Pathology - Research and Practice 215 (2019) 265–271

Contents lists available at ScienceDirect

Pathology - Research and Practice

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

Expression and significance of CD47, PD1 and PDL1 in T-cell acute lymphoblastic lymphoma/leukemia

Kun Yang[a], Jing Xu[a], Qinghang Liu[b], Jing Li[c], Yanfeng Xi[c,*]

[a] Department of Pathology, Shanxi Medical University, Taiyuan, Shanxi Province, China
[b] Department of Thoracic Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, China
[c] Department of Pathology, Shanxi Cancer Hospital, Taiyuan, Shanxi Province, China

ARTICLE INFO

Keywords:
Lymphoma
T-cell
Leukemia
PD1
PDL1
CD47

ABSTRACT

Although dose intensification strategies achieve a favorable prognosis for pediatric patients of T-lmphoblastic lymphoma/leukemia (T-LBL/ALL), numerous side effects have been followed. Molecular targeted therapies will be needed to optimize the current treatment strategy for T-LBL/ALL. The aim of this study was to analyse expression and significance of CD47, PD1 and PDL1 in. T-LBL/ALL. We performed immunohistochemistry staining and real time fluorescence quantitative PCR (qRT-PCR) on FFPE tissues. Immunohistochemistry results showed that the high expression rate of CD47 protein was 46.4% (26/56) and the positive expression rate of PDL1 protein was 37.5% (21/56). PD1 expression was observed in tumor infiltrating lymphocytes in approximately 20% of T-LBL/ALL patients, but not expressed on tumor cells of T-LBL/ALL. And the results of qRT-PCR showed that the relative expression levels of CD47, PDL1 and PD1 mRNA in 56 cases of T LBL/ALL were significantly higher than those in control group (6.915 vs 4.050, 12.255 vs 2.575, 37.990 vs 3.615), and the differences were all statistically significant (p all < 0.05). Univariate analysis showed that age, CD47 protein, CD47 mRNA,PDL1 protein and PDL1 mRNA expression were closely correlated with prognosis (P all < 0.05). We found that the overall one-year survival rates of patients with a high expression ($\geq$M) of CD47 and PDL1 mRNA were higher than in patients with low expression ($<$ M). However, the overall one-year survival rate of patients with a high expression ($\geq$M) of CD47 and PDL1 protein were lower than in patients with low expression ($<$ M). And patients with $\leq$25 years old had a worse prognosis than with $>$ 25 years old. Multivariate Cox regression analysis showed that the high expression of CD47 and PDL1 protein were independent prognostic factors (both $p < 0.05$). In a word, PD1/PDL1 and CD47 may be involved in the disease progression and prognosis of T-LBL/ALL, and detection and targeting of CD47 and PD1/PDL1 may provide a rational basis to for treatment of T-LBL/ALL.

1. Introduction

T-lmphoblastic lymphoma (T-LBL) and T-Acute lymphoblastic leukemia (T-ALL) are considered to represent different clinical presentations of the same disease [3]. T-lmphoblastic lymphoma/leukemia (T-LBL/ALL), a malignant disorder of T lymphoid progenitor cells, is common in children and adolescents. While dose intensification strategies have led to a favorable prognosis for pediatric patients, high-risk pediatric patients and most adult patients have significantly worse outcomes [5,23]. Furthermore, recurrent and resistant diseases are very difficult to salvage. Therefore, it is extremely urgent to find new treatment regimens based on targeted therapies.

The host prevents tumor formation by adaptive immunity and innate immunity. CD47 (also called integrin-associated protein, IAP), a 50-kd cell surface glycoprotein, is a critical “don’t eat me” signal to the innate immune system and inhibits phagocytosis by binding the inhibitory immunoreceptor, signal regulatory protein alpha (SIRPa) [21]. Whereas, Programmed death-ligand 1 (PD-L1) delivers a “don’t find me” signal to the adaptive immune system by binding PD1 (programmed cell death protein 1), which is an inhibitor of T cells [4]. PD-1/PD-L1, as immune checkpoints, as with CD47, play a role not only in normal tissues but also in tumorous tissues.

In the tumor tissue, cancer cells could restrict immune killing through a mechanism of immune evasion mediated by those genes mentioned above, CD47, PD1 and PDL1.Interactions between CD47 expressed on cancer cells and SIRPa on macrophages, inhibit

* Corresponding author at: Department of Pathology, Shanxi Cancer Hospital, No. 3 of Zhigongxin Street, Xinghualing Strict, Taiyuan, 030013, Shanxi Province, China.
E-mail address: xiyanfeng1998@163.com (Y. Xi).

https://doi.org/10.1016/j.prp.2018.10.021
Received 18 June 2018; Received in revised form 4 October 2018; Accepted 19 October 2018

phagocytosis by macrophages and result in tumor immmune evasion. Recent studies found that anti-CD47 antibodies were applied in leukemia [21]. For example, Uno S found that the monoclonal antibody against CD47 (mAb-MABL) expressed antitumor activity in mice implanted with CCRF-CEM (derived from T-ALL) and JOK-1 (B-CLL) cell lines [20]. And Mark P. Chao also demonstrated that anti-CD47 antibody caused the apoptosis of ALL cells in vitro, and inhibited tumor engraftment in vivo [5]. Of note, outcomes obtained from in vivo and in vitro experiments maintain certain limitations, thus the clinical application of anti-CD47 antibodies still requires verification of clinical trials.

PD1/PDL1 was found to be more highly expressed on solid and leukemia tumors compared to normal counterparts and to be an independent predictor of survival in some experiments [15,18,22]. At present, various antibodies against PD1 and PD-L1 are in the preclinical phase in diverse malignancies. And anti-PD1 antibodies have been approved for the treatment of relapsed and refractory HL in May 2016 [11]. However, to date, the expression of PD1/PDL1 in. T- LBL/ALL is still rarely reported.

Our previous article published in the Chinese Journal of *Leukemia and Lymphoma* have summarized the relationship between the expression of PD1 and PDL1 and clinicopathological factors [27]. Results showed the expression of PDL1 protein was correlated with age, international prognostic score (IPI), lactate dehydrogenase (LDH) level and clinical symptoms (p all < 0.05). The relative expression of PDL1 mRNA was positively correlated with age ($p < 0.05$) and negatively associated with LDH level and IPI score (p all < 0.05). Furthermore statistical analysis showed that PD1 protein was related to KPS score, and the expression of PD1 mRNA correlated with IPI score (P both < 0.05).

In this study, we analysed expression and significance of CD47, PD1 and PDL1 in. T-cell acute lymphoblastic leukemia/lymphoma by IHC and qRT-PCR, and to explore the correlation between the expression of CD47, PDL1 and PD1.

2. Materials and methods

2.1. Case samples

We collected 56 lymph nodes diagnosed with T-LBL/ALL in Shanxi Cancer Hospital from 2003 to 2016. All cases were confirmed by immunohistochemistry: CD1a, CD3, εCD3, CD7, CD10, CD34, CD43, CD45RO, CD99, TDT, CD20, CD23, MPO, Ki-67. Inadequate/insufficient samples were excluded, and clinical follow-up information of 56 T-LBL/ALL cases were available. These cases were reclassified according to the 2016 World Health Organization classification by experienced hematopathologists (authors J.L and YF.X) [3].And additional 20 reactive hyperplasia of lymph nodes were used as control. The use of materials and clinical information was approved by the Research Ethics Committee of Shanxi Cancer Hospital (code: 2016LL101). Human tonsil and ovarian cancer were used as positive controls for PDL1, PD1 and CD47 immunohistochemical markers, and no primary antibody was used as a negative control.

2.2. Immunohistochemistry

Immunohistochemistry (IHC) was performed on formalin-fixed paraffin-embedded (FFPE) lymph node specimens by using a Roche BenchMark®XT autostainer (Roche, Switzerland). The antibodies were used according to the manufacturer's instructions: anti-CD47 (the monoclonal mouse anti-human antibody, 1:200, Abcam, UK); anti-PD1 antibody (mouse anti-PD1 monoclonal antibody) and anti-PDL1 antibody (the monoclonal rabbit anti-human antibody) were purchased from Beijing Zhongshan Golden bridge biotechnology company in China.

For PD1, PDL1 and CD47, the cell was considered positive if membranous staining was observed with/without a variably strong component of cytoplasmic staining in tumor cells and tumour-infiltrating immune cells. For PD1, staining in more than 5% of tumor cells or tumour-infiltrating immune cells was assigned a positive score. The PDL1 protein expression was considered positive if membranous staining was observed in 5% or more of the lymphoma cells. Specimens were scored as IHC 0, 1, 2, or 3,and 3 + > 0% cells, 2 + > 5% cells, 0–1 + < 5% cells. 0–1 + was considered as negative, while 2–3 + > 10% cells was positive [10]. For CD47, intensity of staining was categorized as 0 (no immunostaining), 1 (weak), 2 (moderate) and 3 (strong); the percentage of chromatosis cells as 0 (none), 1 (1–10%), 2 (11–50%), 3 (51–80%) and 4 (> 80%). Multiply these 2 numbers: 0–2 is considered (−); 3–4 (+); 5–8 (++); and 9–12 (+++). 0–1 + was considered as low expression, while 2–3 + was high expression [1]. Each slide was independently assessed twice by J.L and YF.X in a blinded fashion.

2.3. Isolation of total RNA and reverse transcription

Total RNA was extracted from FFPE specimens using the Total RNA Extraction Kit (AmoyDx, China) according to the manufacturer's instructions. RNA was reverse transcribed using the RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific™ Fermentas), according to the manufacturer's instructions.

2.4. Real time fluorescence quantitative PCR (qRT-PCR)

For quantitative measurements of the mRNA expression of PD1, PDL1 and CD47 gene, real time RT-PCR was performed using the Fast SYBR® Green Master Mix (Applied Biosystems) in a 20 μl reaction volume, according to the manufacturer's instructions. All reactions were run in triplicate on Step One™ PCR amplifier (Applied Biosystems), and GAPDH gene as endogenous control. The primer sequences for PD1 gene were: forward 5′ -CTC AGG GTG ACA GAG AGA AG-3′and reverse: 5′-GAC ACC AAC CAC CAG GGT TT-3′; Primers sequences for PDL1 gene were: forward 5′-TAT GGT GGT GCC GAC TAC AA-3′and reverse: 5′ -TGC TTG TCC AGA TGA CTT CG-3′; The primer sequences for CD47 gene were: forward 5′-GGCAAT GACGAAGGAGGTTA-3′and reverse: 5-ATCCGGTGGTATGGATGAGA-3′; The primer sequences for GAPDH gene were: forward 5′-GCA CCG TCA AGG CTG AGA AC-3′and reverse:5′-TGG TGA AGA CGC CAG TGG A -3′. A thermocycling program was set for 40 cycles of 3 s at 95 °C, and 30 s at 60 °C with an initial denaturation step at 95°Cfor 20 s. Gene expression values were normalized to endogenous control GAPGH, and calibrated to sample with the lowest expression. Relative quantification (RQ) of mRNA expression was calculated by using the $2^{-\Delta\Delta Ct}$ method described by Kenneth J. Livak et al [16], RQ = 2-ΔΔCt, ($\Delta\Delta Ct = \Delta Ct_{\text{sample}} - \Delta Ct_{\text{calibrater}}$, where$\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{GAPDH}}$).

2.5. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software 17.0, differences in mRNA levels were compared using the Mann–Whitney U test. Correlations of variables were computed with the Spearman rank correlation coefficient. The overall survival of T-ALL/LBL was examined by the Kaplan-Meier method, while differences were examined by the log-rank test. Multivariate Cox proportional hazard regression analysis was used to evaluate independent prognostic factors associated with patient survival. $P < 0.05$ were considered statistically significant.

3. Results

The cohort of 56 T-LBL/ALL patients is amenable to analysis with a median age of 22 years (range, 3–70 years) including 43 male and 13 female patients. According to the Ann Arbor staging system for

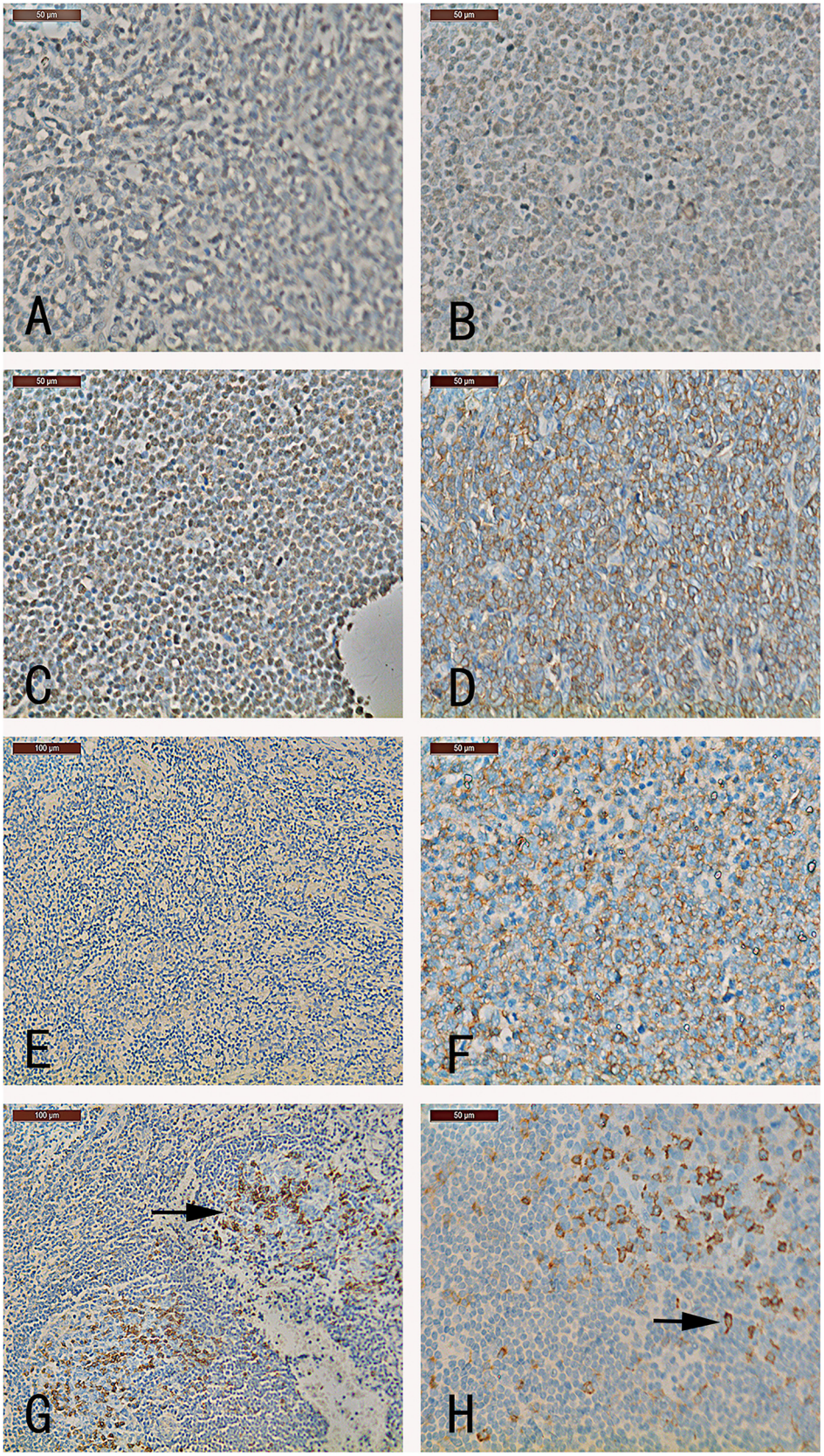

Fig. 1. Representative immunohistochemical micrographs of CD47, PD1 and PDL1 expression in T-LBL/ALL Panels A–D show four levels of CD47 expression as determined by grading criteria, where − ~ + is low expression of CD47 protein and + + ~ + + + is high expression of CD47 protein. (A) : − of CD47 expression; (B) : + of CD47 expression; (C) : + + of CD47 expression; (D) : + + + of CD47 expression; (E): PDL1- tumor cells of T-LBL/ALL.(F) PDL1 + tumor cells of T-LBL/ALL. (G) PD1 is mainly expressed in lymphoid nodule region of LH tissues(Arrow refers to $PD1^+$ immune cells). (H) Arrow refers to $PD1^+$ tumor infiltrating immune cells.

lymphoma, 9 patients were at stages I and II, and 47 patients were at stages III and IV.

3.1. Expression of CD47, PDL1 and PD1

3.1.1. Expression of CD47, PDL1 and PD1 by IHC

In T-LBL/ALL tissues, the expression of the CD47 protein was mainly found in the cell membrane. The high expression rate of CD47 in the experimental group was 46.4% (26/56), which was significantly higher than in the control group, 15% (3/20) ($p = 0.027$). PDL1 stainning was observed on tumor cells in the T-LBL/ALL tissues and on activated lymphocytes in the reactive lymph nodes. The positive expression rate of PDL1 in the T-LBL/ALL tissues was 37.5% (21/56), which was significant compared with 10% (2/20) in the control group ($p = 0.044$). PD1 stainning was not detected on tumor cells, however, was mainly expressed in tumor infiltrating lymphocytes in the T-LBL/ALL tissues. In control group, PD1 was found on activated lymphocytes. The positive expression rate of PD1 in the T-LBL/ALL tissues were 17.9% (10/56), which was significantly lower than in the control group, 90%(18/20) ($P < 0.001$) (Fig. 1).

3.1.2. Expression of CD47, PDL1 and PD1 by qRT-PCR

Comparing the expression of CD47, PDL1 and PD1 in. T-LBL/ALL tissues versus in reactive lymph nodes, the differences were statistically significant (P all < 0.05) (Fig.2).

3.2. Relationship between the expression of CD47 and clinicopathological factors of T-LBL/ALL

Table1 summarizes the relationship between the expression of CD47 and clinicopathological factors of the 56 patients with T-LBL/ALL. The high expression rate of CD47 protein in patients with ≤25 years old was 61.9% (19/31), which was significantly higher than that in patients with > 25 years old, 28% (7/25) ($p = 0.013$). And the relative expression of CD47 mRNA was correlated with age ($p < 0.05$). Nevertheless, there was no correlationship between the expression of CD47 protein and CD47 mRNA and other listed clinicopathological factors (p all > 0.05).

3.3. Survival analysis

As of December 2016, the median follow-up time for the 56 patients was 11.36 months (ranged from 0.57 to 80.01 months). A Kaplan-Meier method and Log-rank test were performed to analyze survival data and plot survival curves. Regarding the median as a cutoff value, the gene of CD47, PDL1 and PD1 were divided into high and low expression of two groups respectively. We found that the overall one-year survival rates of patients with a high expression (≥M) of CD47 and PDL1 mRNA were higher than in patients with low expression (< M), 62.2% vs 57.1%, 63.5% vs 26.7%, respectively (p all < 0.05), see Fig. 3. There was no statistically significant difference between low and high PD1 mRNA expression ($p = 0.958$, Fig. 3). However, the overall one-year survival rate of patients with a high/positive expression (≥M) of CD47 and PDL1 protein were lower than in patients with low/negative expression (< M), (57.4% vs 60%,27.8% vs 54.3%,p all < 0.05). And results showed that the overall 1-year survival rate in patients with ≤25 years old was 29.0%, but was 66.9% for patients with > 25 years old, ($p = 0.001$, Fig. 3). No significant differences were found between the other clinical factors and the prognosis.

Cox's regression analysis revealed that the high expression of CD47 and PDL1 protein were independent prognostic factors ($p < 0.05$), see Table 2.

Table 1
Relationship between the expression of CD47 and clinicopathological factors of T-LBL/ALL.

Characteristics	Cases	CD47 protein expression			CD47 mRNA	
		high	low	p-value	relative expression	p-value
Sex				0.33		0.174
Male	43	22	21		7.460 (4.960, 16.910)	
female	13	4	9		4.260 (2.735, 14.340)	
Age				**0.013**		**0.001**
≤25	31	19	12		5.060 (3.010, 7.570)	
> 25	25	7	18		10.930 (6.750, 24.675)	
Ann Arbor stage				0.621		0.867
I–II	9	3	6		8.540 (3.270, 12.665)	
III–IV	47	23	24		6.630 (3.660, 18.380)	
Serum LDH(U/L)				0.197		0.09
≤250	31	12	19		7.620 (5.060, 16.910)	
> 250	25	14	11		5.310 (2.615, 14.840)	
IPI				0.505		0.08
0-2	37	16	21		7.620 (4.185, 19.770)	
3–4	19	10	9		5.310 (2.220, 8.690)	
KPS score				0.952		0.905
< 80	11	5	6		7.315 (2.093, 19.940)	
≥80	45	20	25		6.750 (3.773, 13.348)	
Mediastinum widened				0.361		0.191
No	23	9	14		7.620 (5.310, 19.430)	
Yes	33	17	16		5.700 (2.292, 15.715)	
Bone marrow infiltration				0.338		0.521
No	20	11	9		6.580 (3.198, 11.208)	
Yes	36	15	21		6.195 (4.148, 19.168)	
Clinical symptoms				0.224		0.369
No	34	18	16		5.580 (2.966, 13.503)	
Yes	22	8	14		8.130 (4.853, 17.278)	

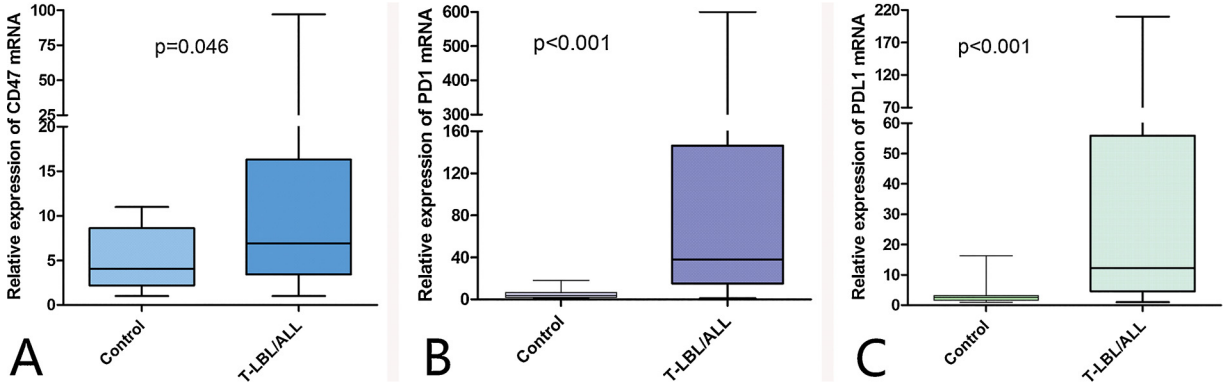

Fig. 2. The results of qRT-PCR showed that the relative expression levels of CD47, PD L1 and PD1 mRNA in 56 cases of T LBL/ALL were significantly higher than those in control group (6.915 vs 4.050, 12.255 vs 2.575, 37.990 vs 3.615), and the differences were all statistically significant (p all < 0.05).

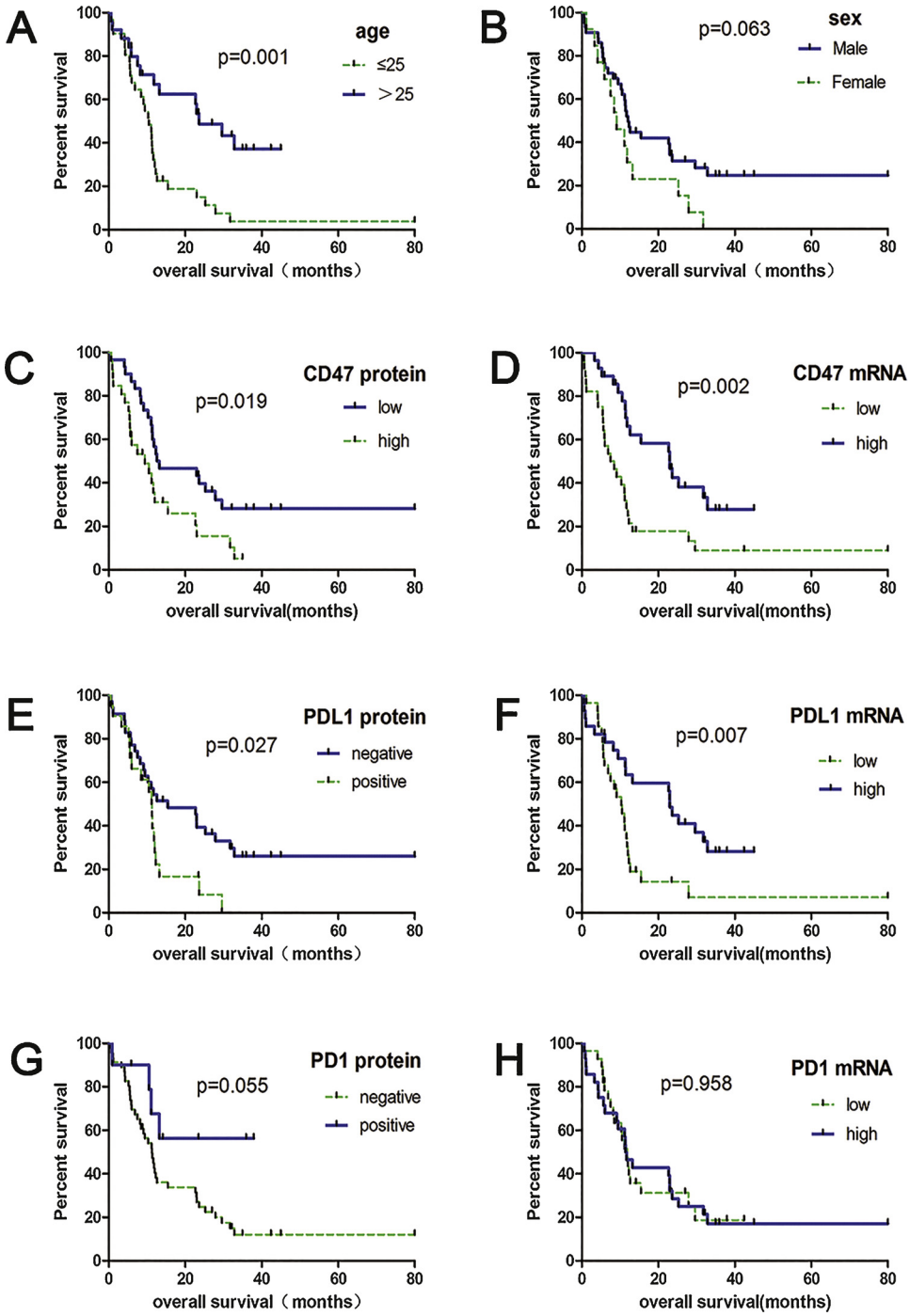

Fig. 3. Overall survival (OS) analysis for T-LBL/ALL patients.

Table 2
Cox multivariate regression analysis of CD47, PDL1 and PD1 expression and related factors.

Related factors	B	SE	Wald	P value	HR	95%CI
Sex	0.631	0.354	3.178	0.075	1.88	0.939–3.761
Age	−0.171	0.462	0,137	0.711	0.843	0.341–2.083
CD47 protein	0.83	0.361	5.283	**0.022**	2.293	1.130–4.655
CD47 mRNA	−0.623	0.406	2.351	0.125	0.536	0.242–1.189
PD-L1 protein	0.752	0.36	4.375	**0.036**	2.122	1.049–4.296
PD-L1 mRNA	−0.342	0.481	0.507	0.476	0.71	0.277–1.821
PD-1 protein	−0.734	0.546	1.805	0.179	0.48	0.165–1.400
PD-1 mRNA	0.286	0.383	0.56	0.454	1.332	0.629–2.821

3.4. Correlation between CD47, PDL1 and PD1 expression

There was a positive correlation between CD47 mRNA, and PDL1 mRNA, and similarly, PDL1 mRNA and PD1 mRNA were also positively correlated (rs = 0.540, P < 0.001; rs = 0.727, P < 0.001, respectively, see Fig. 4B,C). No correlation was found between CD47 and PD1 mRNA (rs = 0.193, p = 0.153, see Fig. 4A). Furthermore, There were no correlation between CD47 protein (or PDL1)and PD1 protein, the same as between PDL1 and CD47 protein. Finally, we did not find statistically significant differences in the three paired combinations, CD47 protein- mRNA, PDL1 protein- mRNA and PD1 protein – mRNA (rs = −0.215, p = 0.112; rs = −0.037, p = 0.787; rs = −0.187, p = 0.169, respectively).

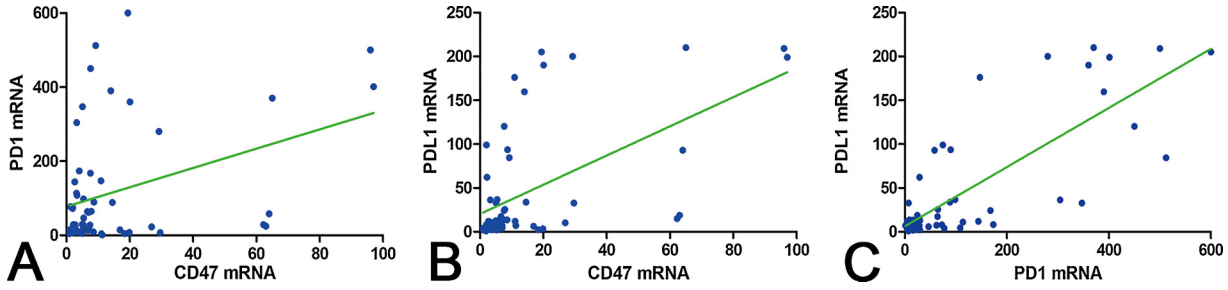

Fig. 4. Correlation among CD47 mRNA, PDL1 mRNA and PD1 mRNA expression. Picture 4 A showed that there was no correlation between CD47 mRNA and PD1 mRNA ($p = 0.153$); Picture 4B showed that a positive correlation was observed between CD47 mRNA and PDL1 mRNA ($P < 0.001$); Picture 4C showed there was a positive correlation between PDL1 mRNA and PD1 mRNA ($P < 0.001$).

4. Discussion

In this study, we explored the expression and clinical significance of CD47, PDL1 and PD1 in. T lymphoblastic lymphoma, and demonstrated that CD47 protein and PDL1 protein are independent prognostic factors of the disease. We observed the relationship between the three factors and the clinicopathological factors of the disease from two aspects of protein level and gene expression, which will provide a basis for the future diagnosis and treatment of the disease.

Our study found that the high expression rate of CD47 protein accounts for about half of all T-LBL tissues, and indicated a poor prognosis. This ratio is consistent with the expression of Wenhua Fu [8] in melanoma, but higher than that of Serena Galli [9] in AML tissues. Many studies have shown that CD47 protein could be highly expressed in a variety of cancers [2,17], in which unfavourable prognosis was also been observed. And this poor prognosis is mainly related to the immune escape of the tumor caused by the combination of the CD47 expressed on tumor cells and SIRPa on macrophages. The probable reason is that high expression of CD47 on the surface of tumor cells will give phagocytic cells a "don't eat me" signal, so that these tumor cells are protected from the phagocytosis of phagocytic cells, further promoting the occurrence and development of tumors. In addition, we found that the expression of CD47 mRNA in T-LBL/ALL patients was higher than that in the counterpart, which was consistent with the high protein expression we observed. And Stephanie C. Casey et al [4] found that the expression of CD47 mRNA and protein levels could be regulated by MYC gene, MYC Genes can bind to the promoter region of the CD47 gene sequence to regulate the expression of CD47 genes and proteins. However, the correlation between CD47 mRNA and protein expression was not observed in our experiment, which may have a certain relationship with our sample size, and we still need to further expand the sample to verify.

The mechanisms leading to tumor immune escape also include the PD1/PDL1 signaling pathway, which is primarily a negative regulatory process of T cell activation mediated by the binding of PDL1 on the surface of tumor cells to PD1 on the surface of tumor-infiltrating immune cells. Our study found that PDL1 protein and mRNA levels in tumor tissue increased, and the positive expression of PDL1 protein in T-LBL disease prompted a poor prognosis. Currently, studies found that the overexpression of PDL1 protein was a poor prognostic indicator in a variety of lymphomas [15,18,22], however, studies have also shown that high expression of PDL1 protein suggests a good prognosis [12,14]. For this issue, Lorenzo Falchi1 [7] gave explanations: 1. Different cut-off values for the positive expression of PD-L1, or the application of different antibodies Clones ; 2. Intrinsic variability of PD-L1 expression in different tumor sites or different regions of the same tumor sample.And in this study, 5% tumor cells were selected as the cut-off value for PDL1 protein interpretation, which was consistent with the criteria of Herbst RS [10] and Topalian SL [24].

In addition, our results showed that PD1 protein was not expressed in tumor cells of T-LBL/ALL, however, mainly in a small number of tumor infiltrating immune cells, which is consistent with the results of Dorfman.DM [6]. In their experiments, PD1 is not expressed in T-LBL/ALL and a series of other lymphoma tumor cells but is a new prognosis in immunoblastic lymphoma index. Also, the expression rate of PD1 + tumor infiltrating immune cells was only 11%–20% in other lymphoma tissues [13,19], which was consistent with the low expression of PD1 protein in experimental infiltrating immune cells in our study. In view of the evaluation criteria of PD1 protein, although 20% tumor infiltrating immune cells are selected as cut-off values in the Hodgkin lymphoma tissue, we refer to the NK/T cell lymphoma experiment with Jo JC [12], which is similar to this study. It is considered that the selection of 5% criteria is of greater reference significance. However, PD1 mRNA was highly expressed in T-LBL/ALL group, which might be related to post-transcriptional regulation mechanism, and there was likely to be degradation of the protein. Furthermore, Zhang G [26] found that MIR-4717 could regulate the expression of PD1 gene at translational level. Transfection of MIR-4717 mimics into lymphocytes had no effect on the expression of PD1 mRNA but significantly inhibited the expression of PD1 protein on lymphocytes. Some studies have also shown that the low expression of PD1 protein in the tumor microenvironment may be related to the depletion of T cells, and PD1/PDL1 pathway may be involved in [13]. And various antibodies against PD1 and PD-L1 are under clinical evaluation in different malignancies. Therefore, antibodies against PD1/PDL1 may be a new strategy for the treatment of T-LBL/ALL.

Correlation analysis showed that the correlation between PDL1mRNA and PD1mRNA, which also suggested that PD1/PDL1 signaling pathway might be involved in disease progression. And there was a certain correlation between CD47mRNA and PDL1mRNA, but no corresponding correlation between the protein. In addition, although studies such as Guang-Tao Yu et al in HPV negative head and neck squamous cell carcinoma [25] showed that down-regulation of expression of CD47/SIRPa signaling pathway by blockade PD-1 may improve phagocytosis of macrophage to enhance antitumor immunity. However, no correlation between PD1 and CD47 was found in our study. Furthermore, no correlation was found between the protein expression and gene levels of PD1, PDL1, or CD47, indicating the complexity of the expression of CD47, PD1, and PDL1 in. T-LBL/ALL. Further experiments are needed to investigate the occurrence of disease and the expression mechanism, which provides a theoretical basis for the treatment of diseases.

To sum up, we found that CD47 protein and PDL1 protein were independent prognostic risk factors, and the mRNA levels of PD1 and PDL1 were highly expressed and correlated, further suggesting that PD1/PDL1 is involved in the disease progression and prognosis of T-LBL/ALL. Anti-CD47 as well as anti-PD1/PDL1 treatment is expected to become a new target for the treatment of disease.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

This research was supported by Natural Science Foundation of Shanxi Province, P. R. China (No. 201601D011129). The authors would like to thank you to all those who work in Department of Pathology, Shanxi Cancer Hospital for their support.

References

[1] I. Baccelli, A. Stenzinger, V. Vogel, B.M. Pfitzner, C. Klein, M. Wallwiener, M. Scharpff, M. Saini, T. HollandLetz, H. Sinn, Co-expression of MET and CD47 is a novel prognosticator for survival of luminal-type breast cancer patients, Oncotarget 5 (2014) 8147.
[2] L. Barrera, E. Montes-Servín, J.-M. Hernandez-Martinez, Mdl.Á. García-Vicente, E. Montes-Servín, M. Herrera-Martínez, J.C. Crispín, J.R. Borbolla-Escoboza, O. Arrieta, CD47 overexpression is associated with decreased neutrophil apoptosis/phagocytosis and poor prognosis in non-small-cell lung cancer patients, Br. J. Cancer 117 (2017) 385–397.
[3] J.M. Bennett, Changes in the updated 2016: WHO classification of the myelodysplastic syndromes and related myeloid neoplasms, Clin. Lymphoma Myeloma Leuk. 16 (2016) 607–609.
[4] S.C. Casey, L. Tong, Y. Li, R. Do, S. Walz, K.N. Fitzgerald, A. Gouw, V. Baylot, I. Gutgemann, M. Eilers, MYC regulates the anti-tumor immune response through CD47 and PD-L1, Science 352 (2016) 227.
[5] M.P. Chao, A.A. Alizadeh, C. Tang, M. Jan, R. Weissman-Tsukamoto, F. Zhao, C.Y. Park, I.L. Weissman, R. Majeti, Therapeutic antibody targeting of CD47 eliminates human acute lymphoblastic leukemia, Cancer Res. 71 (2011) 1374–1384.
[6] D.M. Dorfman, J.A. Brown, A. Shahsafaei, G.J. Freeman, Programmed death-1 (PD-1) is a marker of germinal center-associated T cells and angioimmunoblastic T-cell lymphoma, Am. J. Surg. Pathol. 30 (2006) 802–810.
[7] L. Falchi, Immune dysfunction in non-Hodgkin lymphoma: avenues for new immunotherapy-based strategies, Curr. Hematol. Malig. Rep. 12 (2017) 484–494.
[8] W. Fu, J. Li, W. Zhang, P. Li, High expression of CD47 predicts adverse prognosis in Chinese patients and suppresses immune response in melanoma, Biomed. Pharmacother. 93 (2017) 1190–1196.
[9] S. Galli, I. Zlobec, C. Schurch, A. Perren, A.F. Ochsenbein, Y. Banz, CD47 protein expression in acute myeloid leukemia: a tissue microarray-based analysis, Leuk. Res. 39 (2015) 749–756.
[10] R.S. Herbst, J.C. Soria, M. Kowanetz, G.D. Fine, O. Hamid, M.S. Gordon, J.A. Sosman, D.F. McDermott, J.D. Powderly, S.N. Gettinger, H.E. Kohrt, L. Horn, D.P. Lawrence, S. Rost, M. Leabman, Y. Xiao, A. Mokatrin, H. Koeppen, P.S. Hegde, I. Mellman, D.S. Chen, F.S. Hodi, Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients, Nature 515 (2014) 563–567.
[11] I. Hude, S. Sasse, A. Engert, P.J. Bröckelmann, The emerging role of immune checkpoint inhibition in malignant lymphoma, Haematologica 102 (2017) 30–42.
[12] J.C. Jo, M. Kim, Y. Choi, H.J. Kim, J.E. Kim, S.W. Chae, H. Kim, H.J. Cha, Expression of programmed cell death 1 and programmed cell death ligand 1 in extranodal NK/T-cell lymphoma, nasal type, Ann. Hematol. (2015) 1–7.
[13] J.C. Jo, M. Kim, Y. Choi, H.J. Kim, J.E. Kim, S.W. Chae, H. Kim, H.J. Cha, Expression of programmed cell death 1 and programmed cell death ligand 1 in extranodal NK/T-cell lymphoma, nasal type, Ann. Hematol. (2017) 1–7.
[14] W.Y. Kim, H.Y. Jung, S.J. Nam, T.M. Kim, D.S. Heo, C.-W. Kim, Y.K. Jeon, Expression of programmed cell death ligand 1 (PD-L1) in advanced stage EBV-associated extranodal NK/T cell lymphoma is associated with better prognosis, Virchows Arch. 469 (2016) 581–590.
[15] J. Kiyasu, H. Miyoshi, A. Hirata, F. Arakawa, A. Ichikawa, D. Niino, Y. Sugita, Y. Yufu, I. Choi, Y. Abe, Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma, Blood 126 (2015) 2193.
[16] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method, Methods 25 (2001) 402–408.
[17] R. Majeti, M.P. Chao, A.A. Alizadeh, W.W. Pang, S. Jaiswal, K.D. Gibbs, N. van Rooijen, I.L. Weissman, CD47 Is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells, Cell 138 (2009) 286–299.
[18] H. Miyoshi, J. Kiyasu, T. Kato, N. Yoshida, J. Shimono, S. Yokoyama, H. Taniguchi, Y. Sasaki, D. Kurita, K. Kawamoto, PD-L1 expression on neoplastic or stromal cell is respectively poor or good prognostic factor for adult T-cell leukemia/lymphoma, Blood (2016).
[19] S. Paydas, E. Bagir, G. Seydaoglu, V. Ercolak, M. Ergin, Programmed death-1 (PD-1), programmed death-ligand 1 (PD-L1), and EBV-encoded RNA (EBER) expression in Hodgkin lymphoma, Ann. Hematol. 94 (2015) 1545–1552.
[20] S. Uno, Y. Kinoshita, Y. Azuma, T. Tsunenari, Y. Yoshimura, S. Iida, Y. Kikuchi, H. Yamada-Okabe, N. Fukushima, Antitumor activity of a monoclonal antibody against CD47 in xenograft models of human leukemia, Oncol. Rep. 17 (2007) 1189.
[21] E. Sick, A. Jeanne, C. Schneider, S. Dedieu, K. Takeda, L. Martiny, CD47 update: a multifaceted actor in the tumour microenvironment of potential therapeutic interest, Br. J. Pharmacol. 167 (2012) 1415–1430.
[22] M. Sznol, L. Chen, Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer, Clin. Cancer Res. 19 (2013) 1021–1034.
[23] T. Terwilliger, M. Abdul-Hay, Acute lymphoblastic leukemia: a comprehensive review and 2017 update, Blood Cancer J. 7 (2017) e577.
[24] S.L. Topalian, F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, J.D. Powderly, R.D. Carvajal, J.A. Sosman, M.B. Atkins, P.D. Leming, D.R. Spigel, S.J. Antonia, L. Horn, C.G. Drake, D.M. Pardoll, L. Chen, W.H. Sharfman, R.A. Anders, J.M. Taube, T.L. McMiller, H. Xu, A.J. Korman, M. Jure-Kunkel, S. Agrawal, D. McDonald, G.D. Kollia, A. Gupta, J.M. Wigginton, M. Sznol, Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, N. Engl. J. Med. 366 (2012) 2443–2454.
[25] G.T. Yu, L.L. Bu, C.F. Huang, W.F. Zhang, W.J. Chen, J.S. Gutkind, A.B. Kulkarni, Z.J. Sun, PD-1 blockade attenuates immunosuppressive myeloid cells due to inhibition of CD47/SIRPÎ ± axis in HPV negative head and neck squamous cell carcinoma, Oncotarget 6 (2015) 42067–42080.
[26] G. Zhang, N. Li, Z. Li, Q. Zhu, F. Li, C. Yang, Q. Han, Y. Lv, Z. Zhou, Z. Liu, microRNA-4717 differentially interacts with its polymorphic target in thePD13′ untranslated region: a mechanism for regulating PD-1 expression and function in HBV-associated liver diseases, Oncotarget 6 (2015) 18933–18944.
[27] 杨坤, 郗彦凤, 高宁, 步鹏, 徐菁, 程序性死亡受体1与其配体在T淋巴母细胞淋巴瘤/白血病中的表达及其临床意义. 白血病·淋巴瘤 26 (2017) 589-595.