

The relationship between Lin28 and the chemotherapy response of gastric cancer

Rong Yue Teng^{1,*}
 Ji Chun Zhou^{1,*}
 Zi Nong Jiang²
 Chao Yang Xu³
 Zi Duo Li¹
 Qing Chuan Wang¹
 Chen Pu Xu¹
 Ju Feng Guo¹
 Jian Guo Shen¹
 Lin Bo Wang^{1,4}

¹Department of Surgical Oncology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, ²Department of Pathology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, ³Department of Breast and Thyroid Surgery, Shaoxing People's Hospital, The First Affiliated Hospital of Shaoxing Liberal Art College, Shaoxing, ⁴Biomedical 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 histoscore 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 widely 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 protein⁴ 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 patients⁸ 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% H₂O₂ 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; weakly 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 chemotherapy response

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

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

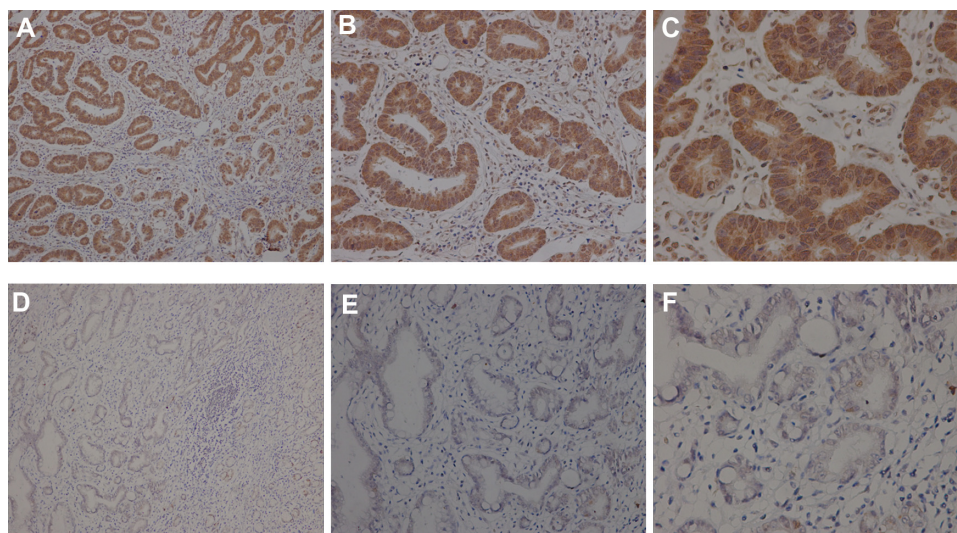


Figure 1 Lin28 Immunohistochemical staining of gastroscopically obtained tissues in gastric patients (A–C) Lin28 expression are strong positive in representative slide (A, B, C is separately shown at 100 \times , 200 \times , 400 \times magnification). (D–F) Lin28 expression are almost negative in representative slide (D, E, F is separately shown at 100 \times , 200 \times , 400 \times magnification).

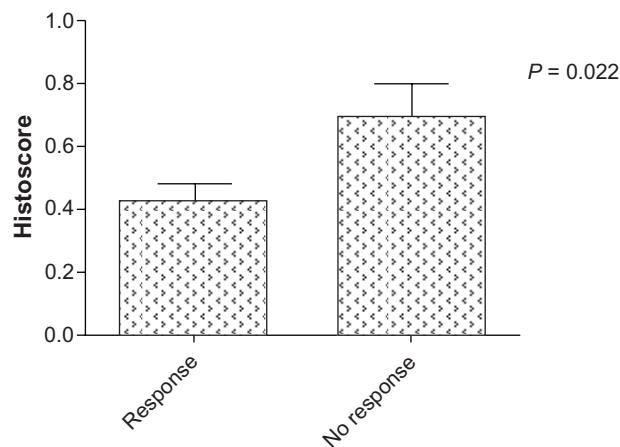


Figure 2 Lin28 average histoscore between two groups which divide patients with chemo-response. Patients who had better chemotherapy pathological response (TRG2-3), have lower level of Lin28 histoscore.

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 al¹² 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%)	0.001
>0.325	13 (27.7%)	16 (34.0%)	

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

This study was supported by grants from the National Natural Science Foundation of China (grant no 81101659/H1609, 81101659), Natural Science Foundation of Zhejiang Province (grant numbers Y2110073 and Q13H160024), Science and Health Care Foundation of Zhejiang Province (grant no 2011KYA086), and the Program for Innovative Research Team in Zhejiang Province (grant no 2010R50046).

Disclosure

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

References

- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69–90.
- Napierski R, Ott K, Kremer M, Specht K, Vogelsang H, Becker K, Müller M, Lordick F, et al. Combined GADD45A and thymidine phosphorylase expression levels predict response and survival of neoadjuvant-treated gastric cancer patients. *Clin Cancer Res*. 2005;11:3025–3031.

3. Hirakawa M, Sato Y, Ohnuma H, Takayama T, Sagawa T, Nobuoka T, Harada K et al. A phase II study of neoadjuvant combination chemotherapy with docetaxel, cisplatin, and S-1 for locally advanced resectable gastric cancer: nucleotide excision repair (NER) as potential chemoresistance marker. *Cancer Chemother Pharmacol*. 2013;71:789–797.
4. Moss EG, Lee RC, Ambros V. The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the *lin-4* RNA. *Cell*. 1997;88(5):637–646.
5. Yang DH, Moss EG. Temporally regulated expression of Lin-28 in diverse tissues of the developing mouse. *Gene Expr Patterns*. 2003;3(6):719–726.
6. Viswanathan SR, Powers JT, Einhorn W, et al. Lin28 promotes transformation and is associated with advanced human malignancies. *Nat Genet*. 2009;41(7):843–848.
7. Guo Y, Chen Y, Ito H, et al. Identification and characterization of lin-28 homolog B (LIN28B) in human hepatocellular carcinoma. *Gene*. 2006;384:51–61.
8. Xu C, Shen J, Xie S, Jiang Z, Huang L, Wang L. Positive expression of Lin28 is correlated with poor survival in gastric carcinoma. *Med Oncol*. 2013;30(1):382.
9. Oh JS, Kim JJ, Byun JY, Kim IA. Lin28-let7 modulates radiosensitivity of human cancer cells with activation of K-Ras. *Int J Radiat Oncol Biol Phys*. 2010;76(1):5–8.
10. Wang LB, Teng RY, Jiang ZN, et al. Clinicopathologic variables predicting tumor response to neoadjuvant chemotherapy in patients with locally advanced gastric cancer. *J Surg Oncol*. 2012;105(3):293–296.
11. Becker K, Mueller JD, Schulmacher C, et al. Histomorphology and grading of regression in gastric carcinoma treated with neoadjuvant chemotherapy. *Cancer*. 2003;98(7):1521–1530.
12. Liu K, Qian T, Tang L, Wang J, Yang H, Ren J. Decreased expression of microRNA let-7i and its association with chemotherapeutic response in human gastric cancer. *World J Surg Oncol*. 2012;10:225.
13. Teo K, Gemmell L, Mukherjee R, Traynor P, Edwards J. Bad expression influences time to androgen escape in prostate cancer. *BJU Int*. 2007;100(3):691–696.
14. Wagner AD, Unverzagt S, Grothe W, et al. Chemotherapy for advanced gastric cancer. *Cochrane Database Syst Rev*. 2010;(3):CD004064.
15. Li W, Qin J, Sun YH, Liu TS. Neoadjuvant chemotherapy for advanced gastric cancer: a meta-analysis. *World J Gastroenterol*. 2010;16(44):5621–5628.
16. Saigusa S, Tanaka K, Toiyama Y, et al. Gene expression profiles of tumor regression grade in locally advanced rectal cancer after neoadjuvant chemoradiotherapy. *Oncol Rep*. 2012;28(3):855–861.
17. Lv K, Liu L, Wang L, et al. Lin28 mediates paclitaxel resistance by modulating p21, Rb and Let-7a miRNA in breast cancer cells. *PLoS One*. 2012;7(7):e40008.
18. Heo I, Joo C, Kim YK, et al. TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. *Cell*. 2009;138(4):696–708.
19. Yang X, Lin X, Zhong X, Kaur S, Li N, Liang S, Lassus H et al. Double-negative feedback loop between reprogramming factor LIN28 and microRNA let-7 regulates aldehyde dehydrogenase 1-positive cancer stem cells. *Cancer Res*. 2010;70(22):9463–9472.
20. Viswanathan SR, Daley GQ. Lin28: A microRNA regulator with a macro role. *Cell*. 2010;140(4):445–449.
21. Boyerinas B, Park SM, Hau A, Murmann AE, Peter ME. The role of let-7 in cell differentiation and cancer. *Endocr Relat Cancer*. 2010;17(1):F19–F36.
22. Thornton JE, Gregory RI. How does Lin28 let-7 control development and disease? *Trends Cell Biol*. 2012;22(9):474–482.

OncoTargets and Therapy

Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic agents and protocols on

Submit your manuscript here: <http://www.dovepress.com/oncotargets-and-therapy-journal>

patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Dovepress