentiation			
Differentiated	8 (17.0%)	8 (17.0%)	
Undifferentiated	21 (44.7%)	10 (21.3%)	0.192
Tumor location			
Upper	6 (12.8%)	4 (8.5%)	
Middle and lower	23 (48.9%)	12 (25.5%)	
Diffuse type	0 (0.0%)	2 (4.3%)	0.176
Serosa invasion			
Yes	22 (46.8%)	16 (34.0%)	
No	7 (14.9%)	2 (4.3%)	0.239
Lymph metastasis			
Yes	14 (29.8%)	14 (29.8%)	
No	15 (31.9%)	4 (8.5%)	0.043

both tumor size ($P = 0.01$) and lymph metastasis ($P = 0.043$) were found to be related with patient chemotherapy response (Table 1).

Lin28 expression predicting pathologic tumor response

The average Lin28 expression histoscore was 0.53 and the range was 0.05–1.65 (Figure 1). Compared with patients who had a lower Lin28 histoscore (0.423 ± 0.059), patients with a higher Lin28 histoscore (0.695 ± 0.112) seemed to have a worse pathological response to chemotherapy (TRG 1–2; Figure 2).

Using receiver-operating characteristic analysis, a Lin28 histoscore cutoff level of 0.325 was found. The sensitivity and specificity of this cutoff value in predicting tumor regression were 88.9% and 58.6%, respectively. Consequently, the correlation between Lin28 histoscores (≤0.325 and >0.325) and pathological response were investigated by Chi-square statistical analysis. We found that high Lin28 expression levels were more frequent in nonresponse patients ($P = 0.001$; Table 2).

In multivariate analysis, controlling for other variables studied on univariate analysis, Lin28 expression was found to be an independent predictive factor for pathologic tumor regression response ($P < 0.05$) and tumor size ($P < 0.05$; Table 3).

Discussion

Neoadjuvant chemotherapy is an important adjunctive treatment to increase the radical resection rate in gastric cancer and prolong the overall survival (OS) of gastric cancer patients.[14] However, as chemotherapy is less efficient due to tumor heterogeneity, the screening of a factor which could predict the chemosensitivity of a gastric cancer patient to neoadjuvant chemotherapy is particularly important. Neoadjuvant chemotherapy is a good platform that provides a chance to filter tumor molecular markers for predicting the efficacy of chemotherapy for gastric cancer.[15]

Tumor regression grading reportedly has prognostic value in patients with various cancers after preoperative
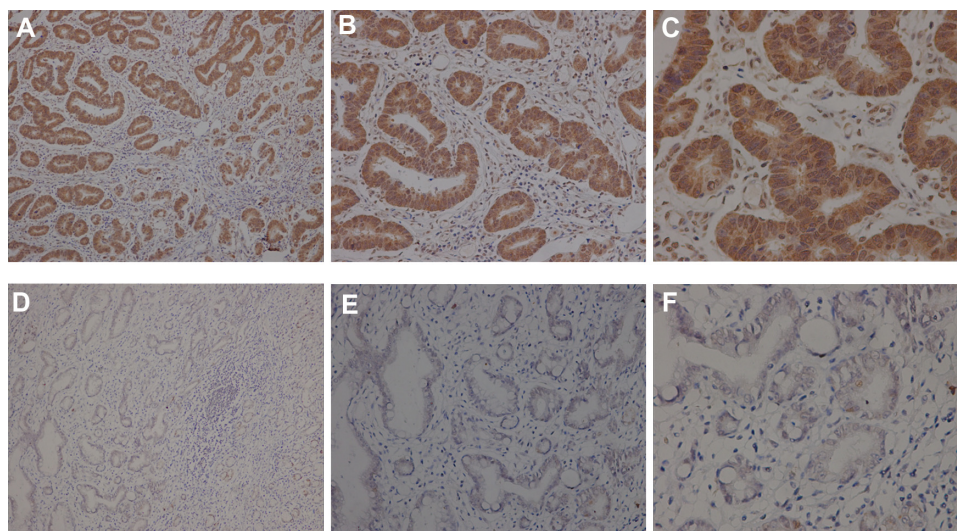
Figure 1 Lin28 Immunohistochemical staining of gastroscope tissues in gastric patients (**A-C**) Lin28 expression are strong positive in representative slide (A, B, C is separately shown at 100×, 200×, 400× magnification). (**D-F**) Lin28 expression are almost negative in representative slide (D, E, F is separately shown at 100×, 200×, 400× magnification).
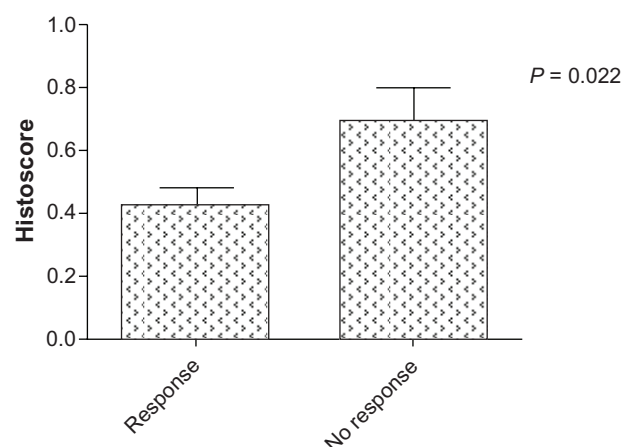
Figure 2 Lin28 average histoscore between two groups which divide patients with chemo-response. Patients who had better chemotherapy pathological response (TRG2-3), have lower level of Lin28 histoscore.

chemoradiotherapy, such as rectal,[16] breast,[17] and gastric.[11] Lin28 is a highly conserved RNA-binding protein that has emerged as a modulator of the processing of microRNAs (eg, let-7, miR-107, miR-143, and miR-200c).[18] Lin28 recruits terminal uridylyltransferase 4 (TUT4) to pre-let-7 by recognizing a tetra-nucleotide sequence motif (GGAG) in the terminal loop. In turn, TUT4 adds an oligo uridine tail to the pre-let-7, which blocks Dicer processing.[18] This role for Lin28 has important implications for our mechanistic understanding of pluripotency, the timing of development, and oncogenesis.[20] "Let-7" is a family of small noncoding RNAs regulating the expression of many genes that control important cellular activities. Loss of let-7 increases resistance to certain chemotherapeutic drugs and radiation.[21] In agreement with our result, Lv et al found that Lin28 expression is a possible mechanism of chemoresistance in breast cancer and that overexpression of Lin28 effectively induced p21 and Rb expression and inhibited let-7 miRNA.[13] Meanwhile, Liu et al[12] revealed that a low level of let-7i is associated with chemotherapy resistance and shorter OS in gastric cancer patients treated with neoadjuvant chemotherapy. Besides, accumulating data have proved the double negative feedback between Lin28 and let-7.[19] The Lin28/let-7 pathway plays an important role in cancer progress.[22] Consistent with this observation, the

Table 2 Chi-square statistical analysis to identify Lin28 expression and pathologic tumor response

Lin28 histoscore	Pathologic tumor response		P value
	No Response	**Response**	
≤0.325	16 (34.0%)	2 (4.3%)	
>0.325	13 (27.7%)	16 (34.0%)	0.001

Table 3 Multivariate logistic analysis to identify Lin28 and other variables

Variable	Exp	95% CI	P value
Lin28 histoscore (≤0.325 vs >0.325)	13.334	2.095~84.871	0.006
Tumor size (≤4.25 cm vs >4.25 cm)	4.809	1.056~21.904	0.042
Lymph metastasis (yes vs no)	4.739	0.955~23.529	0.057

Abbreviations: CI, confidence interval; Exp, expectations.

study presented here showed a relationship between Lin28 and the chemotherapy response of gastric cancer patients. This indicates that Lin28 might also play a role in gastric cancer patient's chemoresistance by downregulating let-7. However, although the lin28 histoscore cutoff value of 0.325 was found to differentiate pathologic nonresponse and response, the sensitivity and specificity were still low, which might be due to our small sample size. Moreover, the potential molecular mechanism of how Lin28 mediated chemoresistance needs to be further studied in vitro.

Conclusion

To our knowledge, this is the first study to have investigated the role of Lin28 in predicting chemosensitivity in gastric cancer patients treated with neoadjuvant chemotherapy. Our findings indicate that lin28 could serve as a predictive biomarker for neoadjuvant chemotherapy in patients with gastric cancer, warranting further study.

Acknowledgments

Disclosure

Other than the grants outlined in the "Acknowledgments" section, the authors declare no conflicts of interest in this work.

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