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Original Article

Tumor-associated macrophages increase the proportion of cancer stem cells in lymphoma by secreting pleiotrophin

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Abstract: Tumor-associated macrophages (TAMs) are closely related to the occurrence and development of lymphoma, but their mechanism is still unclear. Here we collected peripheral blood and lymphoma tissue from patients with diffuse large B lymphoma. Results showed that the proportion of TAMs in high-risk group was significantly higher than that in low-risk group. Moreover, the expressions of pleiotrophin (PTN), PTPRZ1 (PTN receptor) and β-catenin in lymphoma tissues of high-risk group were also significantly higher than those in low-risk group. Correlation analysis showed that the proportion of TAMs in lymphocyte was positively correlated with the expression of PTN and PTPRZ1 in lymphoma tissue. In vitro experimental results showed that TAM promoted the invasion and proliferation of lymphoma cells by secreting PTN. We also found that TAMs increased the proportion of cancer stem cells in lymphoma. Animal experiments showed that TAMs promoted lymphoma growth. Both Ki-67 proliferation index and CD44+cancer stem cells increased significantly in TAM group. Blocking PTN or β-catenin partly inhibited these effects of TAMs. In conclusion, TAMs increased the proportion of cancer stem cells through PTN/β-catenin pathway in lymphoma.

Keywords: Lymphoma, tumor-associated macrophage, pleiotrophin, cancer stem cell

Introduction

In recent years, the incidence of lymphoma has been increasing rapidly [1]. Hematopoietic stem cell transplantation (HSCT) is currently the most effective treatment for lymphoma. However, due to the high cost or physical intolerance, many patients can not undergo HSCT [2]. With the development of gene detection and immunotherapy, the treatment of malignant tumors has entered an era of precise treatment [3]. Finding new therapeutic targets is of great significance for the precise treatment of lymphoma.

Tumor-associated macrophages (TAMs) have a large proportion in tumor stromal cells. TAMs are differentiated from monocytes under the influence of tumor cells and microenvironment [4, 5]. Many studies found that TAMs were closely related to the occurrence and development of lymphoma. TAMs affected the chemotherapy effect, and could be used as an independent predictor for the prognosis of patients with lymphoma [6-8]. Therefore, inhibiting the function of TAMs is very important for the treatment of lymphoma.

Pleiotrophin (PTN) is a secretory growth factor that can bind to heparin. It has important functions such as promoting cell proliferation, migration and inducing angiogenesis [9]. Many studies found that PTN was highly expressed in breast cancer, gastric cancer and other malignant tumors [10, 11]. PTN can be used as a multifunctional tumor promoter for the occurrence and development of tumors by reconstructing tumor microenvironment [12]. Recent study found that TAMs promoted the invasion and metastasis of glioma by secreting PTN [13].

This study was to investigate the expression and mechanism of PTN in lymphoma, and provide a new target for the precise treatment of lymphoma.

Materials and methods

Clinical data of patients

Patients with diffuse large B lymphoma treated in our hospital from October 2017 to April 2019 were collected. Exclusion criteria: age < 18 years old, with diabetes or uremia, drug abuse, alcoholism, with systemic autoimmune diseases or other malignant tumors, with AIDS or viral hepatitis. According to the International Prognostic Index of Lymphoma (IPI), the patients were divided into low-risk group (score ≤ 2) and high-risk group (score > 2). There were 30 patients in the low-risk group, with an average age of 46.3 ± 8.6 years, and 30 patients in the high-risk group, with an average age of 48.6 ± 9.3 years. 30 cases of normal lymph nodes were collected as the control group. All experiments were performed in accordance with the regulations of The Third Affiliated Hospital of Chongqing Medical University (Chongqing, China) and were approved by the Ethics Committee of The Third Affiliated Hospital of Chongqing Medical University.

Histological staining

Lymphoma tissues in each group were collected for paraffin sectioning. Some slices were stained with hematoxylin & eosin dyes (H&E) (ZsBio) to detect the pathological changes, and others were conducted to immunohistochemical staining. Briefly, 3% H_2O_2 and sheep serum blocking solution (ZsBio) were applied to the dewaxed paraffin sections which then were washed three times with PBS. Subsequently, these sections were incubated with primary antibodies including PTN, PTPRZ1 (PTN receptor), Ki-67, β-catenin and CD163 (antibodies were all purchased from Abcam) overnight at 4°C and washed three times with PBS. Afterwards, Biotin-labeled secondary antibody and streptavidin working solution (ZsBio) were sequentially added. Finally, sections were counterstained with hematoxylin (ZsBio) for 10 min after colored staining agents were developed and observed under a microscope.

Detection and sorting of TAMs

Peripheral blood in each group was collected and lymphocyte was separated by human lymphocyte separating fluid (TBD Company). CD14, CD3 and CD163 antibodies (BD Company) were added and incubated at room temperature for 15 minutes. After washed three times with PBS, flow cytometry was used to detect the proportion of CD14+CD3-CD163+TAMs in lymphocyte. In addition, CD14+CD3-CD163+TAMs were sorted by flow cytometry and cultured in RPMI1640 medium containing 10% fetal bovine serum (Hyclone).

Invasive experiment

Diffuse large B lymphoma cells (U2932, ATCC) were cultured in RPMI1640 medium containing 10% fetal bovine serum. We used Transwell chamber (Corning) to carry out the invasion experiment, and 2×10^4 CM-DiI (Sigma) labeled U2932 was added into the upper chamber. The experiment was divided into four groups: control group, co-culture group (TAMs were added in the lower chamber), PTN blocking group (PTN neutral antibodies and TAMs were added in the lower chamber) and β-catenin blocking group (β-catenin neutral antibodies and TAMs were added in the lower chamber). After 12 hours, the cells in the lower chamber were collected and the proportion of CM-DiI labeled cells was detected by flow cytometry.

Proliferation experiment

We used Transwell chamber (Corning) to co-culture U2932 and TAMs. After 24 hours of co-culture, U2932 was collected and planted into 96-well plates at a density of 4×10^3/well. After 24 hours, MTT solution (Sigma) was added. After 4 hours of continuous culture, the supernatant was discarded and 150 uL DMSO was added. After mixing for 10 minutes, the absorbance (OD: 490 nm) of each group was measured.

Detection of lymphoma stem cells

CD34 and CD44 are specific markers of cancer stem cells. After 24 hours of co-culture, the cells in each group were collected. Then CD34 and CD44 antibodies (BD) were added and incubated at room temperature for 15 minutes. Flow cytometry was used to detect the proportion of CD34+CD44+cells in lymphocyte after three washings with PBS.

Animal experiments

Mouse tumorigenesis experiment was operated according to the methods reported in previ-

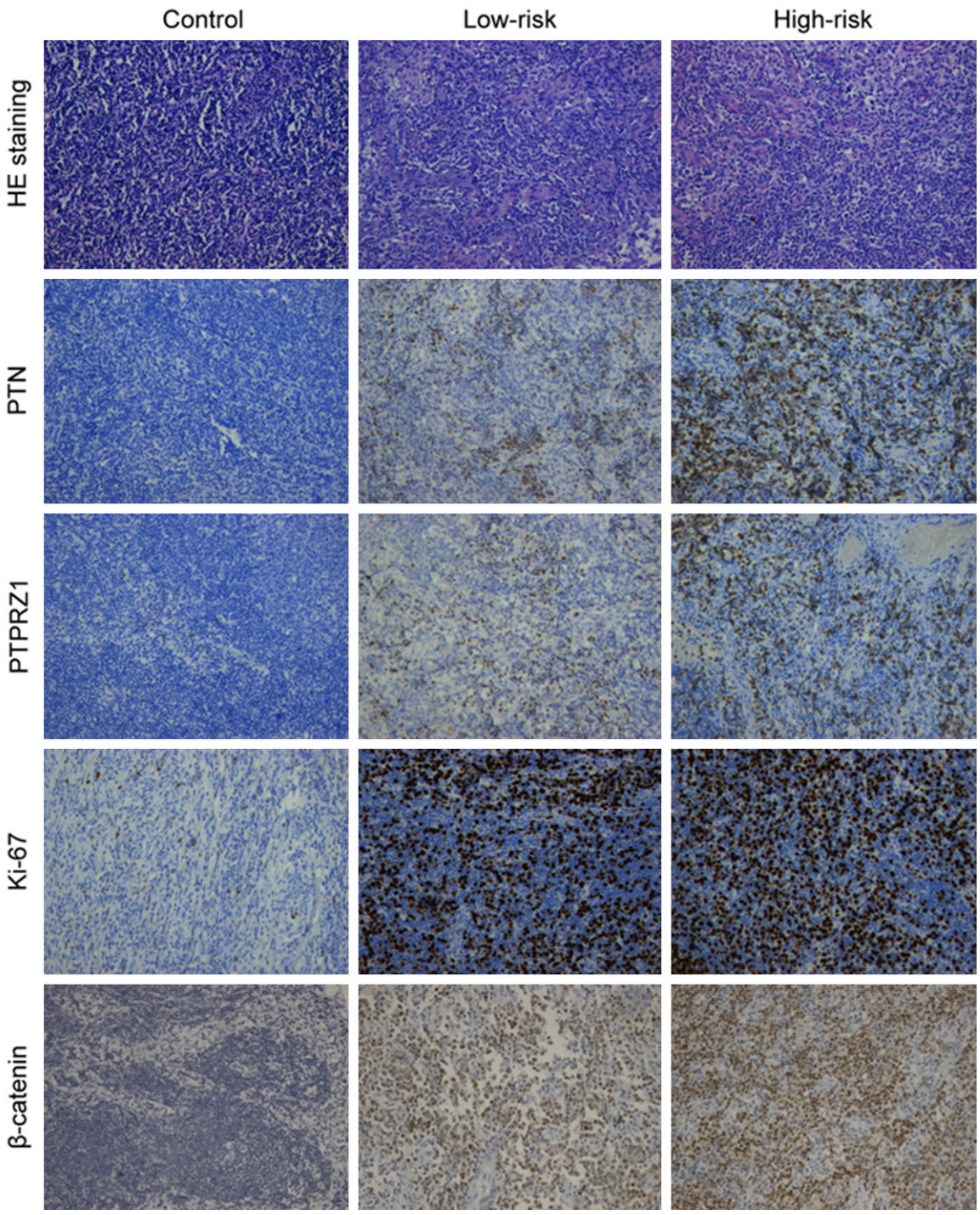

Figure 1. The expression of PTN, PTPRZ1 and β-catenin in lymphoma tissue. PTN, PTPRZ1 and β-catenin could not be detected in the lymphoid tissues of control group. The expression of PTN, PTPRZ1 and β-catenin in lymphoma tissue of low-risk group was significantly lower than that of high-risk group.

ous paper [14]. 1 × 10^6 U2932 in 30 ml PBS were transplanted into the subcutaneous tissue of C57Bl/6 mice. The experiment was divided into four groups: control group (subcutaneous transplantation of U2932 cells), TAM group (subcutaneous transplantation of U2932 cells and TAMs), PTN blocking group (subcutaneous transplantation of U2932 cells and TAMs, tail vein injection of PTN antibody) and β-catenin blocking group (subcutaneous transplantation

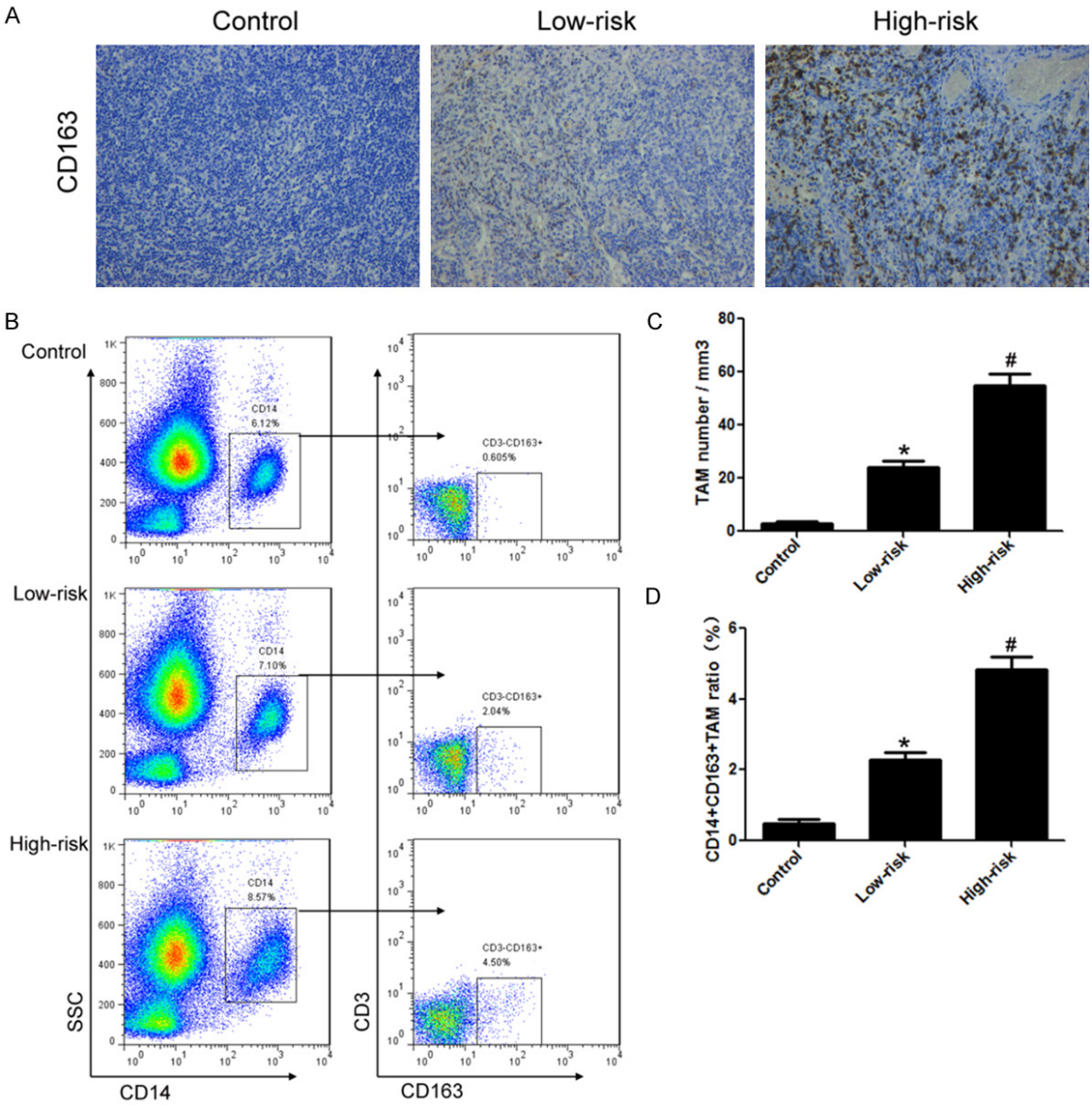

Figure 2. The proportion of TAMs in peripheral blood and lymphoma tissue of patients. A, C. CD163+TAMs in lymphoma tissues was detected by immunohistochemistry. The number of CD163+TAMs in high-risk group was 54.80 ± 4.389/mm^3, which was significantly different from that in low-risk group; B, D. CD14+CD3-CD163+TAMs in lymphocyte was detected by flow cytometry. The proportion of TAMs in high-risk group was 4.840 ± 0.3436%, which was significantly different from that in low-risk group. *P < 0.05 (n = 30) versus Control; #P < 0.05 (n = 30) versus Low-risk. Values are mean ± SD.

of U2932 cells and TAMs, tail vein injection of β-catenin antibody). Two weeks after transplantation, mice were anesthetized with 0.1% sodium pentobarbital and euthanized by cervical dislocation. Tumor tissues were collected and fixed in 4% paraformaldehyde. After paraffin sectioning, some tissues were stained with H&E, and other tissues were contucted to immunohistochemistry to detect the expressions of Ki-67 and CD44.

Statistical analysis

Statistical analyses were carried out using SPSS 19.0. Data values were presented as mean ± standard deviations. Multiple data comparisons were analyzed via ANOVA and Bonferroni *post hoc* test. Correlation analyses were performed via Spearman's rank correlation. A P < 0.05 is considered statistically significant.

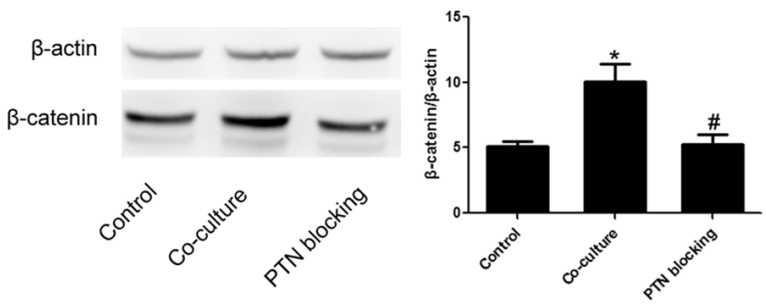

Figure 3. TAMs promoted the expression of β-catenin in diffuse large B cells. Western blotting results showed that the expression of β-catenin in co-culture group was significantly higher than that in control group. PTN blockade inhibited the effect of TAMs. *P < 0.05 (n = 6) versus Control; #P < 0.05 (n = 6) versus Co-culture. Values are mean ± SD.

Results

The expression of PTN and PTPRZ1 in lymphoma tissue increased significantly

Immunohistochemical results showed that PTN, PTPRZ1 and β-catenin could not be detected in the lymphoid tissues of control group. The proliferation index of Ki-67 in control group was 3.400 ± 0.5207%. The expressions of PTN, PTPRZ1 and β-catenin in lymphoma tissues of low-risk group and high-risk group were significantly increased. The average optical densities of PTN, PTPRZ1 and β-catenin in low-risk group were 30.20 ± 4.008, 26.23 ± 3.016 and 56.90 ± 4.900, and the proliferation index of Ki-67 was 37.01 ± 2.678%. The average optical densities of PTN, PTPRZ1 and β-catenin in high-risk group were 80.11 ± 8.171, 66.26 ± 5.824 and 150.7 ± 6.941, and the proliferation index of Ki-67 was 55.59 ± 4.022. There were significant statistical differences between them (t = 5.483, P < 0.0001; t = 6.104, P < 0.0001; t = 11.04, P < 0.0001; t = 3.845, P = 0.0012) (**Figure 1**).

The proportion of TAMs in patients with lymphoma increased significantly

The number of CD163+TAMs in the control group was 2.700 ± 0.7895/mm^3 and that in the low-risk group was 24.00 ± 2.490/mm^3 (t = 8.154, P < 0.0001). The number of CD163+TAMs in high-risk group was 54.80 ± 4.389/mm^3, which was significantly different from that in low-risk group (t = 6.104, P < 0.0001).

Flow cytometry showed that the proportion of CD14+CD3-CD163+TAMs in the control group was 0.4805 ± 0.1105%, and that in the low-risk group was 2.294 ± 0.2084% (t = 7.691, P < 0.0001). The proportion of CD14+CD3-CD163+TAMs in the high-risk group was 4.840 ± 0.3436%, which was significantly different from that in the low-risk group (t = 6.336, P < 0.0001). The results of correlation analysis showed that the proportion of TAMs in lymphocyte was positively correlated with the expression of PTN and PTPRZ1 in lymphoma tissue (r = 0.6591, P = 0.0016; r = 0.7012, P = 0.0006) (**Figure 2**).

TAMs promoted the expression of β-catenin in diffuse large B cells

It has been reported that PTN can promote the expression of β-catenin, which is closely related to cancer stem cells. Western blotting results showed that the expression of β-catenin in co-culture group was significantly higher than that in control group (t = 3.426, P = 0.0065). PTN blockade completely inhibited the effect of TAMs (t = 3.060, P = 0.0120) (**Figure 3**).

TAMs promoted the invasion of diffuse large B lymphoma cells

The proportion of invasive lymphoma cells in the co-culture group was 78.20 ± 3.333%. Compared with the control group, the results showed significant statistical difference (t = 6.598, P < 0.0001). The proportion of invasive lymphoma cells in PTN blocking group and β-catenin blocking group was 63.42 ± 2.968% and 59.53 ± 2.764%. Compared with co-culture group, the results showed significant statistical differences (t = 3.313, P = 0.0078; t = 4.311, P = 0.0015) (**Figure 4**).

TAMs promoted the proliferation of diffuse large B lymphoma cells

The proliferation rate of lymphoma cells in co-culture group was 49.67 ± 3.303%. Compared with the control group, the results showed significant statistical difference (t = 4.961, P = 0.0006). The proliferation rate of lymphoma cells in PTN blocking group and β-catenin blocking group was 35.33 ± 2.155% and 33.10 ± 1.868%. Compared with co-cultured group, the results showed significant statistical differences (t = 3.634, P = 0.0046; t = 4.366, P = 0.0014) (**Figure 5**).

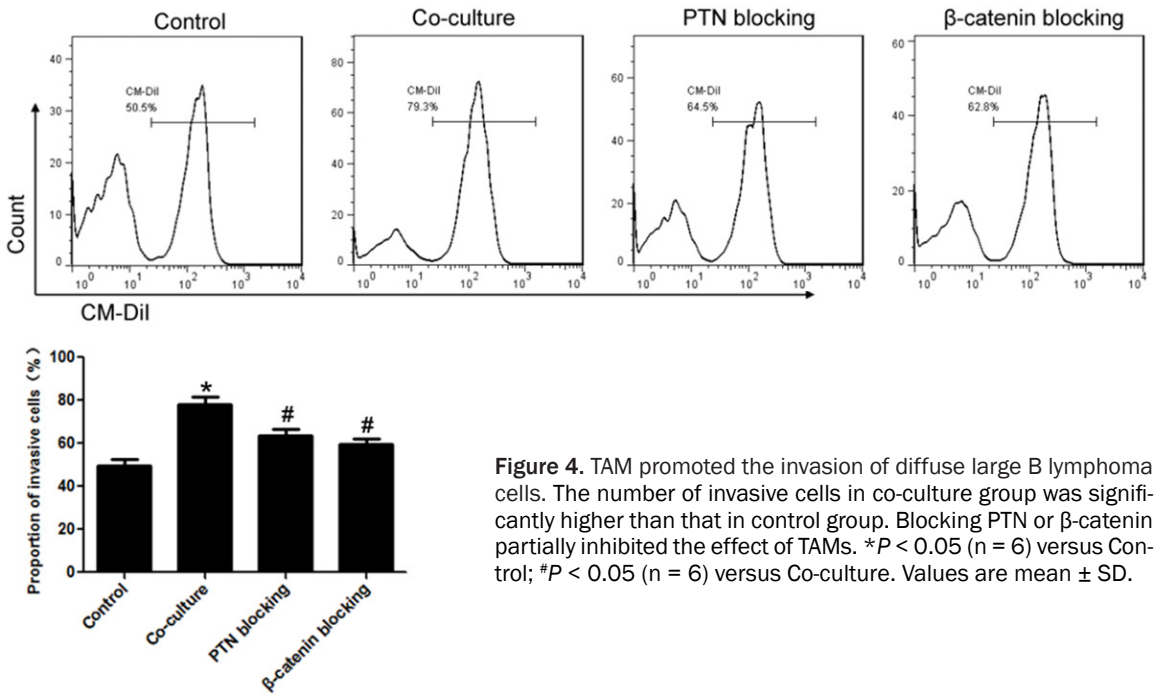

Figure 4. TAM promoted the invasion of diffuse large B lymphoma cells. The number of invasive cells in co-culture group was significantly higher than that in control group. Blocking PTN or β-catenin partially inhibited the effect of TAMs. **P* < 0.05 (n = 6) versus Control; #*P* < 0.05 (n = 6) versus Co-culture. Values are mean ± SD.

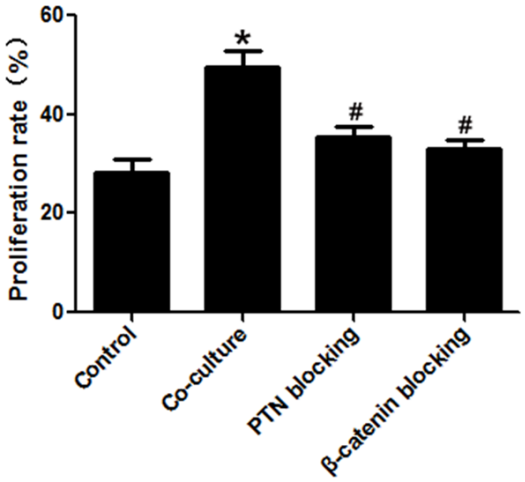

Figure 5. TAM promoted the proliferation of diffuse large B lymphoma cells. Cell proliferation rate in co-culture group was significantly higher than that in control group. Blocking PTN or β-catenin partially inhibited this effect of TAMs. **P* < 0.05 (n = 6) versus Control; #*P* < 0.05 (n = 6) versus Co-culture. Values are mean ± SD.

TAMs increased the proportion of cancer stem cells in lymphoma

The results of flow cytometry showed that the proportion of CD34+CD44+cells in the co-culture group was 0.7842 ± 0.03555%. Compared with the control group, the results showed significant statistical difference (t = 13.73, P < 0.0001). The proportion of CD34+CD44+cells in PTN blocking group and β-catenin blocking group was 0.2115 ± 0.03627% and 0.1178 ± 0.02861%. Compared with co-culture group, the results showed significant statistical differences (t = 11.28, P < 0.0001; t = 14.60, P < 0.0001) (**Figure 6**).

TAMs promoted lymphoma progression in vivo

Animal experiments showed that the volume and weight of lymphoma in the control group was 1299 mm^3 and 1.38 g, respectively, and those in the TAM group were 2217 mm^3 and 2.75 g. There were significant differences between two groups. Blocking PTN or β-catenin could partly inhibit the effect of TAMs. Compared with TAM group, the volume and weight of tumors in PTN blocking group was reduced by 27.56% and 38.25%, respectively. The volume and weight of tumors in β-catenin blocking group was reduced by 26.70% and 47.83%, respectively.

Immunohistochemical results showed that Ki-67 proliferation index in TAM group was 54.70%. Compared with the control group, it increased by 68.31%. Blocking PTN or β-catenin could partly inhibit the pro-proliferation effect of TAMs. Compared with the TAM group, Ki-67

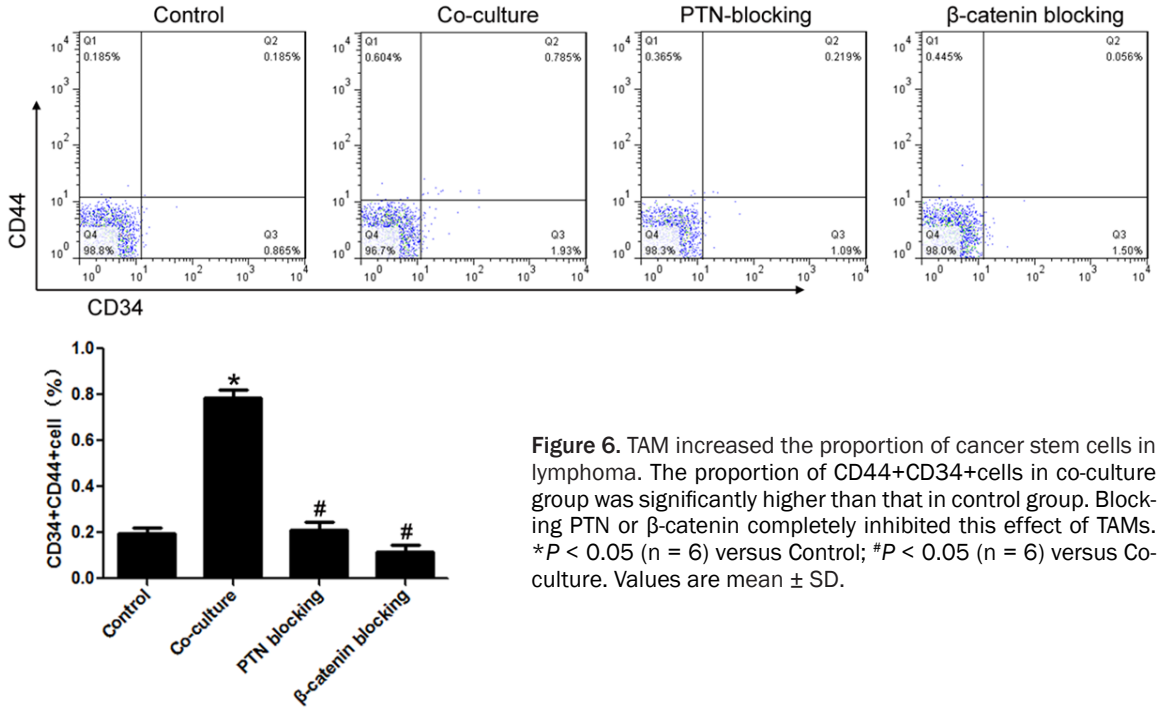

Figure 6. TAM increased the proportion of cancer stem cells in lymphoma. The proportion of CD44+CD34+cells in co-culture group was significantly higher than that in control group. Blocking PTN or β-catenin completely inhibited this effect of TAMs. $^{*}P < 0.05$ (n = 6) versus Control; $^{\#}P < 0.05$ (n = 6) versus Co-culture. Values are mean ± SD.

ratio decreased by 27.23% and 21.01%. The number of CD44+cancer stem cells in TAM group was 24.8/HPF, which increased by 7.27 times, compared with the control group. The number of CD44+cancer stem cells in PTN blocking group and β-catenin blocking group was 5.2 and 5.6/HPF, which had significant statistical significances compared with TAM group (**Figure 7**).

Discussion

In recent years, with the development of gene detection and immunotherapy technology, the treatment of malignant tumors has entered an era of precise treatment. Many targeted drugs have been used in clinic. The results of clinical treatment showed that these drugs significantly prolonged the survival time of patients and had fewer side effects [15]. However, molecular targeted drugs are only effective for specific populations, so it is very important to find new targets. PTN is a heparin-binding cytokine that can be expressed in embryonic tissues and seldom in normal human tissues [16]. Many studies found that PTN was highly expressed in gastric and oral cancer tissues, and was closely related to prognosis and tumor grade [17-19]. Our study found that PTN was highly expressed in lymphoma tissues, and PTN expression in high-risk group was significantly higher than that in low-risk group. These indicated that PTN had a very important relationship with the occurrence and development of lymphoma and can be used as a potential target in the treatment of lymphoma.

Macrophages play an important role in tumor immunity. Macrophages can be divided into M1 type and M2 type according to their functions and phenotypes. M1 macrophages are pro-inflammatory, secreting inflammatory factors such as IL-6, killing tumor cells and activating local immune response [20]. M2 macrophages are tumor-related and can promote tumor growth and escape immune surveillance. Recent studies found that TAMs promoted the proliferation and invasion of glioma by secreting PTN [13]. We also found that the proportion of TAMs in patients with lymphoma was significantly increased, which was closely related to the malignant degree of lymphoma. In vitro and in vivo experiments showed that TAMs could promote the proliferation and invasion of lymphoma cells. The blockade of PTN inhibited these effects of TAMs, which indicated that PTN may mediate the occurrence and development of lymphoma induced by TAMs.

Many studies have found that there is a class of cancer stem cells in malignant tumors, which are closely related to metastasis, proliferation,

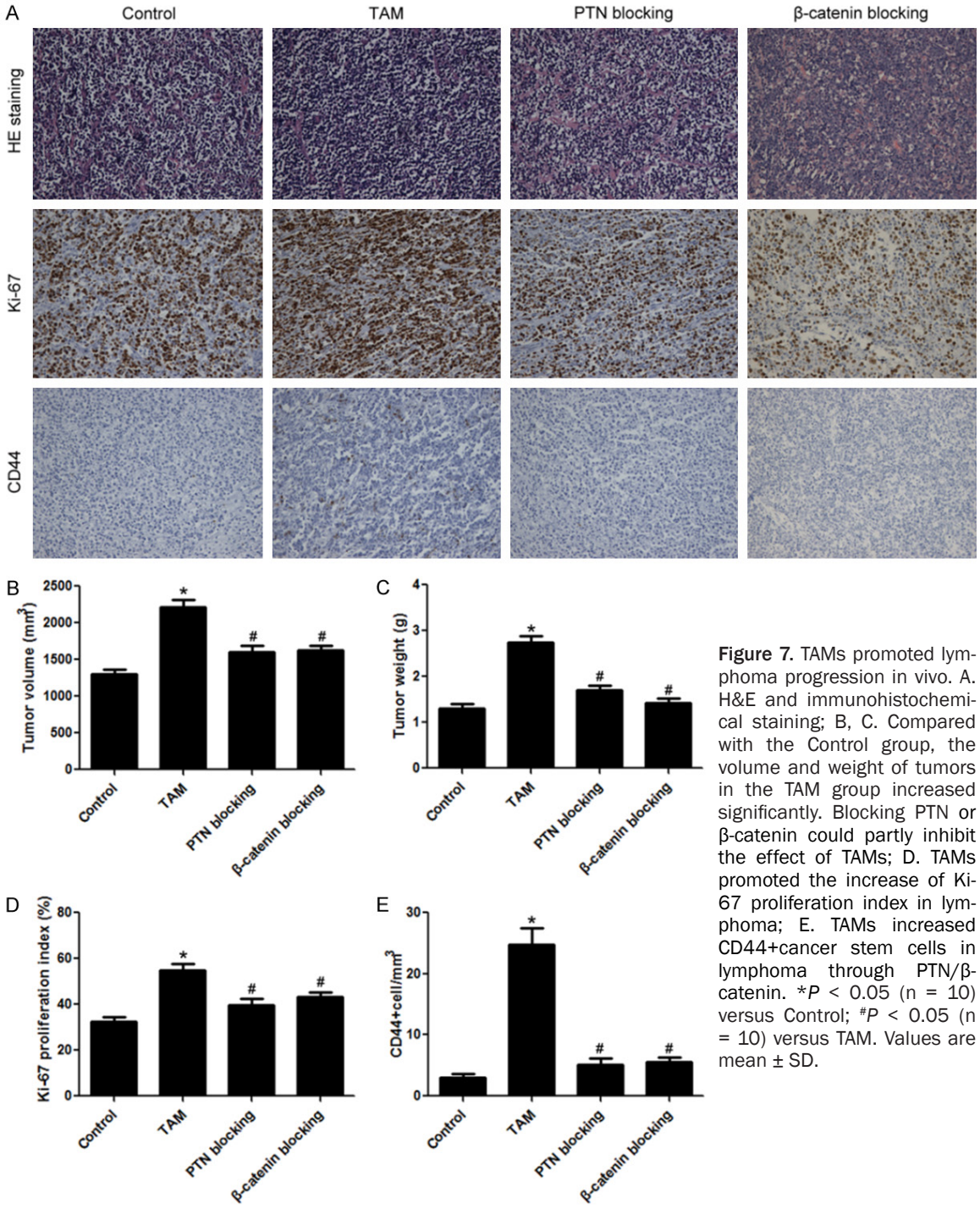

Figure 7. TAMs promoted lymphoma progression in vivo. A. H&E and immunohistochemical staining; B, C. Compared with the Control group, the volume and weight of tumors in the TAM group increased significantly. Blocking PTN or β-catenin could partly inhibit the effect of TAMs; D. TAMs promoted the increase of Ki-67 proliferation index in lymphoma; E. TAMs increased CD44+cancer stem cells in lymphoma through PTN/β-catenin. $^{*}P < 0.05$ (n = 10) versus Control; $^{\#}P < 0.05$ (n = 10) versus TAM. Values are mean ± SD.

recurrence and insensitivity to radiotherapy and chemotherapy [21, 22]. Lee et al. found that the insensitivity of follicular lymphoma patients to chemotherapeutic drugs was closely related to the role of cancer stem cells, which was mediated by CXCL12/CXCR4 [23]. Our study found that TAMs increased the proportion of cancer stem cells in lymphoma through PTN pathway. The increase of cancer stem cells can significantly affect the therapeutic effect and prognosis of lymphoma patients.

In conclusion, PTN and its receptors were highly expressed in lymphoma tissues. TAMs promoted the proliferation and invasion of lymphoma cells and increased the proportion of can-

cer stem cells through PTN/β-catenin pathway. Our study provided a new target for precise treatment of lymphoma.

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Disclosure of conflict of interest

None.

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References

[1] Wang C, Xia B, Ning Q, Zhao H, Yang H, Zhao Z, Wang X, Wang Y, Yu Y and Zhang Y. High prevalence of hepatitis B virus infection in patients with aggressive B cell non-Hodgkin's lymphoma in China. Ann Hematol 2018; 97: 453-457.

[2] Weber LL, Dunbar NM, Meehan KR, Szczepiorkowski ZM and Lansigan F. Successful implementation of a rural extracorporeal photopheresis program for the treatment of cutaneous T-cell lymphoma and chronic graft-versus-host disease in a rural hospital. J Clin Apher 2015; 30: 359-363.

[3] Rigter LS, Snaebjornsson P, Rosenberg EH, Atmodimedjo PN, Aleman BM, Ten Hoeve J, Geurts-Giele WR, van Ravesteyn TW, Hoeksel J, Meijer GA, Te Riele H, van Leeuwen FE, Dinjens WN and van Leerdam ME. Double somatic mutations in mismatch repair genes are frequent in colorectal cancer after Hodgkin's lymphoma treatment. Gut 2018; 67: 447-455.

[4] Li Q, Hao Z, Hong Y, He W and Zhao W. Reprogramming tumor associated macrophage phenotype by a polysaccharide from ilex asprella for sarcoma immunotherapy. Int J Mol Sci 2018; 19.

[5] Fu Q, Xu L, Wang Y, Jiang Q, Liu Z, Zhang J, Zhou Q, Zeng H, Tong S, Wang T, Qi Y, Hu B, Fu H, Xie H, Zhou L, Chang Y, Zhu Y, Dai B, Zhang W and Xu J. Tumor-associated macrophage-derived interleukin-23 interlinks kidney cancer glutamine addiction with immune evasion. Eur Urol 2019; 75: 752-763.

[6] Taskinen M, Karjalainen-Lindsberg ML, Nyman H, Eerola LM and Leppa S. A high tumor-associated macrophage content predicts favorable outcome in follicular lymphoma patients treated with rituximab and cyclophosphamide-doxorubicin-vincristine-prednisone. Clin Cancer Res 2007; 13: 5784-5789.

[7] Pham LV, Vang MT, Tamayo AT, Lu G, Challagundla P, Jorgensen JL, Rollo AA, Ou Z, Zhang L, Wang M and Ford RJ. Involvement of tumor-associated macrophage activation in vitro during development of a novel mantle cell lymphoma cell line, PF-1, derived from a typical patient with relapsed disease. Leuk Lymphoma 2015; 56: 186-193.

[8] Kayal S, Mathur S, Karak AK, Kumar L, Sharma A, Bakhshi S and Raina V. CD68 tumor-associated macrophage marker is not prognostic of clinical outcome in classical Hodgkin lymphoma. Leuk Lymphoma 2014; 55: 1031-1037.

[9] Fang W, Hartmann N, Chow DT, Riegel AT and Wellstein A. Pleiotrophin stimulates fibroblasts and endothelial and epithelial cells and is expressed in human cancer. J Biol Chem 1992; 267: 25889-25897.

[10] Kadomatsu K and Muramatsu T. Midkine and pleiotrophin in neural development and cancer. Cancer Lett 2004; 204: 127-143.

[11] Moon HS, Park WI, Sung SH, Choi EA, Chung HW and Woo BH. Immunohistochemical and quantitative competitive PCR analyses of midkine and pleiotrophin expression in cervical cancer. Gynecol Oncol 2003; 88: 289-297.

[12] Jager R, List B, Knabbe C, Souttou B, Raulais D, Zeiler T, Wellstein A, Aigner A, Neubauer A and Zugmaier G. Serum levels of the angiogenic factor pleiotrophin in relation to disease stage in lung cancer patients. Br J Cancer 2002; 86: 858-863.

[13] Shi Y, Ping YF, Zhou W, He ZC, Chen C, Bian BS, Zhang L, Chen L, Lan X, Zhang XC, Zhou K, Liu Q, Long H, Fu TW, Zhang XN, Cao MF, Huang Z, Fang X, Wang X, Feng H, Yao XH, Yu SC, Cui YH, Zhang X, Rich JN, Bao S and Bian XW. Tumour-associated macrophages secrete pleiotrophin to promote PTPRZ1 signalling in glioblastoma stem cells for tumour growth. Nat Commun 2017; 8: 15080.

[14] Iwamoto H, Abe M, Yang Y, Cui D, Seki T, Nakamura M, Hosaka K, Lim S, Wu J, He X, Sun X, Lu Y, Zhou Q, Shi W, Torimura T, Nie G, Li Q and Cao Y. Cancer lipid metabolism confers antiangiogenic drug resistance. Cell Metab 2018; 28: 104-117, e105.

[15] Li Z, Wang M, Yao X, Li H, Li S, Liu L, Yu D, Li X, Fang J and Huang C. Development of novel anti-CD19 antibody-drug conjugates for B-cell lymphoma treatment. Int Immunopharmacol 2018; 62: 299-308.

[16] Muramatsu T. Midkine and pleiotrophin: two related proteins involved in development, sur-

vival, inflammation and tumorigenesis. J Biochem 2002; 132: 359-371.
[17] Ma J, Kong Y, Nan H, Qu S, Fu X, Jiang L, Wang W, Guo H, Zhao S, He J and Nan K. Pleiotrophin as a potential biomarker in breast cancer patients. Clin Chim Acta 2017; 466: 6-12.
[18] Mikelis C, Koutsioumpa M and Papadimitriou E. Pleiotrophin as a possible new target for angiogenesis-related diseases and cancer. Recent Pat Anticancer Drug Discov 2007; 2: 175-186.
[19] Jee YH, Sadowski SM, Celi FS, Xi L, Raffeld M, Sacks DB, Remaley AT, Wellstein A, Kebebew E and Baron J. Increased pleiotrophin concentrations in papillary thyroid cancer. PLoS One 2016; 11: e0149383.
[20] Ye L, Liang S, Guo C, Yu X, Zhao J, Zhang H and Shang W. Inhibition of M1 macrophage activation in adipose tissue by berberine improves insulin resistance. Life Sci 2016; 166: 82-91.
[21] Wang M, Ren D, Guo W, Huang S, Wang Z, Li Q, Du H, Song L and Peng X. N-cadherin promotes epithelial-mesenchymal transition and cancer stem cell-like traits via ErbB signaling in prostate cancer cells. Int J Oncol 2016; 48: 595-606.
[22] Shen S, Xia JX and Wang J. Nanomedicine-mediated cancer stem cell therapy. Biomaterials 2016; 74: 1-18.
[23] Lee CG, Das B, Lin TL, Grimes C, Zhang X, Lavezzi T, Huang L, Cole J, Yau L and Li L. A rare fraction of drug-resistant follicular lymphoma cancer stem cells interacts with follicular dendritic cells to maintain tumourigenic potential. Br J Haematol 2012; 158: 79-90.