Biochemical and Biophysical Research Communications 456 (2015) 879–883

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Expression and prognostic value of Mycl1 in gastric cancer

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ARTICLE INFO

Article history:
Received 9 December 2014
Available online 18 December 2014

Keywords:
Mycl1
Biomarker
Prognostic factor
Gastric cancer

ABSTRACT

As a member of the Myc proto-oncogene family, *MYCL1* has been found to be amplified and overexpressed in some malignancies. However, the clinical significance of Mycl1 expression in gastric cancer is still unknown. Mycl1 expression was detected on tissue microarrays of gastric cancer samples in 176 cases using immunohistochemical staining, and its association with clinicopathological factors and overall survival was also analyzed. Mycl1 showed greater expression in gastric cancer tissue than in adjacent normal tissue (62.5% vs 46.0%, respectively, $P = 0.002$), and its expression was correlated with patient age, tumor differentiation, and TNM stage ($P = 0.007$, 0.003, and 0.002, respectively). The Mycl1 positive group had an unfavorable outcome compared with the negative group ($P < 0.001$). Multivariate analysis showed that Mycl1 expression was an independent prognostic factor of gastric cancer ($P = 0.009$). These results suggest that Mycl1 expression might be useful as a biomarker to predict prognosis and is a promising therapeutic target for patients with gastric cancer.

1. Introduction

Gastric cancer ranks as the fourth most common cancer in men and fifth in women and it is the second leading cause of cancer-related death in the world [1]. There are approximately 300,000 deaths in the People's Republic of China annually. Most of patients are in advanced stages when diagnosed. Because of the high incidence of recurrence and metastasis, overall survival still remains poor even after surgical resection and/or systemic chemotherapy. Therefore, detecting gastric cancer early and preventing cancer from dissemination is very important. Furthermore, predicting patient prognosis and providing promising therapeutic targets is also crucial for decreasing mortality. Thus, many scientists have devoted their work to finding valuable biomarkers for gastric cancer.

MYCL1, first isolated from human small-cell lung cancer cell lines [2], is an important member of the *MYC* family. *MYCL1* is located on chromosome 1p34.2, contains three exons, two introns, and has a genomic length of 6.59 kb. *MYCL1* encodes a nuclear phosphoprotein with 364 amino acids, heterodimerizing with partner protein Max and acts as a transcription factor to control the expression of target genes [3]. *MYCL1* is involved in many biological processes, including cell proliferation, differentiation, apoptosis [4,5] and has been found to be amplified and overexpressed in some malignancies [2,6–8]. Therefore, *MYCL1* is thought to be a proto-oncogene. In our previous study, we found that one intron single-nucleotide polymorphism (SNP) in *MYCL1* was associated with the risk of diffuse-type gastric cancer and with gastric cancer differentiation [9]. However, *MYCL1* expression and its clinical significance in gastric cancer is still unknown.

In the present study, we investigated Mycl1 expression in paired gastric cancer tissue and adjacent normal gastric tissue by immunohistochemical staining. Moreover, we analyzed the correlation of Mycl1 expression with clinicopathological characteristics and evaluated the possibility of using Mycl1 as a prognostic biomarker for gastric cancer.

2. Materials and methods

2.1. Study population

All patients ($n = 176$) were diagnosed with primary gastric cancer between May 2007 and August 2008 in southeast China. They had not received chemotherapy or radiotherapy and showed no evidence of distant metastasis before surgery. Among the 176 patients, the average age was 64 years and 71% were male. Overall, 72 (40.9%) patients were in early stages (stage I and stage II), 104 (59.1%) were in advanced stages (all in stage III, none in stage

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http://dx.doi.org/10.1016/j.bbrc.2014.12.060
0006-291X/

IV), and 132 (75%) had lymph node metastases at the time of surgery. Tumor differentiation was classified based on World Health Organization criteria, and patient clinical stage was determined according to the seventh American Joint Committee on Cancer (AJCC) TNM cancer classification system [10]. Overall survival was defined as the interval between the date of surgery and cancer-related death. Follow-up duration ranged from 1 to 75 months (median, 61.4 months), and 111 patients (63.1%) died during this period. Informed consent was obtained from each patient before use of their resected sample. The project was approved by the Institutional Review Board of Fujian Medical University.

2.2. Sample collection and tissue microarray (TMA) construction

Gastric cancer tissue and corresponding normal gastric tissue adjacent to the tumor tissue (distance >4 cm) were both collected from each patient shortly after surgery. These tissues were formalin-fixed and paraffin-embedded. Tissue microarrays (TMA) were constructed by Shanghai Outdo Biotech Limited Company (Shanghai, People's Republic of China). The 1.5 mm cores of cancer tissue and its adjacent normal tissue, identified by a pathologist, were removed from representative areas of each paraffin block, and precisely arrayed onto a new paraffin block (TMA block). TMA blocks were serially sectioned (4 μm), fixed on slides, and baked overnight at 56 °C in preparation for immunohistochemistry staining.

2.3. Immunohistochemistry

For immunohistochemistry, 4 μm TMA slides were deparaffinised and rehydrated through a graded ethanol series. Next, the slides were boiled in a pressure cooker until gas discharging for 3 min. Endogenous peroxidase was blocked with 3% hydrogen peroxide (H_2O_2) for 10 min. Subsequently, slides were incubated at 37 °C with anti-Mycl1 (dilution, 1:150, rabbit polyclonal, Abcam, Cambridge, MA, USA), positive and negative controls were also used. After washing with phosphate-buffered saline (PBS), tissues were incubated with horseradish peroxidase-conjugated anti-rabbit Ig polymer as a second antibody (Elivision kit, Maixin_Bio, Fuzhou, People's Republic of China) for 30 min at 37 °C. Immunoreactions were visualized using a diaminobenzidine (DAB) staining kit. After washing, the slides were counterstained with hematoxylin.

Each slide was evaluated independently by two pathologists who were blinded to clinical information. In cancer or in corresponding adjacent normal tissue, ⩾10% cells (either tumor cells or normal cell or combined) showing brown color was defined as "positive expression"; no staining or positive staining in less than 10% cells was defined as "negative expression". When different interpretations occurred, slides were reviewed until consensus was obtained.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA). Correlation between MYCL1 expression and other clinicopathological characteristics (including age, gender, tumor size, differentiation, lymph node metastasis, and TNM stage) was evaluated by Pearson Chi-square test. Survival curves were plotted by the Kaplan–Meier method and differences in survival rate were assessed using the log-rank test. Univariate and multivariate analysis of prognostic factors was performed using the Cox proportional hazards model. A two-sided *P*-value <0.05 was considered statistically significant.

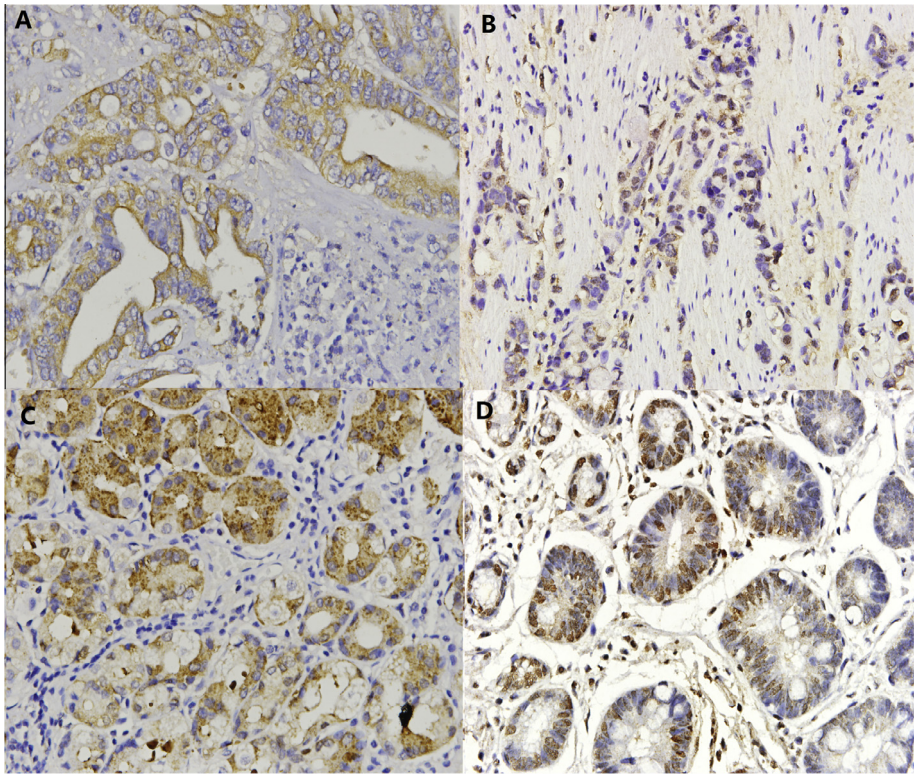

Fig. 1. (A) Mycl1 protein was expressed in the cytoplasm of gastric cancer tissue (×400). (B) Mycl1 protein was expressed both in the nucleus and cytoplasm of gastric cancer tissue (×400). (C) Mycl1 protein was expressed in the cytoplasm of normal gastric tissue (×400). (D) Mycl1 protein was expressed in the nucleus of normal gastric tissue (×400).

Table 1
Comparison of Mycl1 expression between gastric cancer tissue and adjacent normal tissue.

Groups	Total	Mycl1 expression (+)		Mycl1 expression (−)		*P*-value
		n	%	*n*	%	
Gastric cancer	176	110	62.5	66	37.5	0.002*
Adjacent normal tissue	176	81	46.0	95	54.0	

* $P < 0.05$ indicates statistically significant.

3. Results

3.1. Mycl1 expression in gastric cancer tissue and adjacent normal tissue

Mycl1 protein was expressed in gastric cancer cells as well as in normal gastric mucous membrane epithelium and glandular epithelium, but no immunoreactivity was found in stromal cells. The protein was located either in the cytoplasm or nucleus or both in the cytoplasm and nucleus. Fig. 1 shows representative images of Mycl1 expression in gastric cancer tissue and adjacent normal tissue.

Of 176 cases, only 76 (43.2%) cases expressed Mycl1 both in cancer tissue and paired adjacent normal tissue; 34 (19.3%) of cases expressed Myc11 only in cancer tissue; and 5 (2.8%) cases expressed Myc11 only in normal tissues. As Table 1 shows, the positive expression rate in tumor tissue was significantly higher than in adjacent normal tissue (62.5% vs 46.0%, respectively; $P = 0.002$).

3.2. Association of Mycl1 expression with clinicopathological characteristics

The relationship between Mycl1 expression and clinicopathological features was analyzed. As shown in Table 2, Mycl1 was more highly expressed in the younger age group (⩽64), poorly differentiated group, and advanced stage group, compared to the older age group (>64), moderately/well differentiated group, and early stage group (73.4% vs 53.6%, $P = 0.007$; 71.4% vs 49.3%, $P = 0.003$; and 72.1% vs 48.6%, $P = 0.002$, respectively). However, no significant correlation between Mycl1 expression and other parameters, such as gender, tumor size, and lymph node status was observed (all $P > 0.05$).

3.3. Univariate and multivariate analysis of overall survival

Table 3 shows the univariate and multivariate analyses of factors related to patient prognosis in gastric cancer. Univariate analysis showed that tumor differentiation, TNM stage, and Mycl1 expression were significantly related to overall survival (all $P < 0.01$). As shown in Fig. 2, the group with positive Mycl1 expression ($n = 110$) had a significantly unfavorable outcome compared with the Mycl1 expression negative group ($n = 66$; $P < 0.001$). Multivariate analysis also suggested that Mycl1 expression (hazard ratio [HR] = 3.785, 95% confidence interval [CI] 1.158–4.108, $P = 0.009$), tumor differentiation (HR = 2.318, 95% CI 1.543–5.442, $P = 0.017$), and TNM stage (HR = 3.556, 95% CI 1.693–6.035, $P = 0.011$) were independent prognostic factors for overall survival in gastric cancer patients.

4. Discussion

In this study, we demonstrated that Mycl1 expression was associated with the development and progression of gastric cancer, and the evidence for this is as follows: the rate of Mycl1 expression in cancer tissue was significantly higher than that in adjacent normal tissue; and Mycl1 was more highly expressed in poorly differentiated cancers and those with higher TNM staging. Furthermore, our study also indicated that Mycl1 expression may be used as an independent prognostic factor for predicting patient overall survival, as positive expression was correlated with unfavorable outcome.

As with two other well-characterized MYC family members, *c-MYC* and *n-MYC*, *MYCL1* (*L-MYC*) is also thought to be a critical element of several carcinogenic processes in humans. *MYCL1* was first reported to be amplified in human primary small cell lung cancer and lung carcinoma cell lines [2]; thereafter, *MYCL1* amplification and overexpression has been described in many other

Table 2
Association between Mycl1 expression and clinicopathological characteristics in gastric cancer.

Characteristics	Total	Mycl1 expression		*P*-value
		Positive ($n = 110$) (%)	Negative ($n = 66$) (%)	
Age (years)	64 (34–83)			0.007*
⩽64	79	58 (73.4)	21 (26.6)	
>64	97	52 (53.6)	45 (46.4)	
Gender				0.764
Male	125	79 (63.2)	46 (36.8)	
Female	51	31 (60.8)	20 (39.2)	
Size (cm)				0.067
<6	91	51 (56.0)	40 (44.0)	
⩾6	85	59 (69.4)	26 (30.6)	
Differentiation[a]				0.003*
Moderately/well	71	35 (49.3)	36 (50.7	
Poorly	105	75 (71.4)	30 (28.6)	
Lymph node metastasis				0.106
No	44	23 (52.3)	21 (47.7)	
Yes	132	87 (65.9)	45 (34.1)	
TNM stage				0.002*
Early stage (I/II stage)	72	35 (48.6)	37 (51.4)	
Advanced stage (III/IV stage)	104	75 (72.1)	29 (27.9)	

* $P < 0.05$ indicates statistically significant.
[a] Moderately/well, moderately differentiated/well differentiated; Poorly, poorly differentiated, including mucinous carcinoma and signet ring cell carcinoma.

Table 3
Univariate and multivariate analysis of overall survival.

Variables	Univariate analysis	Multivariate analysis	
	P-value	Hazard ratio (95% CI)	*P*-value
Age (year)			
>64 vs. ⩽64	0.494	0.835 (0.486–1.563)	0.537
Gender			
Female vs. male	0.152	0.654 (0.356–1.248)	0.164
Size			
⩾6 vs. <6	0.289	1.465 (0.787–2.643)	0.346
Differentiation			
Poorly vs. moderately/well	0.002*	2.318 (1.543–5.442)	0.017*
Lymph node metastasis			
Yes vs. no	0.528	1.379 (0.767–2.843)	0.633
TNM stage			
III/IV stage vs. I/II stage	0.000*	3.556 (1.693–6.035)	0.011*
Mycl1 expression			
Positive vs. negative	<0.001*	3.785 (1.158–4.108)	0.009*

Abbreviations: CI, confidence interval; vs., versus.
* $P < 0.05$ indicates statistically significant.

malignancies, such as medulloblastoma, Merkel cell carcinoma, and ovarian carcinoma [6–8]. However, to the best of our knowledge, no formal study has previously been performed to investigate the expression pattern of the Mycl1 protein and its clinical significance in most of human tissues, let along in gastric cancer tissue.

Our results showed that Mycl1 positivity is not a cancer-specific phenomenon in gastric tissue, as Mycl1 is not only expressed in cancer tissue but is also expressed in noncancerous tissue. However, the expression rate in the former is significantly higher than in the latter (62.5% vs 46.0%, respectively; $P = 0.002$), and we also noticed that within cancer tissue, even in early detected cases, the level of expression intensity and expression area is not inferior to in advanced cases, which indicates that Mycl1 expression might be involved in gastric cancer pathogenesis. Morgenbesser et al. found that overexpression of *MYCL1* directly affects differentiation processes within cells [11], and Schwab et al. found that the principal function of *MYC* products is to inhibit cell terminal differentiation and promote proliferation [12–14]. Therefore, we believe that Mycl1 might involved in malignant transformation in stomach tumorigenesis. This finding is also in accordance with the "oncogene" role proposed for *MYCL1* in tumors.

Our results also demonstrated that the rate of Mycl1 expression is significantly higher in poorly compared with moderately/well differentiated gastric cancer (71.4% vs 49.3%, respectively; $P = 0.003$). Compared with moderately/well differentiated cancer, poorly differentiated cancer cells have weaker intercellular adhesion and possess a greater growth advantage. Thus, we speculate that Mycl1 expression may be a useful index to judge aggressive clinical biological behavior of tumor cells. Another finding is that Mycl1 is more highly expressed in advanced gastric cancer than in early stage cancer (72.1% vs 48.6%, respectively; $P = 0.002$), which also indicates that Mycl1 may confer cancer cells with a more aggressive phenotype and metastatic potential. In the present study, we also found that the rate of Mycl1 expression is higher in younger patients (⩽64) than in older patients (>64; 73.4% vs 53.6%, respectively; $P = 0.007$), which may partially explain why gastric cancer progresses more rapidly in younger compared to older patients.

The prognostic impact of Mycl1 in gastric cancer was also examined in this study. We found that Mycl1 expression, in addition to tumor differentiation and TNM stage, was an independent prognostic factor for predicting overall survival (HR = 3.785, 95% CI 1.158–4.108, $P = 0.009$); patients with positive Mycl1 expression exhibited significantly poorer clinical outcome compared to those negative for Mycl1 expression ($P < 0.001$). Therefore, Mycl1 can

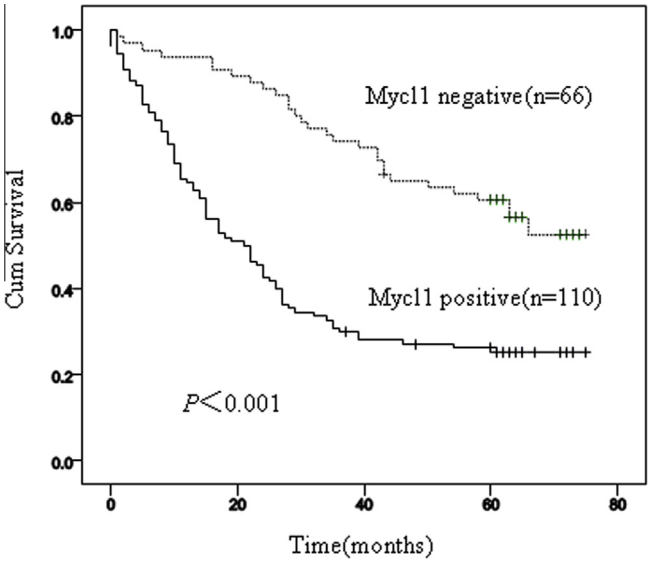

Fig. 2. Kaplan–Meier analysis of overall survival for patients with gastric cancer ($n = 176$). Patients with positive Mycl1 expression have shorter survival time than those with negative Mycl1 expression. ($P < 0.001$ by the log-rank test.)

be used as a molecular biomarker to predict patient prognosis and may also serve as therapeutic target for gastric cancer. Combined with the above findings related to clinicopathologic features, we believe that it is necessary to propose further adjunctive therapy after surgical resection for early stage cases with Mycl1 positive expression.

Mycl1 protein is a nuclear phosphoprotein and, in theory, it should be located in the nucleus. However, in the present study, Mycl1 positive signals were observed more in the cytoplasm than in nucleus. We speculate that this subcellular translocation may lead to abnormal changes in cell structure and physiological function [15], and thus affect tumorigenesis, invasion, and metastasis in cancer, as was reported by Xie et al. [16]. Interestingly, we noticed that there are abundant lymphocytes infiltrating in the stroma of some cases with Mycl1 positive expression, but not with negative expression. Lymphocyte infiltration in tissues usually corresponds with a good local immune response and thus a better prognosis [17–19], which seems paradoxical with the effect that Mycl1 positive expression exerted on survival. It is reported that Mycl1 is selectively expressed in dendritic cells (DCs), and is

required for optimal priming of T cells following bacteria and viral infection [20]. Does Mycl1 produced by cancer cells also attract T cells to mediate anti-cancer immune reactions? How does the interaction between Mycl1 and T cells exert their influence on patient outcome? The answers to these questions remain unknown, and further studies with larger sample sizes are needed to adequately answer them.

In summary, herein we present evidence for the first time that Mycl1 expression is associated with the development and progression of gastric cancer. Our findings suggest that Mycl1 expression can be used as an index to predict patient prognosis, and Mycl1 might be a promising target for gastric cancer therapy. Additional studies should be performed to validate these findings.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank Dr. Colombo N. for comments on the manuscript. This work was supported by the grant from the National Natural Science Foundation of China (Grant No. 81101893).

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