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Original article

Clinicopathologic features and prognostic implications of MYBL2 protein expression in pancreatic ductal adenocarcinoma

Ranjie Yu[a,1], Chengyi Li[b,1], Xiaomei Lin[c], Qun Chen[a], Jie Li[a], Li Song[a], Lin Lin[a], Jingnan Liu[a], Yan Zhang[a], Wencui Kong[a], Xuenong Ouyang[a], Xiong Chen[a,*]

[a] Department of Oncology, Fuzhou General Hospital of Nanjing Military Command, Fuzong Clinical College of Fujian Medical University, Fuzhou 350025, Fujian, China
[b] Department of Surgical Oncology, Affiliated Mindong Hospital of Fujian Medical University, Fuan 355000, Fujian, China
[c] Department of Oncology, Fujian University of Traditional Chinese Medicine Subsidiary Rehabilitation Hospital, Fuzhou 350122, Fujian, China

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ABSTRACT

MYBL2 (B-MYB), a member of the MYB family of transcription factor genes, regulates the expression of genes in the process of tumorigenesis. Many studies have shown that MYBL2 is high expresssion in several human malignancies including pancreatic ductal adenocarcinoma (PDAC). However, its role in PDAC is still unclear. The present study is designed to investigate MYBL2 expression levels and prognostic significance in PDAC patients. We assessed MYBL2 expression level by immunohistochemistry in tumor tissues from 93 PDAC patients undergoing curative resection. The association of MYBL2 expression with clinicopathological parameters was evaluated by Pearson's chi-square ($\chi 2$) test, Fisher's exact test, and Spearman's rank. Kaplan-Meier survival analysis and Cox proportional hazards models were used to estimate the effect of MYBL2 expression on survival. The expression of MYBL2 was significantly higher in PDAC cells compared with adjacent non-cancerous tissues ($P = 0.000$). The overexpression of MYBL2 in the tumor tissues was significantly correlated with a higher T classification ($p = 0.002$), peri-neural invasion (PNI) ($p = 0.013$) and vital status ($p = 0.045$). Kaplan-Meier analysis indicated that high MYBL2 expression was significantly associated with shorter overall survival times in PDAC patients. Moreover, univariate and multivariate analysis confirmed MYBL2 expression ($P = 0.010$), histological grade ($P = 0.001$) as independent prognostic factors in PDAC. These results suggested that overexpression of MYBL2 might serve as a novel prognostic biomarker in PDAC patients.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignant diseases and is the fourth leading cause of cancer-related death in the US and the sixth in China. the overall 5-year survival rate increased slightly from 3% to 8% over the past 40 years even after undergoing curative surgical resection and others therapies [1,2]. These low survival rates are partly because more than 50% cases are diagnosed at a distant stage and resistant to almost all available chemotherapies and radiotherapies, for which 5-year survival is 2% [1,2]. Therefore, there is an urgent need to find biologic markers for early diagnosis and prediction of the survival probabilities and improve treatment outcome in patients with PDAC.

The MYBL2 gene, which is also known as B-MYB, is one of the MYB family of transcription factors which also include A-MYB and C-MYB [3,4]. The homologues of these MYB family genes is present in all vertebrates [5]. C-MYB is highly expressed in the hematopoietic system and in human leukemias [6], whereas A-MYB levels are particularly elevated in the testis and in some subsets of B lymphocytes [7,8]. In contrast to the restricted expression of C-MYB and A-MYB, the overexpression of MYBL2 is found in virtually all proliferative cells, such as embryonal stem cells, developing mammalian tissues and adult haematopietic precursor cells, and the protein level of the MYBL2 is proportional to the degree of cell proliferation [9–11]. In vitro, The transcription factor MYBL2 plays a critical role in regulating gene expression and is implicated in controlling cell cycle, apoptosis, carcinogenesis, and senescence [12,13]. The expression of MYBL2 is barely detectable in G0 and is induced at the G1/S transition of the cell cycle, which protein levels parallel in the expression of mRNA throughout the cell cycle [14,15]. Previous studies showed that MYBL2 is overexpressed in various cancers,

* Corresponding author.
E-mail address: zpccx81@163.com (X. Chen).
[1] These authors have contributed equally to this work.

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such as neuroblastoma, acute myeloid leukaemia, hepatocellular, lung, ovarian and colon cancer [16–21]. In addition, Marion Gayral et al. reported that MYBL2 mRNA is overexpressed in PDAC-derived cell lines and tumor samples diagnosed with PDAC [22].

The correlation between MYBL2 expression and the clinicopathological characteristics of PDAC and its prognostic role in pancreatic ductal adenocarcinoma have not been reported. Our aim was to determine the expression pattern of MYBL2 by immunohistochemical staining and study its correlations with clinicopathologic features and prognosis in patients with PDAC.

2. Materials and methods

2.1. Patients and tissue samples

A total of 93 Paraffin-embedded tissue specimens (surgical resection) diagnosed with pancreatic ductal adenocarcinoma (PDAC) were collected from 2005 to 2014 at the Fuzhou General Hospital, Fujian, China and Xijing Hospital, Fourth Military Medical University, Xi'an, China. These enrolled patients without receiving anti-tumor therapies before surgery. Overall survival data was obtained from the patients' medical records or calls (approximately 0–60 months; median: 9.000 ± 0.677 months), updated march 5, 2016. At the end of follow-up time, 87 patients had died. Overall survival was defined as the survival time after surgery. The staging was based on the seventh edition of the American Joint Commission on Cancer Staging System. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Medical University of Fujian. Informed consent was obtained from all individual participants included in the study.

2.2. Immunohistochemistry staining

Formalin-fixed and paraffin-embedded PDAC samples were cut into 4-μm thick sequent sections. Then the sections were incubated for overnight at 37 °C. After being deparaffinized in formaldehyde and rehydrated with graded ethanol, the sections was treated with 3% hydrogen peroxide for 10 min to block the endogenous peroxidase activity and then boiled with citrate buffer for 5 min in a high-pressure cooker for antigen retrieval. After washing with phosphate-buffered saline (PBS), the sections were incubated with diluted rabbit polyclonal anti-MYBL2 antibody (1:200 dilutions; no. HPA030530, Atlas Antibodies, Sweden) at 37 °C for 1 h, and then washed twice with PBS. After secondary antibody (EliVision kit-9901; Maxim, Fuzhou, China) was added for 30 min, diaminobenzidine (DAB) was used as the chromogen, then the nuclei were counterstained with hematoxylin. The staining score was evaluated according to the extent and intensity of staining (the ratio of positive cells: 0, 0–10%; 1, 10%–24%; 2, 25%–49%; 3, 50%–74%; 4, 75%–100%; the intensity of staining: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) [21]. The final score was designated using the proportion of positive tumor cells multiplied by staining intensity. low expression was defined as a final score <6 and high expression with a final score ≥6. These scores were determined confirmed by 2 pathologists in a double-blind analysis.

2.3. Statistical analysis

Statistical analysis was performed using SPSS software version 18.0.The Pearson's chi-square ($\chi 2$) test was used to analyze the relationship between the MYBL2 expression and clinicopathological parameters of PDAC patients. Survival curves were plotted using the Kaplan-Meier method and differences analyzed by log-rank test. Univariate and multivariate Cox regression analyses were performed to study associations of particular prognostic factors with patient survival. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Expression of MYBL2 is increased in PDAC samples

Immunohistochemistry showed that MYBL2 protein was mainly localized in the cytoplasm and nucleus of the tumor cells and overexpression in the PDAC lesions compared to adjacent normal tissues (Fig. 1). Furthermore, moderately to strong MYBL2 staining was detected in all the adjacent normal pancreatic islet cells (Fig. 1h and j). The higher level of MYBL2 expression was observed in 52/93 (55.91%) of tumor tissues, while only 14/93 (15.05%) in adjacent non-tumor tissues. The results demonstrated that the expression of MYBL2 was significantly higher in PDAC than normal pancreatic ductal cells ($P = 0.000$).

3.2. Relationship between MYBL2 expression and clinical parameters in PDAC patients

The Pearson's chi-square test was used to assess the relationships between MYBL2 protein expression and clinicopathologic parameters in PDAC including gender, age, tumor size, tumor location, histological grade, T classification, N classification, TNM stage, peri-neural invasion(PNI), Surgical margin and vital status. The results showed that MYBL2 expression was significantly correlated with T classification ($p = 0.002$), PNI ($p = 0.013$) and vital status ($p = 0.045$), while no significant associations with gender, age, tumor size, tumor location, histological grade, N classification, TNM stage, surgical margin (Table 1).

3.3. Prognostic significance of MYBL2 expression in PDAC patients

The follow-up time of all the patients (93) ranged from 1 to 60 months, and 87 patients had died at the end of follow-up time. To evaluate the role of MYBL2 in PDAC prognosis, we measured the correlation of MYBL2 expression with overall survival time by Kaplan-Meier method and log-rank test. Our results showed that the Patients with low expression of MYBL2 have a median survival of 12.000 ± 1.098 months compared to 7.000 ± 0.762 months in patients whose tumor was MYBL2-high ($P = 0.000$, Fig. 1k), which indicated that the overall survival was shorter in PDAC patients with higher MYBL2 expression.

Furthermore, the univariate and multivariate analyses were performed to evaluate the risk factors correlated with PDAC patients'prognosis. The results demonstrate that histological grade, T classification, N classification, clinical stage, peri-neural invasion (PNI) and MYBL2 expression were significantly associated with overall survival. And then, the multivariate Cox regression analysis revealed that histological differentiation ($P = 0.001$) and MYBL2 protein expression ($P = 0.010$) were independent predictors of the overall survival in patients with PDAC (Table 2).

4. Discussion

Pancreatic Ductal adenocarcinoma and its variants account for over 90% of pancreatic malignancies [23]. Since it is difficult to diagnose pancreatic cancer at an early stage and poor response to chemotherapy and radiotherapy, the overall 5-year survival rate remains extremely poor [1,2]. Several trials for patients with metastatic PDAC showed that the median overall survival was only 11.1 months for FOLFIRINOX (a combination of oxaliplatin, irinotecan, fluorouracil, and leucovorin), 8.5 months for gemcitabine plus nab-paclitaxel, and 6.24 months for gemcitabine plus erlotinib