



Research Paper

Inhibition of IL-18-mediated myeloid derived suppressor cell accumulation enhances anti-PD1 efficacy against osteosarcoma cancer

Ying Guan^a, Rui Zhang^b, Zhibin Peng^a, Daming Dong^a, Guojun Wei^a, Yansong Wang^{a,*}

^a Department of Orthopedics, the First Affiliated Hospital of Harbin Medical University, Harbin 150001, PR China

^b Department of Neurology, General Hospital of Heilongjiang Province Land Reclamation Headquarter, Harbin 150001, PR China

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ABSTRACT

Myeloid derived suppressor cells (MDSC) are very important in tumor immune evasion and they dramatically increased in peripheral blood of patients with osteosarcoma cancer. The association between MDSC and various cytokines has been studied in the peripheral blood. However, little is known about the mechanism drawing MDSC into tumor parenchyma. This study was to analyze the correlation between MDSC subsets and interleukin 18 (IL-18) level in osteosarcoma tumor model and its effect on the immunotherapy. MDSC were isolated from the blood and parenchyma and analyzed in the osteosarcoma tumor model. IL-18 levels were detected by enzyme-linked immunosorbent assay (ELISA) assay, real-time PCR, western blot and flow cytometry. Moreover, combination treatment with IL-18 inhibition and anti-PD1 was conducted to assess the therapeutic effects of IL-18 blockade. Results showed MDSC levels had a positive correlation with IL-18, suggesting IL-18 may attract MDSC into the parenchyma. IL-18 gene and protein expression significantly increased in blood and tumor lysates of tumor-bearing mice. Anti-IL-18 treatment significantly decreased G-MDSC and M-MDSC in the peripheral blood and tumor. Furthermore, combination therapy decreased the tumor burden and increased CD4⁺ and CD8⁺ T cell infiltration, as well as the production of interferon gamma (IFN γ) and granzyme B. Our study revealed a possible correlation between MDSC subsets and IL-18 inducing MDSC migration into the tumor tissue, in addition to provide the potential target to enhance the efficacy of immunotherapy in patients with osteosarcoma.

1. Introduction

As the most common type of malignant bone tumor in children and young adults, osteosarcoma is characterized by the proliferation of tumor cells producing osteoid or immature bone matrix. Most osteosarcomas are detected at the end of long bone in adolescence when they are rapidly growing [1,2]. Despite the advances in multimodality treatment consisting of chemotherapy and radiation, the survival rate remained low in the past two decades because pulmonary metastasis occurred in approximately 40–50% of patients with osteosarcoma as a major cause of fatal outcome. The effectiveness of conventional therapies for osteosarcoma has remained unchanged with a dismal five-year survival rate of less than 20% [3–5]. Therefore, it is important to search for other effective option for treating osteosarcoma.

Overwhelming evidences have proved that immunotherapy shows to be a promising treatment modality, however, most anti-tumor immune responses are rendered ineffective by tumor-mediated immunosuppression and immune evasion contributing to decreased clinical efficacy [6,7]. There are various types of cells involving in tumor

mediated immune suppression, such as regulatory T cells (Treg), tumor associated macrophages (TAMs), and myeloid derived suppressor cells (MDSC) [8,9]. Among these types of cells, MDSC have become the focus of intense study in recent years because they play a pivotal role in the tumor-associated immune suppression [10].

Arising from myeloid progenitor cells and losing differentiation into mature dendritic cells, granulocytes or macrophages, MDSC possess the capacity to suppress T cell and natural killer cell through down-regulation of CD8 T cell homing to lymph nodes, and induction of FoxP3⁺ Treg cells, etc. [11,12]. In peripheral blood of patients with cancer, accumulation of MDSC have been observed, and increased MDSC levels are associated with a poor prognosis of the tumor-bearing host. Tumor-infiltrated MDSC promoted tumor cell proliferation and facilitated tumor cell dissemination from the primary site [13]. MDSC-induced 'metastatic gene signature' derived from murine syngeneic model predicts poor patient survival in the majority of solid tumors [14]. According to the content of immunosuppressive substances and nuclear morphology, murine MDSC were characterized as granulocytic MDSC (G-MDSC) and monocytic MDSC (M-MDSC).

* Corresponding author.

E-mail address: yansongwang2017@yeah.net (Y. Wang).

Tumor-derived factors and inflammatory cytokines are essential to the differentiation and expansion of MDSC, and associations have been confirmed between MDSC and various cytokines in the peripheral blood [8]. However, little is known about the mechanism drawing MDSC into the tumor parenchyma. Studies showed that interleukin 1 β (IL-1 β) activated the generation and function of MDSC and has been shown to induce the MDSC accumulation [15,16]. Because IL-1 β and interleukin 18 (IL-18) are related closely, we questioned whether IL-18 influences MDSC accumulation [17]. Moreover, the association of this cytokine production with MDSC accumulation in osteosarcoma cancer has not been fully investigated.

In this study, we analyzed the relationship between peripheral or intratumoral levels of MDSC and IL-18 expression in the osteosarcoma tumor model, which would elucidate some of the factors that might promote MDSC accumulation to result in tumor immunosuppression. Our findings suggest that blockade of IL-18 may be a potential strategy to target immune inhibitory MDSCs for combination treatments as methods of enhancing the efficacy of immunotherapy in patients with osteosarcoma.

2. Materials and methods

2.1. Mouse tumor model and treatment

Six-week old female Balb/cJ mice were purchased from Vital River Lab (Beijing, China) and maintained under specific pathogen-free conditions. Mice had free access to food and water during the whole experimental period. All animal experiments were performed in compliance with the Chinese legislation on the use and care of laboratory animals and approved by the Ethical Committee on Animal Care and Use of Harbin Medical University. Anti-PD1 antibody was purchased from Bioexcell. IL-18 binding protein (IL-18BP, 122-BP-100) was purchased from R&D Systems.

The osteosarcoma carcinoma K7M2 cell line was purchased from Shanghai Cell Bank (Shanghai, China) and 5×10^5 cells were injected into Balb/cJ mice subcutaneously [2]. 28 days after cell injection, mice were sacrificed to collect blood and tumor for analysis, normal mice as the control (five mice per group). In the treatment studies, 7 days after cell injection, tumor-bearing mice were treated with IL-18BP (5 mg/kg, once daily I.P.), anti-PD1 antibody (5 mg/kg, once a week I.P.) or the combination. Mice treated with IgG were used as the negative control (five mice per group).

2.2. MDSC subsets from tumor analysis

M-MDSC and G-MDSC were phenotyped using flow cytometry. Tumor was cut into small fragments followed by digestion with tumor disassociation kit for 30 min (Miltenyi Biotec, USA), and then filtered by 70 μ m cell strainers. Mononuclear cells was enriched by subjecting the single cell suspension to percoll gradient. Briefly, 70% percoll (4 ml) was added to the centrifuge tube, 30% percoll (4 ml) was carefully layered, and then tumor suspension (4 ml) was carefully layered on the top. After centrifuging at 1500 rpm for 15 mins, the buffy coat layer in the middle was collected and washed with PBS, followed by staining with anti-CD4 FITC, anti-CD8 Percp, anti-Ly6G AF700, anti-Ly6C APC, anti-CD11b APC-Cy7, anti-IFN γ PE-CF594, and anti-granzyme B PE antibodies, along with appropriate isotype controls (all from BD) for flow cytometry analysis (BD FACSCalibur). M-MDSC were defined as CD11b⁺Ly6C⁺Ly6G⁻ and G-MDSC were defined as CD11b⁺Ly6G⁺Ly6C⁻.

2.3. Analysis of MDSC subsets from peripheral blood

Peripheral blood was obtained from tumor-bearing mice and normal mice. Blood was stained with the same antibodies as the tumor for flow cytometry analysis.

2.4. Cytokine levels from peripheral blood and tumor

IL-18 levels were quantitated by enzyme-linked immunosorbent assay (ELISA) assay in plasma and tumor lysates according to the manufacturer's instructions (R&D systems). Protein was quantitated by bicinchoninic acid (BCA) assay to ensure equal amounts of protein were aliquoted in each well.

2.5. Quantitative real-time RT-PCR

The total RNA was extracted with 1000 μ l TRIzol reagent. Complementary DNA was generated by adding 0.5 μ g total RNA to SuperScript master mix and performing reverse transcription. Quantitative PCR was performed using SYBR green supermix (Bio-Rad, CA). Comparative C_t value method was used to quantify the expression of genes of interest in different samples. The mRNA levels were normalized to the housekeeping gene Gapdh. The gene-specific primer sequences are the following. For IL-18, forward: aatcactctctctggccca; reverse: gttgtacagtgaagtcggcc, NCBI Reference: NM_008360.1. For Gapdh, forward: aagacccttcattgacct; reverse: atgtagtggggctcgcct, NCBI Reference: NM_001289726.1.

2.6. Western blot

After boiling, equal amounts of protein (40 μ g) were subjected to electrophoresis on a 12% (v/v) SDS-polyacrylamide gel. Protein was then electroblotted to a polyvinylidene difluoride (PVDF) membrane from gel. The membrane was blocked with phosphate buffered saline (PBS) containing 5% non-fat milk at room temperature for 1 h, and incubated with indicated primary antibodies (anti-IL-18 rabbit pAb, ab71495, 1:1000; anti-iNOS rabbit pAb, ab15323, 1:250; anti-Arg-1 rabbit pAb, PA5-29645, 1:1000; anti-GAPDH rabbit pAb, SC-25778, 1:3000) at 4 $^{\circ}$ C overnight, followed by incubating with the goat-anti-rabbit horseradish peroxidase-conjugated secondary antibody for 1 h. Membrane was washed three times, and visualized by the enhanced chemiluminescence system.

2.7. Statistical analysis

Data were expressed as mean \pm SD and analyzed by one-way ANOVA with SAS 9.1 software (SAS Institute, USA). The comparison between each two groups is by post hoc analysis. $p < 0.05$ was considered as significant difference.

3. Results

3.1. MDSC subset levels increased in tumor-bearing mice

5×10^5 K7M2 cells were injected into Balb/cJ mice subcutaneously. 28 days after cell injection, mice were sacrificed to collect blood and tumor for flow cytometry analysis, normal mice as the control (five mice per group). MDSC subsets were analyzed based on staining for CD11b, Ly6G and Ly6C. Comparing to normal mice, the amount of G-MDSC and M-MDSC significantly increased in tumor-bearing mice ($p < 0.01$) (Fig. 1).

3.2. IL-18 increased in tumor-bearing mice

5×10^5 K7M2 cells were injected into Balb/cJ mice subcutaneously. 28 days after cell injection, mice were sacrificed to collect blood and tumor for analysis, normal mice as the control (five mice per group). IL-18 levels in blood and tumor were detected by ELISA assay, real-time PCR and western blot. Results showed that IL-18 levels significantly increased in tumor-bearing mice comparing to normal mice. IL-18 surface expression was detected by flow cytometry and results showed that expression of IL-18 significantly increased on MDSC

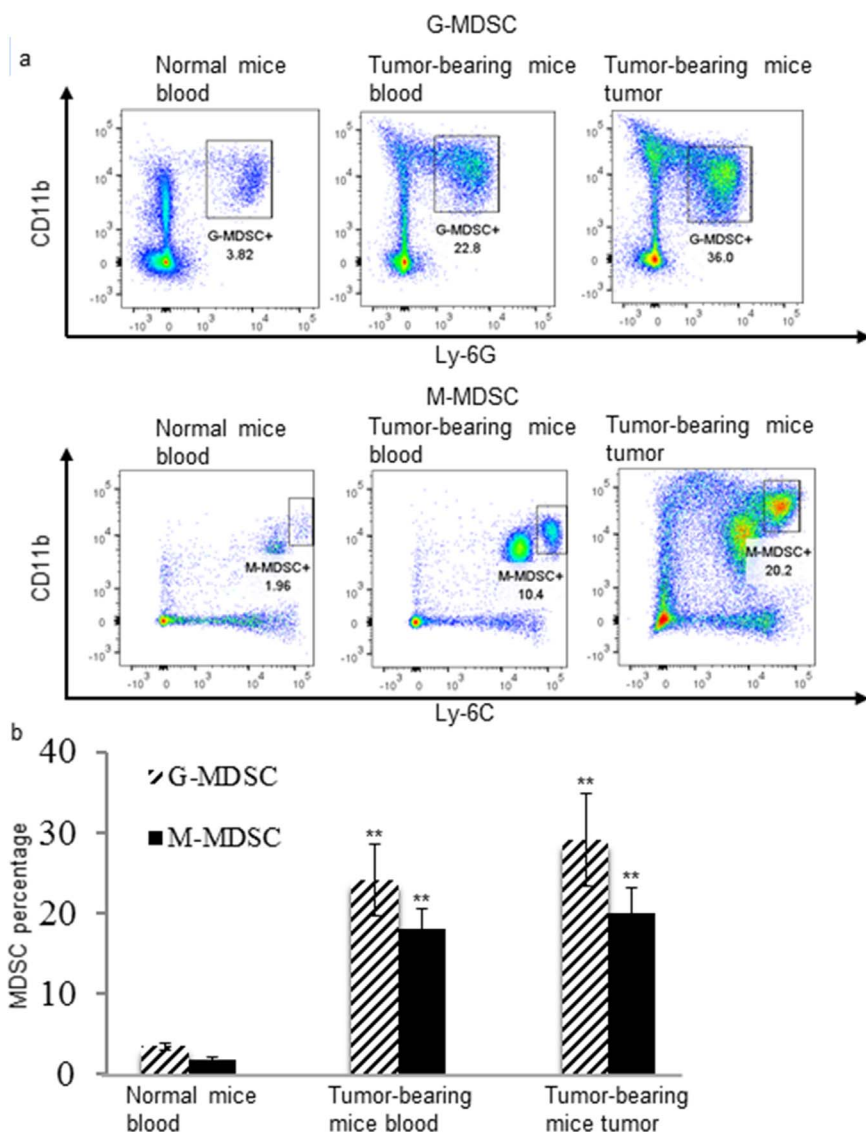


Fig. 1. Myeloid derived suppressor cells (MDSC) subset levels from normal mice and tumor-bearing mice (a). Comparing to normal mice, the percentage of MDSC subsets significantly increased in the tumor-bearing mice (b). Data were expressed as mean \pm SD (n = 5). ***p* < 0.01 vs normal mice.

subsets in the blood and tumor lysates of tumor-bearing mice (*p* < 0.01) (Fig. 2).

3.3. IL-18 blockade inhibited MDSC recruitment

Whether blocking IL-18 activity would impact MDSC subset levels was tested in osteosarcoma tumor model. 5×10^5 K7M2 cells were injected into Balb/cJ mice subcutaneously. 7 days after cell injection, tumor-bearing mice were treated with IL-18BP (5 mg/kg, once daily I.P.). Mice treated with PBS were used as the negative control (five mice per group). Mice were sacrificed to collect blood and tumor for analysis when the tumor volume reached 1500 mm³ in negative control. Anti-IL-18 treatment significantly reduced IL-18 levels, corresponding to the MDSC reduction, both in the blood and tumor. Inhibition of IL-18 also decreased protein expression of iNOS and Arg-1 in the tumor lysates (*p* < 0.01) (Fig. 3).

3.4. IL-18 blockade enhanced the anti-PD1 efficacy

The effects of combination treatment using the IL-18 antagonist with anti-PD1 antibody were explored in this study. 5×10^5 K7M2 cells were injected into Balb/cJ mice subcutaneously. 7 days after cell injection, tumor-bearing mice were treated with IL-18BP (5 mg/kg, once daily I.P.), anti-PD1 antibody (5 mg/kg, once a week I.P.) or the

combination. Mice treated with IgG were used as the negative control (five mice per group). Mice were sacrificed to collect blood and tumor for analysis when the tumor volume reached 1500 mm³ in negative control. Treatment of tumor-bearing mice with anti-PD1 antibody afforded modest effect. The IL-18 antagonist treatment alone also had minimal impact on tumor burden. However, the combination treatment improved the efficacy of the immune checkpoint blockade by increasing both T cell infiltration and the production of IFN γ and granzyme B, and decreasing the tumor burden of tumor-bearing mice (Fig. 4).

4. Discussion

Tumors use various suppressive strategies to escape from antitumor treatment. One common feature is the expansion of MDSC in the tumor microenvironment in many solid tumors, while factors influencing MDSC function and recruitment continue to expand [18,19]. In this study, MDSC subset levels were detected in osteosarcoma tumor-bearing mice and results showed that both G-MDSC and M-MDSC significantly increased comparing to normal mice, which is consistent to the clinical observation that MDSC levels increase in patients with cancer and correlate with metastatic burden, clinical stage and outcome [20].

A variety of mechanisms are involved in MDSC-mediated T cell suppression, including the NO production and arginase activity [21,22].

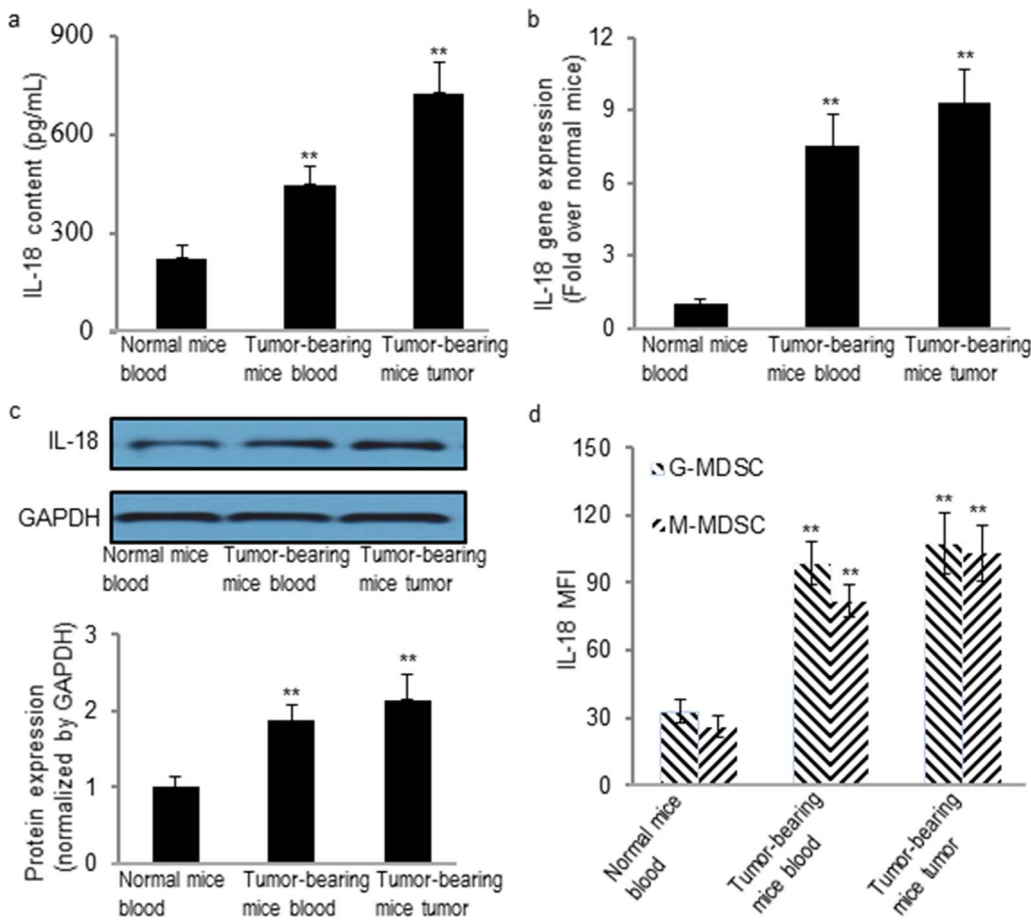


Fig. 2. Interleukin 18 (IL-18) content (a), IL-18 gene (b) and protein (c) expression increased in the blood and tumor lysate of tumor-bearing mice. IL-18 surface expression was detected by flow cytometry and results showed that expression of IL-18 significantly increased on MDSC subsets in the blood and tumor lysates of tumor-bearing mice (d). Data were expressed as mean ± SD (n = 5). **p < 0.01 vs normal mice.

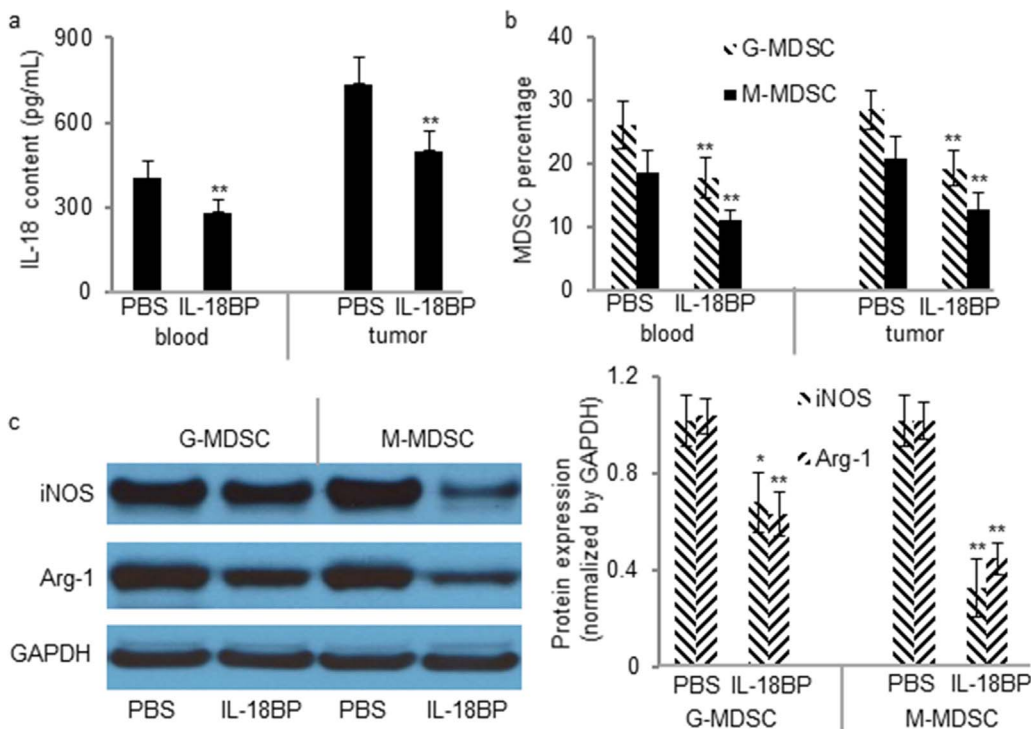


Fig. 3. Tumor-bearing mice were treated with IL-18BP, tumor-bearing mice treated with phosphate buffered saline (PBS) as negative control. Anti-IL-18 treatment significantly reduced IL-18 level (a) corresponding to the MDSC reduction, both in the blood and tumor (b). Anti-IL-18 treatment also decreased protein expression of iNOS and Arg-1 in the tumor lysates (c). Data were expressed as mean ± SD (n = 5). *p < 0.05, **p < 0.01 vs negative control.

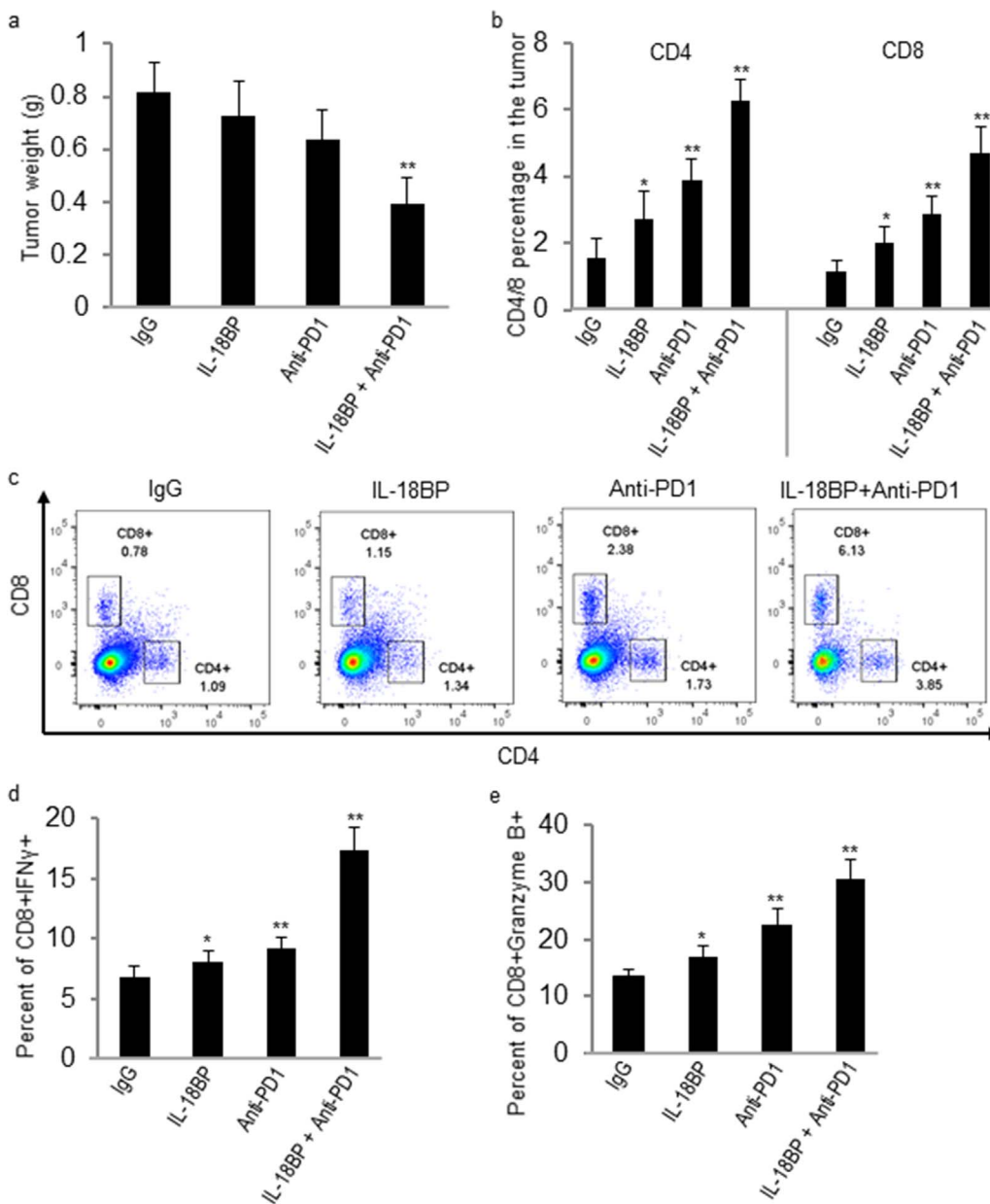


Fig. 4. Tumor-bearing mice were treated with IgG, IL-18BP, anti-PD1 antibody or the combination. The combination treatment decreased the tumor weight (a), increased both CD4⁺ and CD8⁺ T cells tumor infiltration (b, c), and increased the production of interferon gamma (IFN γ) and granzyme B (d, e). Data were expressed as mean \pm SD (n = 5). **p* < 0.05, ***p* < 0.01 vs IgG control.

G-MDSC have been confirmed to block the development of the effective antitumor immunity, promote immune suppression and reduce the effectiveness of anti-PD1 therapy in tumor bearing mice, resultantly promoting tumor progression. G-MDSC reduction delayed tumor growth and significantly improved the efficacy of anti-PD1 treatment [23]. M-MDSC levels inversely correlate with median progression free survival, and are described as an independent risk factor for non-small cell lung cancer and hepatocellular carcinoma [24,25].

Tumor-associated inflammatory factors contribute to cell growth and spread [26]. As a unique cytokine, IL-18 not only enhances both Th1 and Th2 responses to eliminate cancer cells effectively, but also has pro-cancer effects in tumor progression through inducing angiogenesis, metastasis and immune escape [27]. IL-18 alone promotes tumor progression in the absence of Th1 cytokines such as IL-12 [28]. Some cancer cells even secrete IL-18 into the tumor microenvironment [29]. Moreover, IL-18 administration dramatically increased the population of MDSC in the tumor microenvironment and decreased the IFN γ production. IL-18 contributes intratumoral MDSC accumulation and influences MDSC-mediated suppression of CD4⁺ and CD8⁺ T cells [10]. As a result, IL-18-dependent recruitment of myeloid cells promoted

tumor growth and metastasis. Studies demonstrated that targeting IL-18 in the tumor microenvironments may improve the efficiency of cancer immunotherapy.

MDSC were confirmed to promote immune suppression and reduce the effectiveness of anti-PD1 antibody therapy in mice bearing a rhabdomyosarcoma. MDSC reduction with anti-CXCR2 antibody treatment decreased tumor growth and improved the therapeutic effect of anti-PD1 treatment, suggesting that preventing the MDSC trafficking into tumors may enhance the efficacy of anti-PD1 therapy [23]. Our results showed that MDSC significantly increased in the blood and tumor of osteosarcoma tumor-bearing mice. MDSC were found to correlate with IL-18 intratumoral level, suggesting MDSC were drawn into the tumor by IL-18. Blocking IL-18 inhibited MDSC accumulation and simultaneously enhance T cell response to delay tumor progression. Additionally combination treatment using IL-18 antagonist improved the anti-PD1 treatment outcome by increasing the infiltration of T cells and the production of IFN γ and granzyme B, corresponding with the tumor burden decrease.

Checkpoint inhibitor is the most successful strategies developed so far in cancer immunotherapy, which comprises a class of agents

disrupting inhibitory signals to T cells. Antibodies blocking PD1 or PD-L1 mediate objective responses in patients with melanoma, renal cell carcinoma and non-small cell lung cancer [30,31]. Combination treatments appear to be more potent with response rates over 50% in metastatic melanoma [32]. Despite the progress, the percentage is still small for patients with cancer who benefit from checkpoint inhibitors. Therefore, the potential clinical application of our findings is notable because there are currently no clinically applicable approaches to mitigate MDSC-mediated immune escape. The findings raise the prospect that clinical studies of agents blocking IL-18 may be beneficial either as single agent or in combination with checkpoint blockade.

In conclusions, this study provided a potential new target for the osteosarcoma carcinoma treatment, in addition to elucidating a possible association between MDSC subsets and cytokine inducing MDSC migration into the tumor tissue. Targeting IL-18 may reduce MDSC accumulation and function, which could enhance the efficacy of immune checkpoint inhibitor in patients with osteosarcoma cancer.

Conflict of interest statement

The authors declared that there is no conflict of interests in this work.

References

- [1] Y. Li, W. Du, J. Han, J.B. Ge, LAMP3 promotes the invasion of osteosarcoma cells via SPP1 signaling, *Mol. Med. Rep.* (2017), <http://dx.doi.org/10.3892/mmr.2017.7349>.
- [2] D.M. Lussier, J.L. Johnson, P. Hingorani, J.N. Blattman, Combination immunotherapy with α -CTLA-4 and α -PD-L1 antibody blockade prevents immune escape and leads to complete control of metastatic osteosarcoma, *J. Immunother. Cancer* 3 (2015) 21–31.
- [3] S.J. Strauss, T. Ng, A. Mendoza-Naranjo, J. Whelan, P.H. Sorensen, Understanding micrometastatic disease and Anoikis resistance in ewing family of tumors and osteosarcoma, *Oncologist* 15 (2010) 627–635.
- [4] D. Carrle, S. Bielack, Osteosarcoma lung metastases detection and principles of multimodal therapy, *Cancer Treat. Res.* 152 (2009) 165–184.
- [5] M.T. Harting, M.L. Blakely, Management of osteosarcoma pulmonary metastases, *Semin. Pediatr. Surg.* 15 (2006) 25–29.
- [6] T. Schatton, M.H. Frank, Antitumor immunity and cancer stem cells, *Ann. NY Acad. Sci.* 1176 (2009) 154–169.
- [7] K.P. Wilkie, P. Hahnfeldt, Tumor-immune dynamics regulated in the micro-environment inform the transient nature of immune-induced tumor dormancy, *Cancer Res.* 73 (2013) 3534–3544.
- [8] D.I. Gabrilovich, S. Nagaraj, Myeloid-derived suppressor cells as regulators of the immune system, *Nat. Rev. Immunol.* 9 (2009) 162–174.
- [9] S. Ostrand-Rosenberg, Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity, *Cancer Immunol. Immunother.* 59 (2010) 1593–1600.
- [10] H.X. Lim, H.J. Hong, D.H. Cho, T.S. Kim, IL-18 enhances immunosuppressive responses by promoting differentiation into monocytic myeloid-derived suppressor cells, *J. Immunol.* 193 (2014) 5453–5460.
- [11] Y.G. Najjar, J.H. Finke, Clinical perspectives on targeting of myeloid derived suppressor cells in the treatment of cancer, *Front. Oncol.* 3 (2013) 49.
- [12] P.C. Rodriguez, C.P. Hernandez, D. Quiceno, et al., Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma, *J. Exp. Med.* 202 (2005) 931–939.
- [13] C.M. Diaz-Montero, M.L. Salem, M.I. Nishimura, E. Garrett-Mayer, D.J. Cole, A.J. Montero, Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy, *Cancer Immunol. Immunother.* 58 (2009) 49–59.
- [14] M. Ouzounova, E. Lee, R. Piranlioglu, et al., Monocytic and granulocytic myeloid derived suppressor cells differentially regulate spatiotemporal tumour plasticity during metastatic cascade, *Nat. Commun.* 8 (2017) 14979.
- [15] E. Ribechini, V. Greifenberg, S. Sandwick, M.B. Lutz, Subsets, expansion and activation of myeloid-derived suppressor cells, *Med. Microbiol. Immunol.* 199 (2010) 273–281.
- [16] M. Elkabets, V.S. Ribeiro, C.A. Dinarello, et al., IL-1b regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function, *Eur. J. Immunol.* 40 (2010) 3347–3357.
- [17] T. Ogura, H. Ueda, K. Hosohara, et al., Interleukin-18 stimulates hematopoietic cytokine and growth factor formation and augments circulating granulocytes in mice, *Blood* 98 (2001) 2101–2107.
- [18] E. Schlecker, A. Stojanovic, C. Eisen, et al., Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth, *J. Immunol.* 189 (2012) 5602–5611.
- [19] R.F. Gabitass, N.E. Annels, D.D. Stocken, H.A. Pandha, G.W. Middleton, Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13, *Cancer Immunol. Immunother.* 60 (2011) 1419–1430.
- [20] L. Wang, E.W. Chang, S.C. Wong, S.M. Ong, D.Q. Chong, K.L. Ling, Increased myeloid-derived suppressor cells in gastric cancer correlate with cancer stage and plasma S100A8/A9 proinflammatory proteins, *J. Immunol.* 190 (2013) 794–804.
- [21] J.I. Youn, S. Nagaraj, M. Collazo, D.I. Gabrilovich, subsets of myeloid-derived suppressor cells in tumor-bearing mice, *J. Immunol.* 181 (2008) 5791–5802.
- [22] J. Tian, J. Ma, K. Ma, et al., β -glucan enhances antitumor immune responses by regulating differentiation and function of monocytic myeloid-derived suppressor cells, *Eur. J. Immunol.* 43 (2013) 1220–1230.
- [23] S.L. Highfill, Y. Cui, A.J. Giles, et al., Disruption of CXCR2-mediated MDSC tumor trafficking enhances anti-PD1 efficacy, *Sci. Transl. Med.* 6 (2014) 237ra67.
- [24] F. Arihara, E. Mizukoshi, M. Kitahara, et al., Increase in CD14+HLA-DR-/low myeloid-derived suppressor cells in hepatocellular carcinoma patients and its impact on prognosis, *Cancer Immunol. Immunother.* 62 (2013) 1421–1430.
- [25] A. Huang, B. Zhang, B. Wang, F. Zhang, K.X. Fan, Y.J. Guo, Increased CD14(+) HLA-DR (-/low) myeloid-derived suppressor cells correlate with extrathoracic metastasis and poor response to chemotherapy in non-small cell lung cancer patients, *Cancer Immunol. Immunother.* 62 (2013) 1439–1451.
- [26] M. Yu, K. Kang, P. Bu, et al., Deficiency of CC chemokine ligand 2 and decay-accelerating factor causes retinal degeneration in mice, *Exp. Eye Res.* 138 (2015) 126–133.
- [27] S. Park, S. Cheon, D. Cho, The dual effects of interleukin-18 in tumor progression, *Cell. Mol. Immunol.* 4 (2007) 329–335.
- [28] F. Vidal-Vanaclocha, L. Mendoza, N. Telleria, et al., Clinical and experimental approaches to the pathophysiology of interleukin-18 in cancer progression, *Cancer Metastasis Rev.* 25 (2006) 417–434.
- [29] M.K. Jung, H.K. Song, K.E. Kim, et al., IL-18 enhances the migration ability of murine melanoma cells through the generation of ROI and the MAPK pathway, *Immunol. Lett.* 107 (2006) 125–130.
- [30] S.L. Topalian, F.S. Hodi, J.R. Brahmer, et al., Safety, activity, and immune correlates of anti-PD1 antibody in cancer, *N. Engl. J. Med.* 366 (2012) 2443–2454.
- [31] J.R. Brahmer, S.S. Tykodi, L.Q. Chow, et al., Safety and activity of anti-PD-L1 antibody in patients with advanced cancer, *N. Engl. J. Med.* 366 (2012) 2455–2465.
- [32] J.D. Wolchok, H. Kluger, M.K. Callahan, et al., Nivolumab plus ipilimumab in advanced melanoma, *N. Engl. J. Med.* 369 (2013) 122–133.