Int J Clin Exp Pathol 2017;10(6):6918-6923
www.ijcep.com /ISSN:1936-2625/IJCEP0053602

Original Article

Expression of LRP16 in pancreatic ductal adenocarcinoma and its clinicopathological significance and prognostic value

Yong Huang, Xiaojing An, Po Zhao

Department of Pathology, Chinese People's Liberation Army General Hospital, Beijing 100853, China

Received March 23, 2017; Accepted April 23, 2017; Epub June 1, 2017; Published June 15, 2017

Abstract: Background: The aim of this study is to evaluate the expression of leukemia related protein 16 (LRP16) in pancreatic ductal adenocarcinoma, and analyze its correlation with clinicopathologic features and prognosis. Methods: Immunohistochemistry for LRP16 was performed in 118 cases of pancreatic ductal adenocarcinoma and 34 cases of distal normal pancreatic tissue. The relationship between LRP16 expression and patients' gender, age, tumor size, histologic grade, TNM stage and metastatic status was analyzed. Results: LRP16 expression was detected in 64 of 118 cases of the pancreatic ductal adenocarcinoma and in 6 cases of 34 normal pancreatic tissues. The expression of LRP16 was mainly located in cytoplasm and nucleus of tumor cells. The expression of LRP16 in carcinoma was significantly higher than that in normal pancreatic tissue. LRP16 protein expression was found in 37.3% (28/75) of carcinoma at stage I and II, and 83.7% (36/43) of carcinoma at stage III and IV. Correlation between LRP16 expression and clinicopathological factors was significant with regards lymph node, liver metastasis and TNM stage. The difference of median survival between cancer patients with LRP16 positive expression and those with LRP16 negative expression was statistically significant. Conclusion: The expression of LRP16 may be associated with lymph node, liver metastasis and TNM stage. Thusly, it might be used as a potential marker in predicting prognosis of pancreatic ductal adenocarcinomas.

Keywords: Pancreatic ductal adenocarcinoma, immunohistochemistry, leukemia related protein 16, prognosis

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies, which is a major cause of cancer related morbidity and mortality. It is the fourth most common cause of death from cancer worldwide [1]. Even with curative-intent surgery, the majority of patients might develop recurrent or metastatic diseases, and only a small subset of the patients could get long-term survival [2, 3].

Leukemia related protein 16 gene (LRP16) was first recognized and isolated from lymphocytes in 1999. LRP16 is an important estrogen-responsive gene, localized on chromosome 11q12.1 [4]. LRP16 is also an estrogen receptor alpha (ERα) coactivator [5], and it may play an important role in ER signaling pathways. There are few studies, with inconsistent results, evaluating the expression of Estrogen receptors in human pancreatic cancer [6, 7]. What's more, no immunohistochemical and clinicopathological studies of LRP16 have been performed in PDAC. Herein, we investigated the expression of LRP16 in PDAC and normal pancreatic tissue by immunohistochemistry and analyzed the relationships between LRP16 expression and some clinicopathological features in a Chinese cohort with PDAC.

Materials and methods

Patients and specimens

One hundred and eighteen patients who had undergone pancreatic ductal adenocarcinoma operation during 2014-2016 at the Chinese People's Liberation Army General Hospital (Beijing, China) were confirmed histologically and were enrolled in this study. Paraffin tissue of tumor specimens were retrieved from the

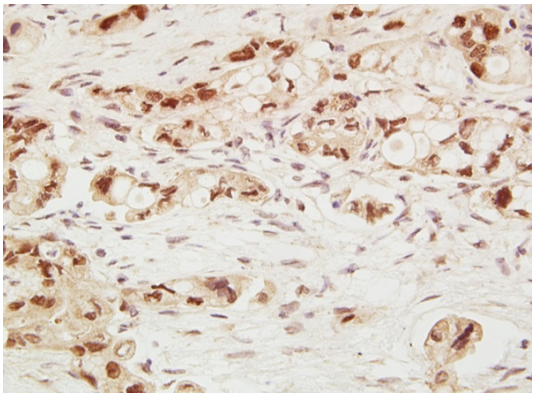

Figure 1. LRP16 expression in poorly differentiated pancreatic ductal adenocarcinomas. LRP16 was stained positively in the nucleus and cytoplasm of cancer cells. (LRP16 × 200).

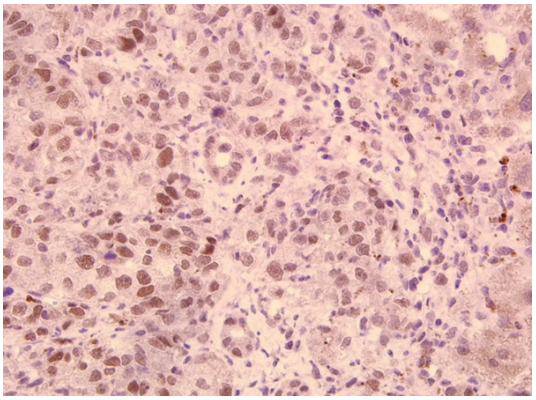

Figure 2. LRP16 expression in hepatic metastatic pancreatic ductal adenocarcinomas. (LRP16 × 200).

archives of the Department of Pathology. Clinical information, such as age, tumor size, grade, lymph node status and liver metastasis were obtained from medical records and the pathology reports.

Immunohistochemical analysis

Immunohistochemical staining was carried on 3-4 μm slides from formalin-fixed, paraffin embedded tissues. Paraffin slides were then deparaffinized inxylene and rehydrated. After rehydrating through a graded ethanol series, the sections were autoclaved in citrate buffer (pH 6.0) at 120°C for 2.5 min for antigen retrieval, then cooled to 30°C and washed with phosphate buffered saline (PBS, pH 7.3). The slides were rinsed with PBS and the endogenous peroxidase was inactivated with 3% hydrogen peroxide. After blocking with 10% goat serum, the slides were incubated with primary polyclonal rabbit antibody to human LRP16 (recognized and isolated in 1999 by the Department of Molecular Biology of our hospital) diluted 1:1000. Finally, the sections were counterstained with hematoxylin and mounted. Negative control sections were incubated with normal rabbit serum instead of the primary antibody. Positive and negative controls were included in each run.

Evaluation of immunohistochemistry

Antigen expression was evaluated independently by two authors using light microscopy. Both observers were blinded to all clinical data. For each sample, at least five fields (inside the tumor and in the area exhibiting tumor invasion). In scoring LRP16 protein expression, both the extent and intensity of positivity in the cell nucleus were considered. Slides were analyzed under light microscopy using manual methods. Staining was graded for intensity of staining (0, negative; 1, weak; 2, moderate; 3, strong) and percentage of cells stained (0, <5%; 1, 6%-25%; 2, 26%-50%; 3, >50%). The final score was got by the combined staining score (extent + intensity). We defined 0 score as negative, 1-3 score as weak positive expression (+) and 4-6 as strong expression (++).

Statistical analysis

Statistical analysis was carried using SPSS 13.0 software (SPSS Inc., Chicago, Illinois). The χ^2 test was used to examine the various clinicopathological characteristics and LRP16 expression. Cumulative survival curves were drawn by the Kaplan-Meier method. The difference between the curves was analyzed by the Log-rank test. Multivariate survival analysis was based on Cox proportional hazard model.

Results

Clinicopathological characterization of pancreatic ductal adenocarcinoma

The mean age of 118 cases at the time of diagnosis was 59.1 years old (range 17-84 years). Thirty-six of 118 demonstrated lymph node metastasis after lymph node sampling, nine cases had liver metastasis. Nineteen cases were categorized into well differentiated PDAC,

Table 1. Comparison of clinicopathological factors between LRP16 positive and LRP16 negative patients with pancreatic ductal adenocarcinoma

Variables	LRP 16		Statistical value
	Positive group	Negative group	
Gender			
Male	40	31	$\chi^2 = 0.317$
Female	24	23	*P>0.05*
Age			
>50	52	44	$\chi^2 = 0.001$
<50	12	10	*P>0.05*
Tumor size			
>5	14	10	$\chi^2 = 0.204$
<5	50	44	*P>0.05*
Histologic grade			
Well differentiated	11	8	$\chi^2 = 1.336$
Moderately differentiated	30	31	*P>0.05*
Poorly differentiated	23	15	
Lymph node metastasis			
Yes	31	5	$\chi^2 = 21.205$
No	33	49	*P<0.01*
Liver metastasis			
Yes	9	0	$\chi^2 = 8.223$
No	55	54	*P<0.05*
TNM stage			
I+II	28	47	$\chi^2 = 23.694$
III+IV	36	7	*P<0.01*

P<0.05, statistically significant. LRP16: Leukemia related protein 16.

61 cases were moderately differentiated PDAC, and 38 cases were poorly differentiated PDAC. Forty-three of 118 cases were in early stage and 75 cases in advanced stage. Follow-up data were recorded and were therefore included in data analysis.

LRP16 expression in pancreatic ductal adenocarcinoma

LRP16 staining was performed in 118 pancreatic ductal adenocarcinoma (**Figures 1**, **2**) and 34 cases of normal tissues by IHC. An evidently significant difference was found in the expression of LRP16 between PDAC and normal pancreatic tissues. The expression result of LRP16 showed that in PDAC, LRP16 staining was negative (-) in 45.8% (54/118) cases, weak positive (+) in 15.3% (18/118) cases and strong positive (++) in 39.0% (46/118) cases; whereas in normal pancreatic tissues, LRP16 was negative in 82.4% (28/34) cases, weak positive in 11.8% (4/34) and strong positive in 5.9% (2/34) cases.

Relationships between LRP16 expression and histological grade, TNM stage and prognosis

A total of 104 cases were followed up for survival to assess LRP16 expression as a prognostic factor. The expression of LRP16 in different histopathological type, tumor grades was analyzed. Samples with advanced TNM stage (36.4%) exhibited positive expression compared to that early TNM stage (63.6%). In addition, the positive expression rate of the cases with lymph node metastasis was significantly higher than cases without lymph node metastasis (86.1% versus 40.2%, P<0.01), as shown in **Table 1**. Significant positive correlations were also found between LRP16 expression and liver metastasis (P<0.05). Kaplan-Meier survival curves and Log-rank test demonstrated that LRP16 positive group showed a significantly shortened median survival in comparison with LRP16 negative patients (**Figure 3**). (Log rank = 25.510; P = 0.0001). Follow-up data presented that there was a significant difference in overall median survival between the tumors with LRP16 positive expression and those with negative expression.

LRP16 expression and Cox proportional hazards model

By Cox proportional hazards model, TNM stage was proved to be statistically significant, LRP16 was also an independent prognostic indicator (**Table 2**).

Discussion

LRP16 was originally recognized and isolated from human lymphocytes in 1999. LRP16 localized on chromosome 11q12.1, its molecular weight was 27.14×10^3. Originally, LRP16 was reported as an estrogen responsive gene. With the research progress, it has been found as a cell cycle regulator. What's more, as a special member of macro domain proteins, LRP16 was reported as a coactivator of both estrogen receptor and androgen receptor, and as an

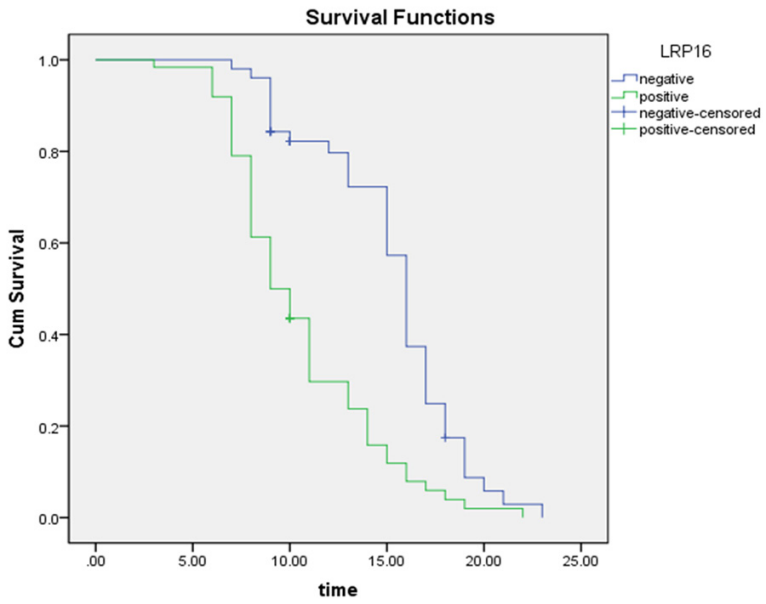

Figure 3. Kaplan-Meier survival analysis by LRP16 status. The y-axis represents the percentage of patients; the x-axis, their survival in months. The green line represents LRP16-positive patients with a trend of worse survival than the blue line representing LRP16-negative patients (Log rank = 25.510; P = 0.0001).

Table 2. Cox regression analysis of prognostic factors in pancreatic ductal adenocarcinoma

	B	SE	Wald value	*P* Value	RR
Gender	0.121	0.225	0.291	0.590	1.129
Age	0.103	0.270	0.147	0.702	1.109
Tumor size	0.078	0.260	0.091	0.763	0.925
TNM stage	2.533	0.592	18.299	0.000	12.594
Lymph node metastasis	0.530	0.527	1.013	0.314	0.589
Liver metastasis	0.039	0.428	0.008	0.928	1.040
Histology grade	0.265	0.150	3.121	0.077	1.303
LRP16	0.642	0.253	6.417	0.011	1.900

P<0.05, statistically significant. B: Partial regression coefficient. RR: Relative risk.

interactor of NF-κB coactivator UXT. The macro domain protein LRP16 was identified as a novel interactor of NF-κB component p65 [8]. Wu et al., found that LRP16 was a crucial regulator for NF-κB activation inside the nucleus, and may be an important contributor to the aberrant activation of NF-κB in tumors [9]. With the research progress, it has been reported over expression in lung endocrine neoplasms, gastric and colorectal adenocarcinomas, which are cancers of hormone-independent. Activation of ER signaling pathway plays an important role in multi-tissue development [10-12]. ERα might contribute to tumorigenesis primarily by stimulating cell proliferation. Thus, ERα can result in the accumulation of random DNA mutations, some of which may be carcinogenic.

ERs were reported as to be transcriptional regulators, which carries potential roles in carcinogenesis and tumor progression that are mediated by estrogen-regulated target genes [13]. Several target genes have been identified in breast and ovarian cancers, most of which were involved in cellular growth control, including cyclin D1 and c-myc, or in cellular motility and invasion. The growth factor pathways found to be modulated by estrogens in these cell lines are also operative in pancreatic cancers [14]. Moreover, recent studies have demonstrated that estrogens are capable of stimulating angiogenesis by upregulating the expression of angiogenic molecules and by direct mitogenic effects on endothelial cells [15]. Estrogen receptor beta/alpha ratio was reported to predict response of pancreatic cancer cells to estrogens [16]. As an ERα coactivator, LRP16 may display an important function in the carcinogenesis and progression of PDAC. Recent studies have shown that blocking estrogen receptor pathways may provide new therapy options for patients. Thus, LRP16 can be used an adjuvant marker to identify tumor cells in cases when the diagnosis of PDAC is in doubt.

In our study, LRP16 expression is related to lymph node metastasis, distant metastasis and TNM stage. The expression of LRP16 in cancerous tissue is significantly higher than that in normal tissue. LRP16 expression was commonly up-regulated in PDAC and was asso-

ciated with decreased survival time of the patients in univariate and multivariate analyses. Thus, LRP16 protein may play an important role in the carcinogenesis, progress and prognosis of PDAC in Chinese patients, and LRP16 expression detected by immunohistochemistry may be a simple and useful molecular marker to predict the prognosis in PDAC patients. We might suggest a hypothesis that LRP16 might regulate the tumorigenesis and progress as an ERα coactivator. This study raises the possibility that anti-estrogen therapy could be used in the patients with high LRP16 expression in PDAC. However, the association between LRP16 and ER in PDAC development needs to be further investigated, from which LRP16 targeting with anti-estrogen therapy may be applied in PDAC.

Conclusions

In conclusion, LRP16 protein may play an important role in the carcinogenesis, progress and prognosis of Chinese PDAC and LRP16 expression detected by immunohistochemistry may be a simple and useful molecular marker to predict the prognosis in pancreatic ductal carcinoma patients.

Acknowledgements

The authors thank all colleagues in the Department of Molecular Biology, Chinese People's Liberation Army General Hospital for their help and support with this study. All authors have contributed significantly, and all authors are in agreement with the content of the manuscript. This research was supported by the grants from the National Natural Science Foundation of China [81230061, 81472612], Beijing Natural Science Foundation [5162027].

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Po Zhao, Department of Pathology, Chinese People's Liberation Army General Hospital, 28 Fuxing Road, Beijing 100853, China. E-mail: path301@163.com

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