Gastric cancer patients have elevated plasmacytoid and CD1c$^+$ dendritic cells in the peripheral blood

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Received June 2, 2017; Accepted December 13, 2017

DOI: 10.3892/ol.2018.7990

Abstract. Dendritic cells (DCs) are important in tumor immunology. Identifying DC subset markers in the peripheral blood, which are informative for gastric cancer stages, is not only useful for prognosis but may also provide mechanistic insights into processes facilitating therapy. The present study investigated plasmacytoid dendritic cells (pDCs) and myeloid CD1c$^+$ dendritic cells (mDC1s) in the peripheral blood of patients with gastric cancer and healthy controls using flow cytometry. Using peripheral DC staining and subset analysis, patients with gastric cancer were identified to have substantially higher numbers of peripheral pDCs and mDC1s. In addition, there was a trend of elevated circulating pDCs with advanced stages and lymph node metastasis in gastric cancer, whereas no differences in circulating mDC1s were observed among the various groups. The results suggested that circulating pDCs are a positive prognostic indicator in patients with gastric cancer of different stages and highlighted the critical role of pDCs immunity in the development of gastric cancer.

Introduction

Gastric cancer is one of the leading causes of cancer-associated mortality in the developing world. Accumulating evidence indicates that dendritic cells (DCs) are important in tumor immunology, including that of gastric cancer (1). Certain DC-associated inflammatory factors are useful in predicting the prognosis of gastric cancer (2). For example, CD83$^+$ DC cells in primary lesions and regional lymph nodes are inversely correlated with the prognosis of gastric cancer (3), and peripheral HLA-G-expressing DC-10 cells are elevated in patients with gastric cancer (4). In addition, the infiltration of certain DC subsets in gastric cancer tissue has been shown to correlate with 5-year survival rate (5,6). Several clinical trials have used DC-based anti-gastric cancer therapy strategies (7). Human DC cells can be divided into four subsets according to the expression of specific markers: CD303$^+$ plasmacytoid DCs (pDCs), CD1c$^+$ classical myeloid DCs (cDCs/mDCs), CD141$^+$ classical myeloid DCs (cDCs/mDCs) and inflammatory DCs (8). The improved characterization of different DC subsets is likely to provide novel avenues for their tumor therapeutic regulation.

pDCs are a multifunctional subset of bone marrow-derived immune cells, which produce interferons (IFNs) and act as antigen-presenting cells (9). High pDC infiltration has been observed in several types of cancer, including melanoma, head and neck cancer, breast cancer, ovarian cancer and prostate cancer, with infiltrated pDCs being involved in tumor promotion and inhibition, which may depend on their maturity/gene expression (9,10). In addition, peripheral pDCs have been reported to show prognostic relevance in certain types of cancer. For example, patients with late-stage breast cancer had significantly lower levels of circulating pDCs (11) and patients suffering from prostate cancer showed a marked reduction in circulating pDCs (12). However, there are few reports concerning circulating pDCs in gastric cancer.

The aim of the present study was to investigate the presence and distribution of circulating pDCs and CD1c$^+$ DCs in patients with gastric cancer. The results showed that patients with gastric cancer had increased numbers of circulating pDCs and CD1c$^+$ DCs. In addition, there was a trend toward elevated circulating pDCs with advanced cancer stage and lymph node metastasis.

Materials and methods

Human subjects. A total of 32 patients with gastric cancer were recruited from Zhongnan Hospital of Wuhan University (Wuhan, China). The patients had no other tumors, trauma, infectious diseases or autoimmune diseases. They had not received radiotherapy, chemotherapy or immunotherapy. In addition, 35 healthy volunteers were recruited as controls. The age range of those recruited was 43-78 years, with an average age of 56 years. Peripheral blood samples were collected from the two groups. All participants signed informed consent and the study was approved by the Ethical Committee of Wuhan University (permit no. 2010-10007).

Peripheral DC staining and subset analysis. The fresh heparinized blood samples were processed within 2 h following

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Key words: dendritic cell, plasmacytoid dendritic cell, myeloid dendritic cell, gastric cancer

Table I. Clinical and pathological characteristics of patients with gastric cancer.

Characteristic	Subcategory	Number
Age (years)	>60	17
	≤60	15
Sex	Male	24
	Female	8
TNM stage	I	5
	II	9
	III	9
	IV	9
Primary tumor	T1	2
	T2	9
	T3	12
	T4	9
Lymph node metastasis	Negative	10
	Positive	22
Distant metastasis	Negative	26
	Positive	6
Histology	Adenocarcinoma	25
	Signet ring cell carcinoma	7

TNM, tumor-node-metastasis.

collection according to the protocol of the human blood dendritic cell enumeration kit (Miltenyi Biotec, Inc., Auburn CA, USA). Briefly, the procedure was as follows: An aliquot (300 μl) of the blood sample was stained with 20 μl anti-BDCA cocktail and PE-Cy7-conjugated anti-PD-L1 or isotype control. Following incubation with dead cell detector and red blood cell lysis solution, the cells were washed and fixed for subsequent flow cytometric analysis (BD FACSAria™ III flow cytometer; BD Biosciences, Franklin Lakes, NJ, USA). A total of 10^5 events in the leukocyte gate were collected.

Absolute enumeration of periphery leukocytes and DC subsets. The absolute number of leukocytes was determined by a hemocytometer (XT-1800i; Sysmex Europe, Norderstedt, Germany). The absolute number of each DC subset per ml of blood was calculated as follows: percentage of DC subset x number of leukocytes per ml blood.

Statistical analysis. All values are expressed as the mean ± standard derivation. Student's t-test was used to compare two groups and a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare multiple groups. $P<0.05$ was considered to indicate a statistically significant difference. The software used was Graphpad Prism version 5 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Number of peripheral leukocytes is increased in patients with gastric cancer. In order to calculate the absolute number

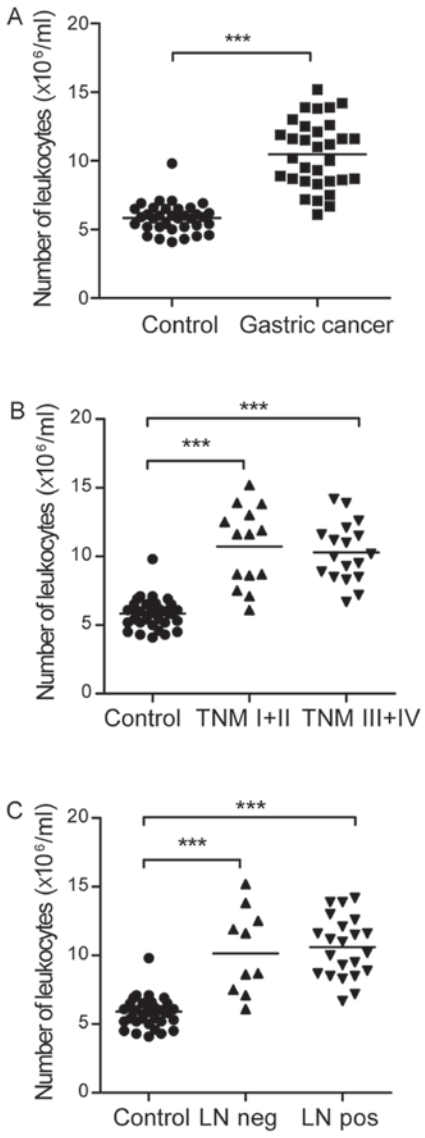

Figure 1. Number of leukocytes in the peripheral blood of different human groups. (A) Patients with gastric cancer and control group. (B) Patients with gastric cancer stage TNM I+II, stage TNM III+IV and control group. (C) Patients with LN-neg gastric cancer, metastatic (LN-pos) gastric cancer and control group. ***P<0.001. TNM, tumor-node-metastasis; LN, lymph node; neg, negative; pos, positive.

of DC subsets, the number of peripheral leukocytes was first determined. It was found that there were a significantly increased number of peripheral leukocytes in the patients with gastric cancer, compared with that in the healthy controls (10.48±2.46 vs. 5.48±1.08x10^6/ml blood; Fig. 1A). There was no significant difference in the number of leukocytic cells between the tumor-node-metastasis (TNM) I+II and TNM III+IV groups (10.73±2.89 vs. 10.29±2.14x10^6/ml blood; Fig. 1B) or the lymph node negative and lymph node metastasis groups (10.30±3.10 vs. 10.56±2.19x10^6/ml blood; Fig. 1C) in patients with gastric cancer. The clinical and pathological characteristics of the patients with gastric cancer are shown in Table I.

Peripheral pDCs and mDC1s are elevated in patients with gastric cancer. To investigate the role of DCs in gastric cancer, the present study evaluated two DC subsets using flow cytometric analysis and the gating strategy, as shown

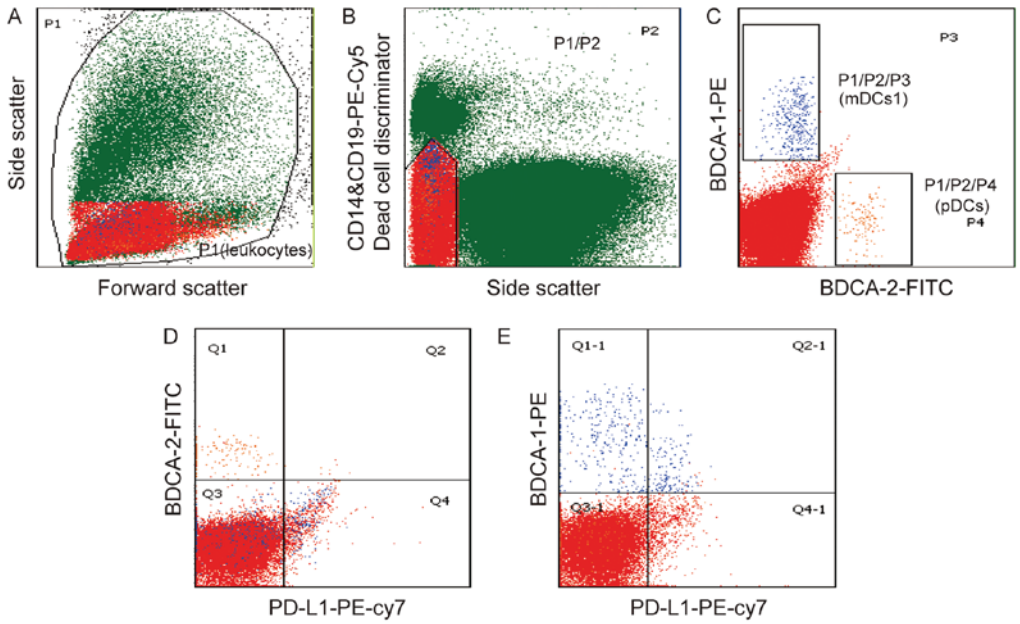

Figure 2. Reprehensive flow cytometric analysis of blood cells and DC subsets. (A) Forward scatter and side scatter dot plot of blood cells with P1 (circled) region gated to exclude debris and platelets. (B) P2 region (red) was gated on P1 to further exclude B cells, monocytes, granulocytes and dead cells. (C) P3 region (blue) was gated on P2 to determine BDCA-1$^+$ mDC1 subset and P4 region (orange) was gated on P2 to determine BDCA-2$^+$ pDC subset. (D) BDCA-2$^+$ pDC subset and PD-L1 analysis of P2 population. (E) BDCA-1$^+$ mDC1 subset and PD-L1 analysis of P2 population. pDC, plasmacytoid dendritic cell; mDC, myeloid dendritic cell.

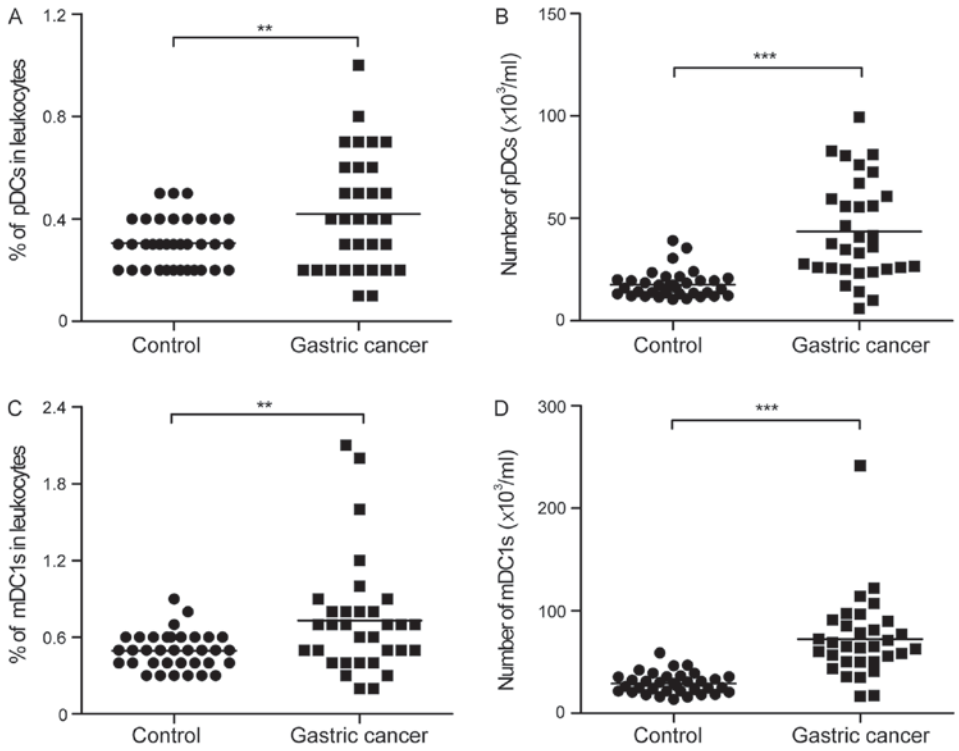

Figure 3. Quantitation of pDC and mDC1 subsets in the peripheral blood of patients with gastric cancer and controls. (A) Percentage of pDCs in the leukocytes. (B) Absolute number of pDCs. (C) Percentage of mDC1s in the leukocytes. (D) Absolute number of mDC1s. **P<0.01; ***P<0.001. pDCs, plasmacytoid dendritic cells; mDC, myeloid dendritic cell.

in Fig. 2. The pDCs were identified as SSC$^{low/-}$CD14$^{low/-}$CD19$^{low/-}$BDCA-2$^+$ (Fig. 2A-C), whereas the mDC1s were identified as SSC$^{low/-}$CD14$^{low/-}$CD19$^{low/-}$BDCA-1$^+$ (Fig. 2A-C). The expression of PD-L1 in the pDCs and mDC1s in gastric cancer was also examined. It was found that the majority of pDCs did not express PD-L1 (Fig. 2D), whereas the mDC1s population showed partial expression of PD-L1 (Fig. 2E). Of note, there was a significant increase in the percentage and number of pDCs in the peripheral leukocytes from the patients with gastric cancer, compared with those from the

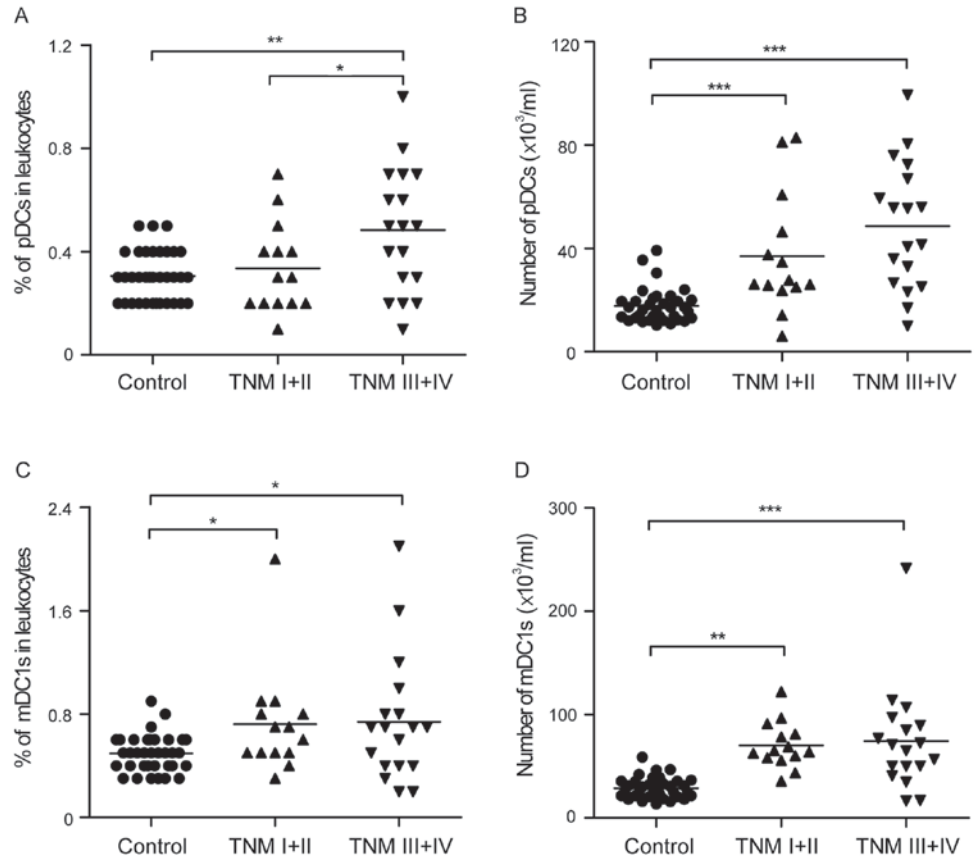

Figure 4. Quantitation of pDC and mDC1 subsets in the peripheral blood of patients with gastric cancer at different TNM stages. (A) Percentage of pDCs in the leukocytes. (B) Absolute number of pDCs. (C) Percentage of mDC1s in the leukocytes. (D) Absolute number of mDC1s. *P<0.05; **P<0.01; ***P<0.001. pDCs, plasmacytoid dendritic cells; mDC, myeloid dendritic cell; TNM, tumor-node-metastasis.

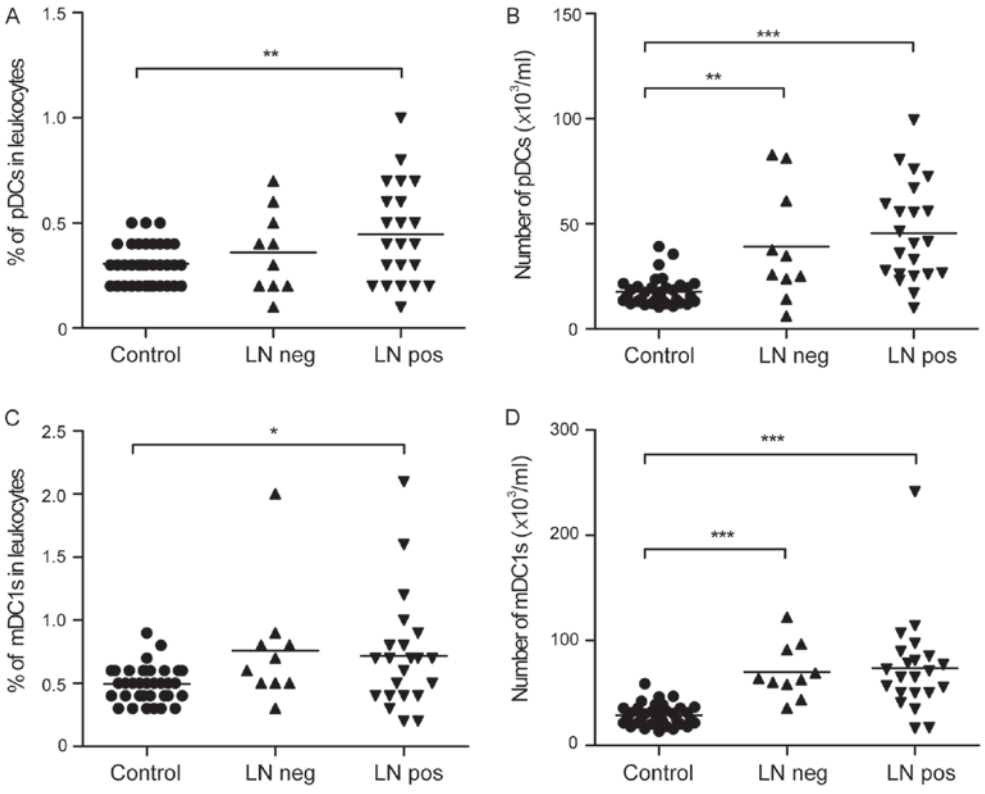

Figure 5. Quantitation of pDC and mDC1 subsets in the peripheral blood of patients with gastric cancer with different LN metastasis status. (A) Percentage of pDCs in the leukocytes. (B) Absolute number of pDCs. (C) Percentage of mDC1s in the leukocytes. (D) Absolute number of mDC1s. *P<0.05; **P<0.01; ***P<0.001. pDCs, plasmacytoid dendritic cells; mDC, myeloid dendritic cell; LN, lymph node; neg, negative; pos, positive.

healthy controls (0.42±0.23 vs. 0.31±0.10%; 43.57±24.25 vs. 17.72±6.64x10^3/ml; Fig. 3A and B). Similarly, the percentage and number of mDC1s was significantly higher in the patients with gastric cancer, compared with that in the healthy controls (0.73±0.45 vs. 0.49±0.14%; 72.49±39.99 vs. 28.91±10.10x10^3/ml; Fig. 3C and D).

Enrichment of peripheral pDCs in patients with gastric cancer at advanced stages. The present study further analyzed the peripheral pDCs and mDC1s in patients with different stages of gastric cancer. Notably, an increase in peripheral pDCs was found as follows: Healthy controls <TNM I+II <TNM III+IV groups (0.31±0.10 vs. 0.34±0.17 vs. 0.48±0.24%, respectively; and 17.72±6.64 vs. 37.02±23.13 vs. 48.66±24.51x10^3/ml, respectively; Fig. 4A and B). Although certain trends did not show statistical significance, the percentage and absolute number of pDCs was significantly higher in the TNM III+IV group, compared with that in the healthy controls. In addition, there were significantly elevated peripheral mDC1 cell percentages (0.72±0.41, vs. 0.74±0.49%; Fig. 4C) and mDC1 cell numbers (70.16±22.37, vs. 74.29±50.25x10^3/ml; Fig. 4D) in the TNM I+II and TNM III+IV groups, compared with the healthy controls, respectively.

Enrichment of peripheral pDCs in patients with gastric cancer with lymph node metastasis. To further investigate the changes of pDCs and mDC1s during tumor invasion, the present study analyzed the peripheral pDCs and mDC1s of patients with different lymph node metastasis status. It was observed that peripheral pDCs increased as follows: Healthy controls <lymph node negative group <lymph node metastasis group in terms of the percentage (0.31±0.10 vs. 0.36±0.20 vs. 0.45±0.24%; Fig. 5A) and number (17.72±6.64 vs. 39.20±26.86 vs. 45.55±23.36x10^3/ml; Fig. 5B) of pDCs. Certain trends were not statistically significant, however, the percentage and absolute number of pDCs were significantly higher in the lymph node metastasis group, compared with those in the healthy controls. No significant differences were found in peripheral mDC1 cell percentages (0.72±0.45 vs. 0.76±0.47%; Fig. 5C) or mDC1 cell numbers (73.52±45.47 vs. 70.22±25.99x10^3/ml; Fig. 5D) between the lymph node metastasis and negative groups.

Discussion

The present study indicated that patients with gastric cancer had markedly higher numbers of peripheral pDCs and mDC1s. pDCs were identified as $SSC^{low/-}CD14^{low/-}CD19^{low/-}BDCA\text{-}2^{+}$. Huang *et al* reported pDCs as Lin^{-} $HLA^{-}DR^{+}CD11c^{-}$ $CD123^{high}$ (13). Although using different surface markers to detect pDCs, the results of these two studies showed a higher proportion of circulating pDCs in patients with gastric cancer, compared with that in healthy controls. Defining circulating pDCs as positive prognostic indicators for gastric cancer is likely to enable easier prediction of disease course without biopsies and also provide useful information on the control of cancer by the immune system.

It has been shown that the pDCs infiltrated in the tumor microenvironment are mainly immature, and appear to be predominantly immunosuppressive/tolerogenic (14). The increased circulating pDCs in patients with gastric cancer may also have an important immunosuppressive role. However, the data obtained in the present study showed the circulating pDCs in patients with gastric cancer did not express PD-L1, which is important in the immunosuppression of gastric cancer (15). Further investigations involving sorting of the pDCs and analysis of their inflammatory cytokine profile, including IFNs and interleukin-10, and function *in vitro* are likely to provide additional clues. In addition, it has been reported that properly activated pDCs can trigger an antitumor response (16,17), therefore, modifying circulating pDCs may be a potentially useful gastric cancer therapeutic strategy.

The present study provided evidence that circulating pDCs were positively correlated with advanced stages and lymph node metastasis in gastric cancer. Although the increase of pDCs in advanced stages and the lymph node metastasis of gastric cancer were not statistically significant, the trends were observed, compared with those of mDC1s. It has been reported that pDCs may have a pathological role in metastasis. pDCs have been shown to be accumulated in positive (with metastasis) sentinel lymph nodes in melanoma (18). In mouse models of breast cancer bone metastasis, the depletion of pDCs inhibited tumor growth and prevented metastasis (19). Therefore, the data in the present study provide a rationale for investigating pDCs in the metastasis of gastric cancer.

In conclusion, the present study suggested that circulating pDCs can be a positive prognostic indicator in patients with gastric cancer of different stages. The future characterization of pDCs is likely to shed light on the systemic understanding of pDC immunity in the development of gastric cancer.

Acknowledgements

This study was supported by grants awarded to Dr. Zan Tong. The authors would like to thank Jieyun Wu of Zhongnan Hospital for collecting blood samples and information from the patients with gastric cancer and healthy controls.

Competing interests

The authors declare that they have no competing interests.

References

1. Veglia F and Gabrilovich DI: Dendritic cells in cancer: The role revisited. Curr Opin Immunol 45: 43-51, 2017.
2. Chang WJ, Du Y, Zhao X, Ma LY and Cao GW: Inflammation-related factors predicting prognosis of gastric cancer. World J Gastroenterol 20: 4586-4596, 2014.
3. Kashimura S, Saze Z, Terashima M, Soeta N, Ohtani S, Osuka F, Kogure M and Gotoh M: CD83(+) dendritic cells and Foxp3(+) regulatory T cells in primary lesions and regional lymph nodes are inversely correlated with prognosis of gastric cancer. Gastric Cancer 15: 144-153, 2012.
4. Xu DP, Shi WW, Zhang TT, Lv HY, Li JB, Lin A and Yan WH: Elevation of HLA-G-expressing DC-10 cells in patients with gastric cancer. Hum Immunol 77: 800-804, 2016.
5. Tsukayama S, Omura K, Yoshida K, Tanaka Y and Watanabe G: Prognostic value of CD83-positive mature dendritic cells and their relation to vascular endothelial growth factor in advanced human gastric cancer. Oncol Rep 14: 369-375, 2005.
6. Ishigami S, Natsugoe S, Tokuda K, Nakajo A, Xiangming C, Iwashige H, Aridome K, Hokita S and Aikou T: Clinical impact of intratumoral natural killer cell and dendritic cell infiltration in gastric cancer. Cancer Lett 159: 103-108, 2000.

7. Niccolai E, Taddei A, Prisco D and Amedei A: Gastric cancer and the epoch of immunotherapy approaches. World J Gastroenterol 21: 5778-5793, 2015.
8. Coutant F and Miossec P: Altered dendritic cell functions in autoimmune diseases: Distinct and overlapping profiles. Nat Rev Rheumatol 12: 703-715, 2016.
9. Swiecki M and Colonna M: The multifaceted biology of plasmacytoid dendritic cells. Nat Rev Immunol 15: 471-485, 2015.
10. Lombardi VC, Khaiboullina SF and Rizvanov AA: Plasmacytoid dendritic cells, a role in neoplastic prevention and progression. Eur J Clin Invest 45 (Suppl 1): S1-S8, 2015.
11. Kini Bailur J, Gueckel B and Pawelec G: Prognostic impact of high levels of circulating plasmacytoid dendritic cells in breast cancer. J Transl Med 14: 151, 2016.
12. Sciarra A, Lichtner M, Autran GA, Mastroianni C, Rossi R, Mengoni F, Cristini C, Gentilucci A, Vullo V and Di Silverio F: Characterization of circulating blood dendritic cell subsets $DC123^+$ (lymphoid) and $DC11C^+$ (myeloid) in prostate adenocarcinoma patients. Prostate 67: 1-7, 2007.
13. Huang XM, Liu XS, Lin XK, Yu H, Sun JY, Liu XK, Chen C, Jin HL, Zhang GE, Shi XX, *et al*: Role of plasmacytoid dendritic cells and inducible costimulator-positive regulatory T cells in the immunosuppression microenvironment of gastric cancer. Cancer Sci 105: 150-158, 2014.
14. Demoulin S, Herfs M, Delvenne P and Hubert P: Tumor microenvironment converts plasmacytoid dendritic cells into immunosuppressive/tolerogenic cells: Insight into the molecular mechanisms. J Leukoc Biol 93: 343-352, 2013.
15. Tamura T, Ohira M, Tanaka H, Muguruma K, Toyokawa T, Kubo N, Sakurai K, Amano R, Kimura K, Shibutani M, *et al*: Programmed death-1 Ligand-1 (PDL1) expression is associated with the prognosis of patients with stage II/III gastric cancer. Anticancer Res 35: 5369-5376, 2015.
16. Kalb ML, Glaser A, Stary G, Koszik F and Stingl G: TRAIL(+) human plasmacytoid dendritic cells kill tumor cells in vitro: Mechanisms of imiquimod- and IFN-α-mediated antitumor reactivity. J Immunol 188: 1583-1591, 2012.
17. Tel J, Smits EL, Anguille S, Joshi RN, Figdor CG and de Vries IJ: Human plasmacytoid dendritic cells are equipped with antigen-presenting and tumoricidal capacities. Blood 120: 3936-3944, 2012.
18. Gerlini G, Urso C, Mariotti G, Di Gennaro P, Palli D, Brandani P, Salvadori A, Pimpinelli N, Reali UM and Borgognoni L: Plasmacytoid dendritic cells represent a major dendritic cell subset in sentinel lymph nodes of melanoma patients and accumulate in metastatic nodes. Clin Immunol 125: 184-193, 2007.
19. Sawant A, Hensel JA, Chanda D, Harris BA, Siegal GP, Maheshwari A and Ponnazhagan S: Depletion of plasmacytoid dendritic cells inhibits tumor growth and prevents bone metastasis of breast cancer cells. J Immunol 189: 4258-4265, 2012.