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Original Article

CD150^{high}Treg cells may attenuate graft versus host disease and intestinal cell apoptosis after hematopoietic stem cell transplantation

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Abstract: Combined transplantation of regulatory T cells (Treg cells) may significantly attenuate graft versus host disease (GVHD) after hematopoietic stem cell transplantation (HSCT). Recent studies indicated that CD150+Treg cells could secret adenosine to maintain the quiescent status of HSCs. However, whether it is attributable to the attenuation of GVHD after HSCT is still unclear. In vitro studies revealed that CD150+Treg cells induced immune tolerance was comparable to that induced by CD150-Treg cells, but CD150+Treg cells can secret more adenosine, increase *P*-AMPK expression and regulate energy metabolism to induce the proliferation of HSC proliferation and inhibit their differentiation into dendritic cells. In this study, GVHD animal model was established, and combined transplantation of Treg cells and HSCs was performed. Results showed the survival time was significantly prolonged, the proliferation rate of HSCs increased significantly and the proportion of undifferentiated HSCs elevated significantly after CD150+Treg transplantation as compared to CD150-Treg transplantation. Immunohistochemistry revealed CD150+Treg cells could secret adenosine, activate AMPK expression and inhibit intestinal cell apoptosis and inflammation after HSCT. Taken together, this study indicates CD150+Treg cells can regulate energy metabolism to attenuate GVHD and intestinal cell apoptosis after HSCT.

Keywords: Regulatory T cells, CD150, hematopoietic stem cell transplantation, graft versus host disease, energy metabolism

Introduction

Hematopoietic stem cell transplantation (HSCT) is still the most effective strategy for the treatment of malignant hematological diseases and a basic way for the treatment of some solid tumors. Graft versus host disease (GVHD) is one of causes of HSCT failure, and severe GVHD may even cause death [1]. Combined transplantation of immunoregulatory cells and HSCs may significantly inhibit the GVHD, and regulatory T cells (Treg cells) have been regarded the most promising cell type for the HSCT. Treg cells are the main negative regulatory cells and can secret transforming growth factor-β (TGF-β) and IL-10 to inhibit the immune rejection after allogeneic transplantation [2, 3]. There is evidence showing that the proportion of peripheral Treg cells is negatively related to the incidence of acute GVHD, and combined transplantation of Treg cells and HSCs can significantly acute GVHD and promote the reconstruction of immune and hematopoietic systems [4-6]. However, the in vitro amplification of Treg cells is difficult and the amount of Treg cells used for transplantation is large. Thus, to improve the function of Treg cells is of great importance in clinical practice.

The bone marrow niche is important for the maintenance of quiescent status, self-renewal and multi-directional differentiation potential of HSCs, in which adenosine signaling pathway plays an important role [7, 8]. After entering the recipients, some HSCs may differentiate to reconstruct the compromised immunity, and others maintain the stemness to reconstruct the hematopoietic system. Therefore, the maintenance of differentiation and self-renewal of HSCs is very important [9, 10]. Recent studies

indicate there are CD150^{high} cells with Treg cells and these cells can secret adenosine to maintain the quiescent status of HSCs [11]. These findings indicate that Treg cells can not only induce immune tolerance, but also inhibit the GVHD after HSCTs via other mechanisms.

Materials and methods

Cell separation and culture

Lymphocyte separation solution (TBD, Tianjin, China) was used for the separation of peripheral blood mononuclear cells (PBMCs) from blood of B6 mice. Treg cell sorting kit (BD company, USA) and magnetic beads were used for the separation of Treg cells from PBMCs. After in vitro amplification of Treg cells, cells were incubated with CD150 antibody (BD company, USA) for 20 min at room temperature, followed by washing in PBS thrice. CD150^{high}Treg cells (CD150+Treg cells) and CD150-Treg cells were sorted by flow cytometry. The femur was collected from B6 mice and the bone marrow was flushed with PBS. The resultant cell suspension was filtered for the sorting of CD34+ HSCs by flow cytometry. HSCs were maintained in RPMI1640 containing 10% fetal bovine serum (FBS).

Detection of HLA-G and CTLA-4

CD150-Treg and CD150+Treg cells were incubated with HLA-G and CTLA-4 antibodies at room temperature in dark for 20 min. After washing in PBS thrice, the mean fluorescence intensity of HLA-G and CTLA-4 was detected by flow cytometry.

Detection of immunosuppressive molecules and adenosine

CD150-Treg cells and CD150+Treg cells were maintained for 48 h and then the supernatant was collected for the detection of IL-10 and TGF-β concentration by ELISA according to the manufacturer's instructions (Xinbo Biotech Co., Ltd). Adenosine concentration was detected according to previously reported [7]. In brief, the solution (1 ml) was collected and added to a centrifuge tube, followed by addition of chloroacetaldehyde (final concentration: 220 μM). After boiling for 20 min, the fluorescence intensity of the mixture was measured by fluorescence spectrophotometry (excitation wavelength = 310 nm, emission wavelength = 410 nm).

EDU proliferation test

Transwell chamber was used for the co-culture of Treg cells and HSCs. HSCs were added to lower chamber, and Treg cells into upper chamber. There were 4 groups in this test: control group (HSCs alone), CD150-Treg group (CD150-Treg cells and HSCs), CD150+Treg group (CD150+Treg cells and HSCs) and adenosine inhibition group (CD150+Treg cells + HSCs + aminophylline [an adenosine inhibitor]). Cells were maintained for 48 h, and then cells in lower chamber were harvested, followed by addition of EDU staining solution. After further incubation for 24 h, 2 mg/mL glycine and 0.5% TritonX-100 were added, followed by washing in PBS thrice. After addition of EDU reaction solution, incubation was done in dark at room temperature for 30 min. Following DAPI staining, the fluorescence was observed under a fluorescence microscope.

Detection of HSCs differentiation

Some studies have reported that IL-6 can induce the differentiation of HSCs into dendritic cells (DCs), and thus IL-6 was used in positive control group. There were 5 groups in this test: control group (HSCs alone), IL-6 group (HSCs + 20 ng/mL IL-6), CD150-Treg group (CD150-Treg cells + HSCs + 20 ng/mL IL-6), CD150+Treg group (CD150+Treg cells + HSCs + 20 ng/mL IL-6) and adenosine inhibition group (CD150+ Treg cells + HSCs + aminophylline + 20 ng/mL IL-6). After incubation for 48 h, cells in lower chamber were harvested, followed by addition of CD1a. Following incubation at room temperature for 20 min, cells were washed in PBS thrice. Flow cytometry was performed for the detection of DCs after HSCs differentiation.

Detection of energy metabolism

After co-culture, HSCs were harvested and then incubated with rhodamine 123 for 30 min for the labeling of mitochondrial membrane potential (MMP). After washing in PBS thrice, the mean fluorescence intensity was detected by flow cytometry. In addition, the ATP concentration of HSCs was also detected by using ATP Bioluminescence Assay Kit. In brief, the boiled 100 nM Tris and 4 nM EDTA were added to the cell suspension, followed by boiling at 100°C for 2 min. After centrifugation, the supernatant was removed, and fluorescence reaction reagent was added. The fluorescence intensity

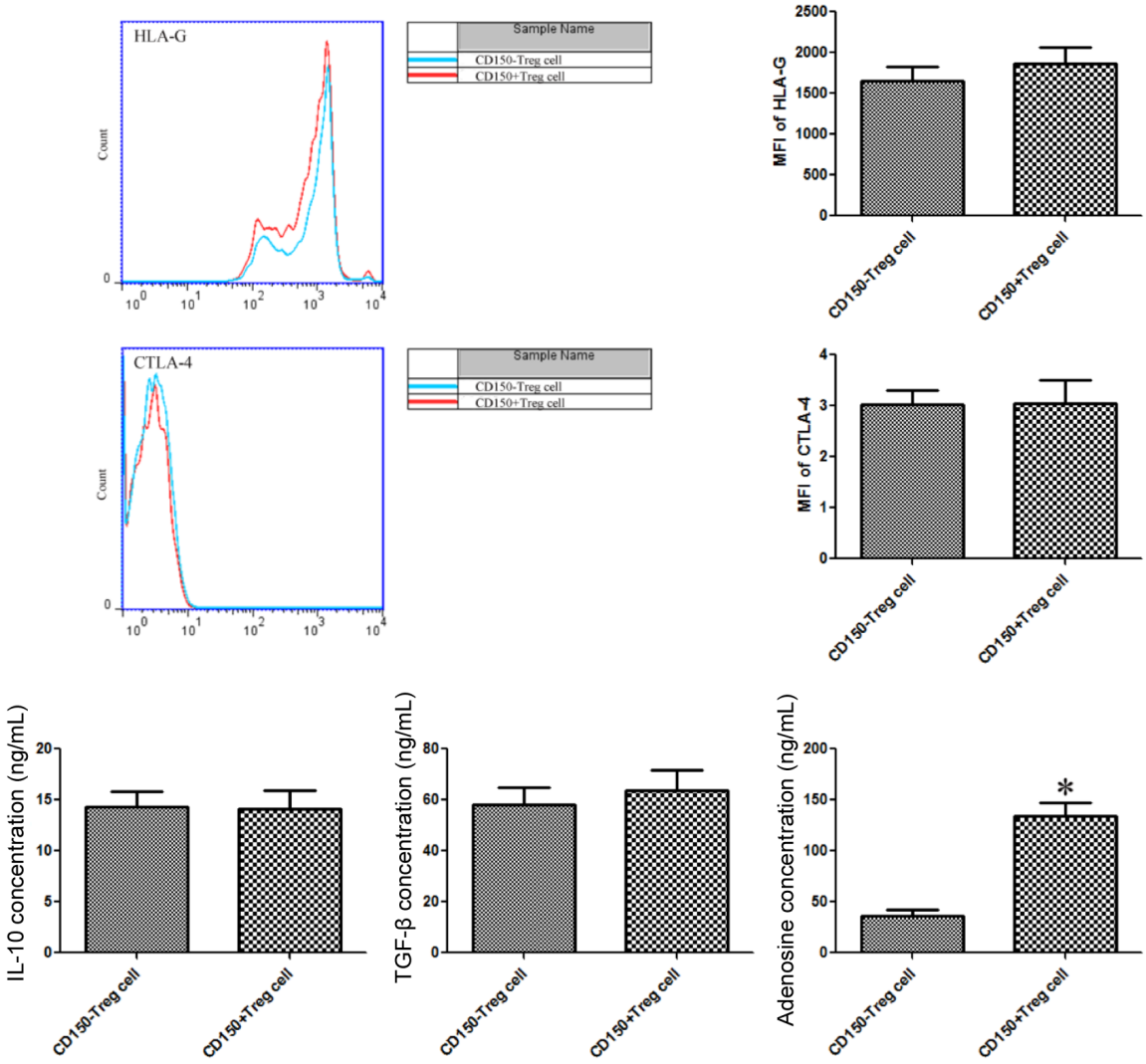

Figure 1. Adenosine concentration of CD150-Treg cells and CD150+Treg cells. Flow cytometry showed the expression of HLA-G and CTLA-4 was comparable between CD150-Treg and CD150+Treg cells. ELISA revealed the concentration of IL-10 and TGF-β in the supernatant was similar between CD150-Treg and CD150+Treg cells. The adenosine concentration in CD150+Treg cells was about 2.66 times that in CD150-Treg cells.

was measured immediately. Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) plays an important role in the energy metabolism regulation and functional maintenance of HSCs. Thus, p-AMPK expression was detected in HSCs of different groups. After fixation, HSCs were embedded in paraffin, followed by processing for immunohistochemistry for p-AMPK and Ki-67.

Construction of GVHD model

GVHD model was established according to previously reported [12]. After fatal irradiation (8 Gy), 8-week aged BALB/c mice were injected with 5 × 10^6 CD34+ HSCs and 1 × 10^6 T cells from B6 mice for the induction of GVHD. There were 5 groups in this test: control group (GVHD), CD150-Treg group (GVHD + injection of 3 × 10^6 CD150-Treg cells via tail vein), CD150+Treg group (GVHD + injection of 3 × 10^6 CD150+Treg cells via tail vein), adenosine inhibition group (GVHD + injection of 3 × 10^6 CD150+Treg cells via tail vein + aminophylline) and AMPK group (GVHD + injection of 3 × 10^6 CD150+Treg cells via tail vein + Compound C [an AMPK inhibitor]).

Assessment of proliferation and differentiation of HSCs in vivo

After labeling of HSCs with DiI, these cells were injected into GVHD mice via the tail vein. At 7

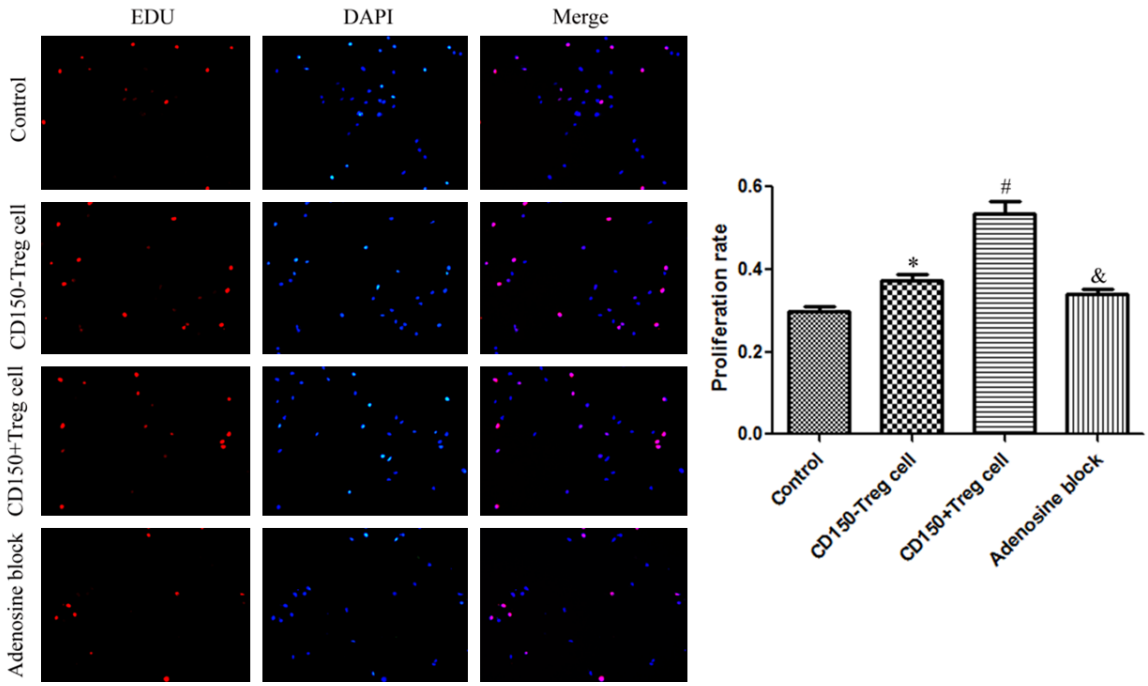

Figure 2. CD150+Treg cells promote HSCs proliferation. EDU proliferation test showed the proliferation rate was significantly different between control group and CD150-Treg group. In CD150+Treg group, the proliferation rate was significantly higher than CD150-Treg group. Adenosine inhibition could significantly reduce the HSCs proliferation induced by CD150+Treg cells.

days after transplantation, peripheral lymphocytes were separated and incubated with CD34 antibody at room temperature for 15 min. After washing in PBS thrice, the DiI positive cells and DiI positive/CD34 negative cells were determined by flow cytometry.

Immunohistochemistry

At 7 days after HSCs transplantation, the intestine was harvested, fixed in 4% paraformaldehyde, dehydrated, and sectioned, followed by HE staining. Some paraffin-embedded sections were subjected to deparaffinization and then incubated with leukocyte common antigen (LCA), Bcl-2 or p-AMPK at 4°C over night. After washing in PBS thrice, biotin conjugated secondary antibody and alkaline phosphatase conjugated streptavidin solution was added, followed by washing in PBS thrice. Visualization was done, sections were flushed with flowing water, followed by counterstaining, dehydration, transparentization and mounting. Sections were then observed under a light microscope.

Statistical analysis

Statistical analysis was performed with SPSS version 19.0, and data are expressed as mean ± standard deviation. Comparisons were done with t test between groups or with analysis of variance followed by Bonferroni method among groups. Correlation analysis was done with Spearman correlation test. A value of $P < 0.05$ was considered statistically significant.

Results

CD150+Treg can secret more adenosine

Flow cytometry showed the expression of HLA-G and CTLA-4 was comparable between CD150-Treg cells and CD150+Treg cells (t = 0.8108, P = 0.4364; t = 0.04628, P = 0.9640). ELISA revealed the concentration of IL-10 and TGF-β in the supernatant was also similar between CD150-Treg cells and CD150+Treg cells (t = 0.1059, P = 0.9178; t = 0.5161, P = 0.6170). However, the adenosine concentration in CD150+Treg cells was about 2.66 times that in CD150-Treg cells, showing significant difference between them (t = 6.728, P < 0.0001) (**Figure 1**).

CD150+Treg cells promote HSCs proliferation

EDU proliferation test showed the proliferation rate was 0.2963 in control group and 0.3740 in

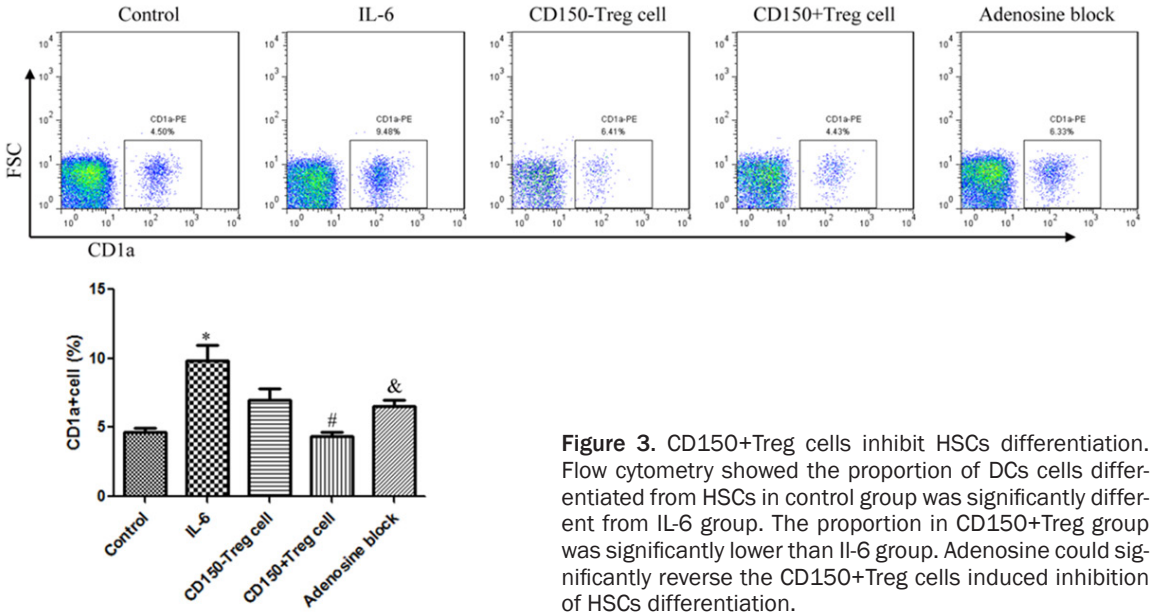

Figure 3. CD150+Treg cells inhibit HSCs differentiation. Flow cytometry showed the proportion of DCs cells differentiated from HSCs in control group was significantly different from IL-6 group. The proportion in CD150+Treg group was significantly lower than Il-6 group. Adenosine could significantly reverse the CD150+Treg cells induced inhibition of HSCs differentiation.

CD150-Treg group, showing significant difference between them (t = 4.547, P = 0.0011). In CD150+Treg group, the proliferation rate was 0.5350, which was significantly higher than in CD150-Treg group (t = 5.015, P = 0.0005). In addition, adenosine inhibition could significantly reduce the HSCs proliferation induced by CD150+Treg cells (t = 6.058, P = 0.0001) (**Figure 2**).

CD150+Treg cells inhibit HSCs differentiation

Flow cytometry showed the proportion of DCs cells differentiated from HSCs was 4.700% in control group and 9.813% in IL-6 group, showing significant difference (t = 4.413, P = 0.0013). This proportion was 6.990% in CD150-Treg group, which was similar to that in IL-6 group (t = 2.023, P = 0.0706), but the proportion in CD150+Treg group (4.343%) was significantly lower than in Il-6 group (t = 4.693, P = 0.0009). Moreover, adenosine could significantly reverse the CD150+Treg cells induced inhibition of HSCs differentiation (t = 4.319, P = 0.0015) (**Figure 3**).

CD150+Treg cells elevate energy metabolism of HSCs

AMPK and energy metabolism play an important role in the maintenance of quiescent status and normal functions of HSCs. This study further investigated the effect of CD150+Treg cells on the energy metabolism of HSCs. These results showed the MMP and intracellular ATP concentration were comparable between CD-150-Treg group and control group (t = 2.111, P = 0.0609; t = 1.584, P = 0.1443). The MMP and intracellular ATP concentration were 8175 and 14.38, respectively, in CD150+Treg group, which were significantly different from those in CD150-Treg group (t = 4.001, P = 0.0025; t = 3.607, P = 0.0048). In addition, adenosine inhibition could significantly reduce the CD150+Treg cells induced increase in energy metabolism of HSCs. Immunohistochemistry was further performed for the detection of p-AMPK and Ki-67. Results showed CD150+Treg cells could significantly increase the p-AMPK expression in HSCs, which however was attenuated by adenosine inhibition. Correlation analysis revealed that p-AMPK expression was positively related to the Ki-67 proliferation index (r = 0.7613, P < 0.0001). These findings indicate that CD150+ Treg cells can secret adenosine to activate AMPK and increase energy metabolism, which elevates the proliferation of HSCs (**Figure 4**).

CD150+Treg cells promote HSCs proliferation and inhibit their differentiation in vivo

Flow cytometry showed the proportion of DiI positive cells was 6.308% in control group and 10.37% in CD150-Treg group, showing significant difference between groups (t = 4.691, P = 0.0009). The proportion of DiI positive cells

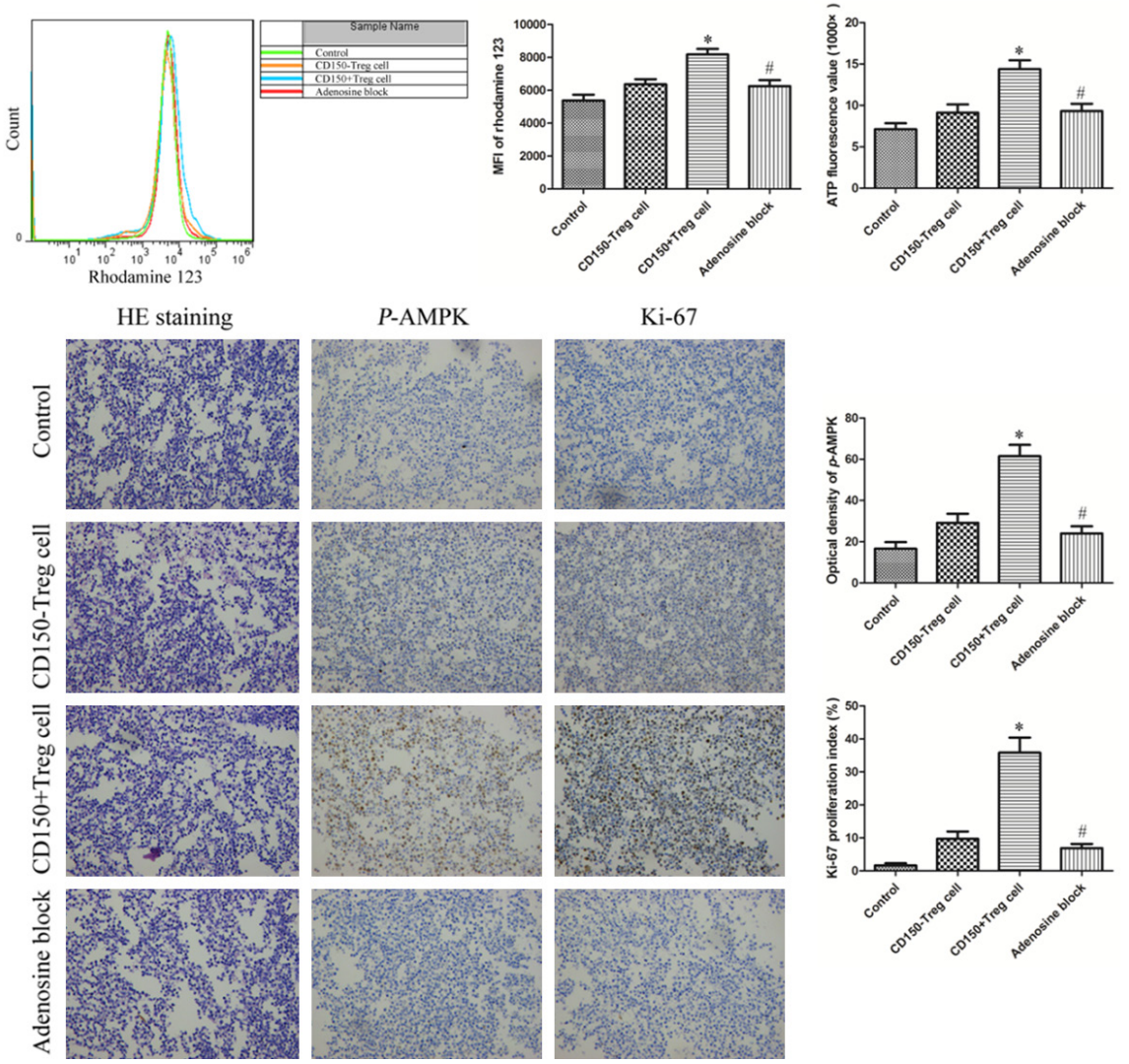

Figure 4. CD150+Treg cells elevate energy metabolism of HSCs. MMP and intracellular ATP concentration were comparable between CD150-Treg group and control group. MMP and intracellular ATP concentration were significantly different from those in CD150-Treg group. Adenosine inhibition could significantly reduce the CD150+Treg cells induced increase in energy metabolism of HSCs. Immunohistochemistry showed CD150+Treg cells could significantly increase the p-AMPK expression in HSCs, which however was attenuated by adenosine inhibition. Correlation analysis revealed that p-AMPK expression was positively related to the Ki-67 proliferation index. These findings indicate that CD150+Treg cells can secret adenosine to activate AMPK and increase energy metabolism, which elevates the proliferation of HSCs.

was 14.10% in CD150+Treg group, which was significantly higher than in CD150-Treg group (t = 3.274, P = 0.0084). Adenosine inhibition or AMPK inhibition could significantly reduce the CD150+Treg induced DiI positive cells (t = 5.422, P = 0.0003; t = 5.474, P = 0.0003).

The proportion of DiI+CD34-differentiated cells was 39.97% in control group and 22.47% in CD150-Treg group, showing significant difference between them (t = 3.561, P = 0.0052). This proportion was 14.65% in CD150+Treg group, which was significantly lower than in CD150-Treg group (t = 2.282, P = 0.0457). In addition, adenosine inhibition or AMPK inhibition could significantly reduce the proportion of differentiated cells as compared to CD150+Treg group (t = 5.765, P = 0.0002; t = 8.045, P < 0.0001) (**Figure 5**).

CD150+Treg cells prolong survival time of mice after HSCs transplantation

Combined transplantation of CD150-Treg cells and HSCs could significantly prolong the survival time of mice as compared to control group,

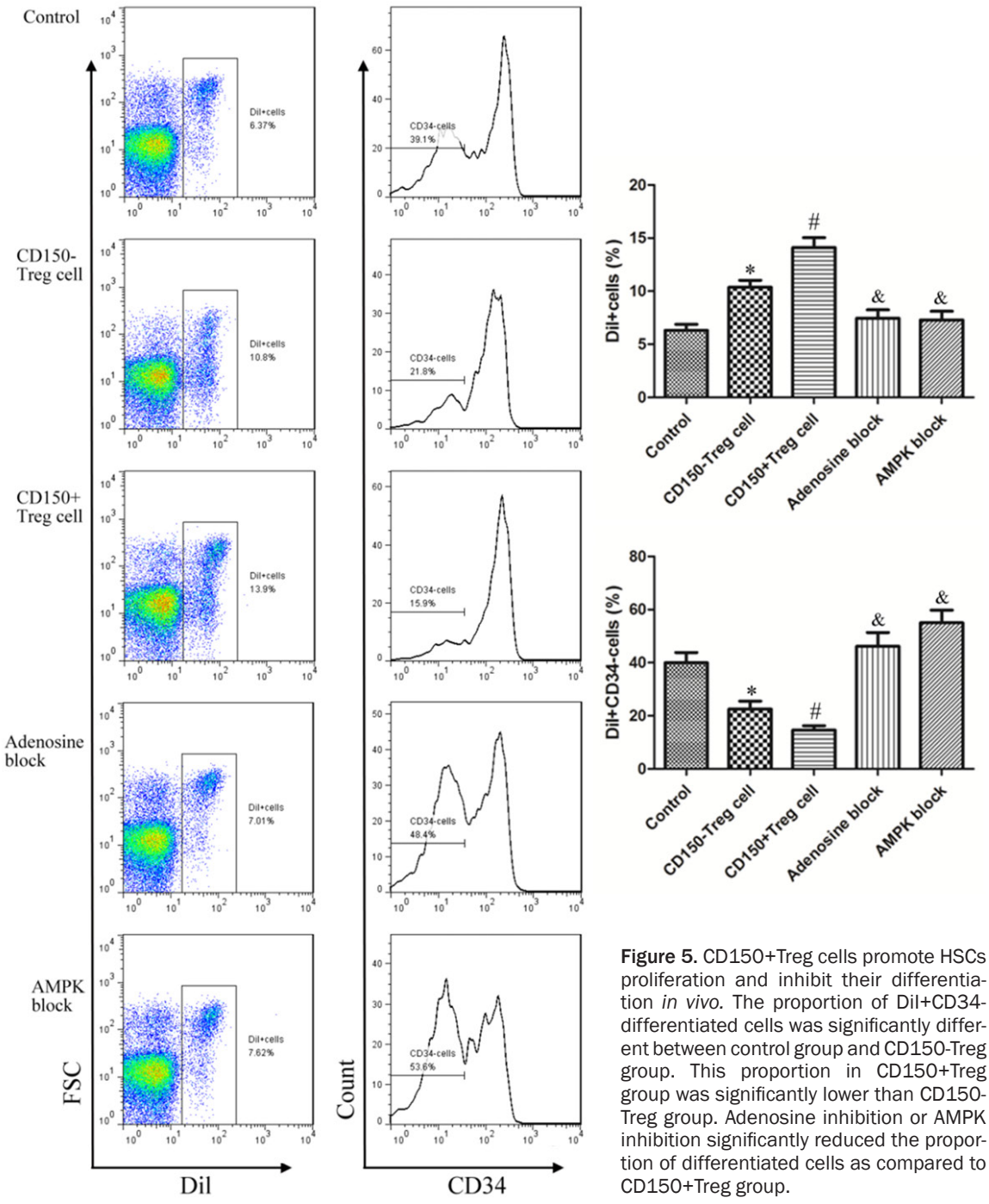

Figure 5. CD150+Treg cells promote HSCs proliferation and inhibit their differentiation *in vivo*. The proportion of DiI+CD34- differentiated cells was significantly different between control group and CD150-Treg group. This proportion in CD150+Treg group was significantly lower than CD150-Treg group. Adenosine inhibition or AMPK inhibition significantly reduced the proportion of differentiated cells as compared to CD150+Treg group.

and the survival time in CD150+Treg group was further prolonged. However, adenosine inhibition or AMPK inhibition significantly shorten the survival time of mice in CD150+Treg group. The intestine is the most common tissue involved in GVHD after HSCs transplantation. Thus, the intestinal tissues were harvested from mice in different groups. These results showed the intestinal injury was significantly attenuated in CD150-Treg group and the number of infiltrated LCA+ lymphocytes reduced significantly as compared to control group ($t = 3.878$, $P = 0.0031$). In CD150+Treg group, the intestinal injury and inflammation were further attenuated, and the number of infiltrated LCA+ lymphocytes reduced significantly by 63% as com-

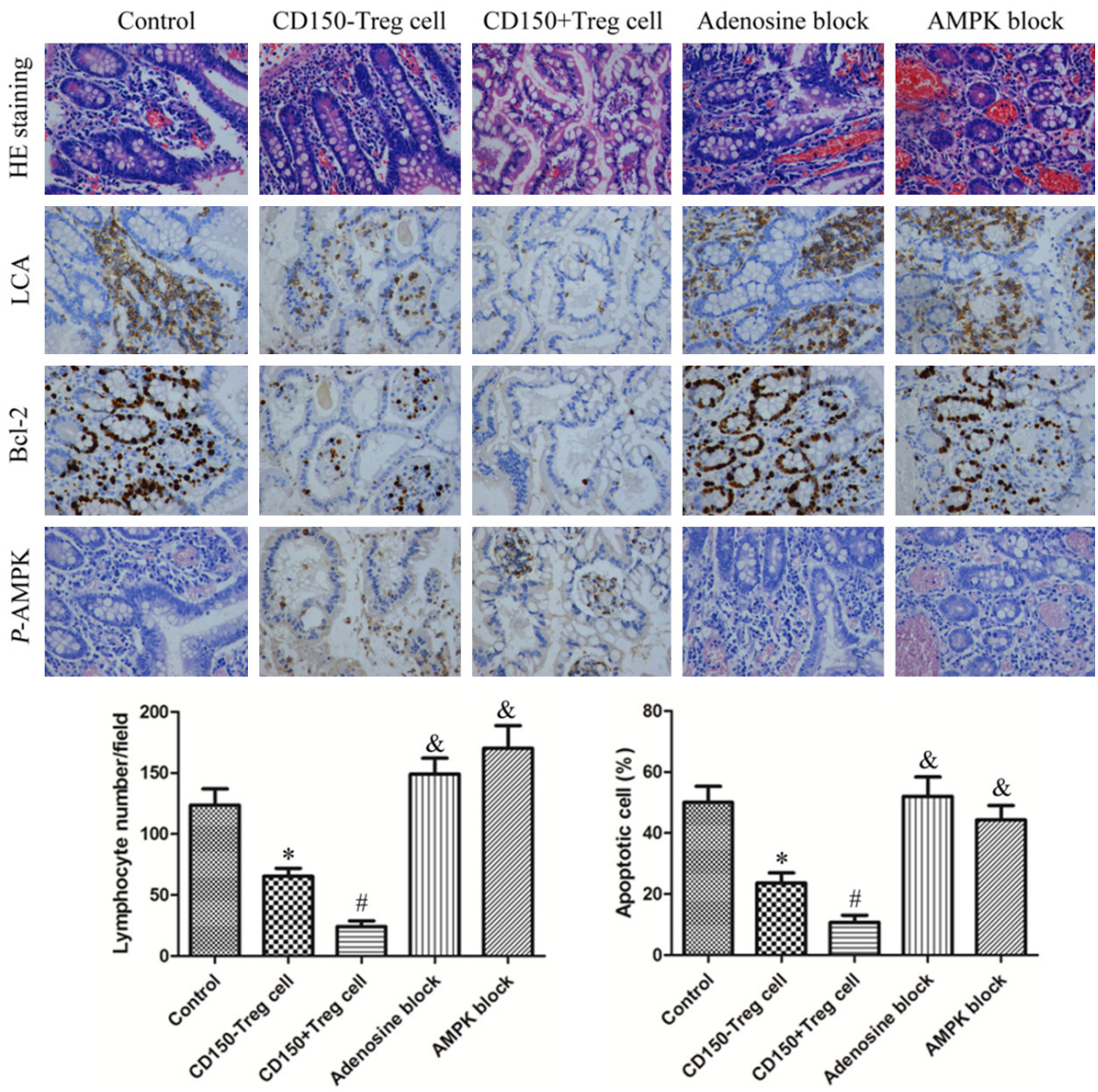

Figure 6. CD150+Treg cells can secret adenosine to activate AMPK expression and regulate energy metabolism, attenuating the intestinal injury and inflammation after HSCs transplantation. Intestinal injury was significantly attenuated in CD150-Treg group and the number of infiltrated LCA+ lymphocytes reduced significantly as compared to control group. In CD150+Treg group, the number of infiltrated LCA+ lymphocytes reduced significantly by 63% as compared to CD150-Treg group.

pared to CD150-Treg group (t = 5.140, P = 0.0004). In addition, adenosine inhibition or AMPK inhibition could compromise the protective effect of CD150+Treg cells on intestinal injury and inflammation (**Figure 6**).

CD150+Treg cells inhibit intestinal cell apoptosis after HSCs transplantation

The proportion of Bcl-2+ cells (apoptotic cells) was 49.89% in control group. CD150-Treg cells could inhibit the GVHD induced intestinal cell apoptosis, and the proportion of apoptotic cells in CD150-Treg group reduced by 52.66% as compared to control group (t = 4.218, P = 0.0018). In CD150+Treg group, the proportion of apoptotic cells further reduced by 54.92% as compared to CD150-Treg group (t = 3.182, P = 0.0098). Adenosine inhibition and AMPK inhibition could significantly increase the proportion of apoptotic intestinal cells by 3.87 times and 3.15 times as compared to CD150+Treg group (t = 6.105, P = 0.0001; t = 6.406, P < 0.0001). The p-AMPK expression was further detected

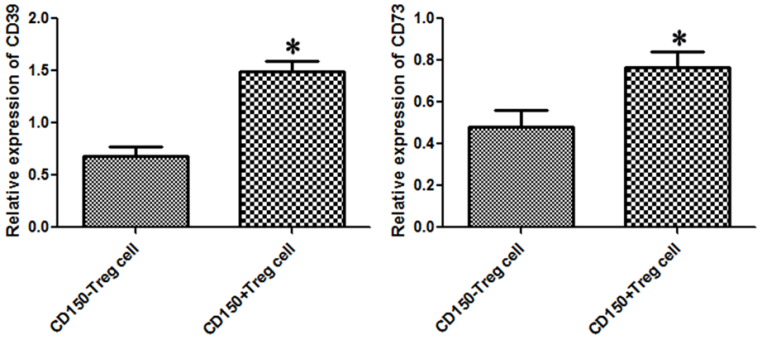

Figure 7. CD39 and CD73 mRNA expression. CD39 and CD73 mRNA expression in CD150+Treg cell were significantly higher than CD150-Treg cell, indicating that CD39-CD73-adenosine signaling pathway was involved in biofunction of CD150+Treg cell.

in the intestine. Results showed CD150+Treg cells could significantly increase p-AMPK expression in the intestine, which however was attenuated by adenosine inhibition. These findings suggest that CD150+Treg cells can secret adenosine to activate AMPK expression and regulate energy metabolism in the intestine, which is related to the attenuation of intestinal injury and inflammation after HSCs transplantation (**Figure 6**).

CD73 and CD39 expression of CD150-Treg and CD150+Treg cell

CD39 and CD73 mRNA expression in CD150+ Treg cell were significantly higher than CD150- Treg cell, indicating that CD39-CD73-adenosine signaling pathway was involved in biofunction of CD150+Treg cell (**Figure 7**). CD39 and CD73 protein expression in CD150+Treg cell was significantly higher than CD150-Treg cell, which confirmed the above results (**Figure 8**).

Discussion

In recent years, the incidence of malignant hematological diseases such as leukemia and lymphoma is increasing over year, bringing significant burden to the family and the society. HSCs transplantation has been the most effective strategy for the treatment of malignant hematological diseases. On one hand, it can reconstruct the immune system and effectively kill the cancer cells in vivo; on the other hand, it may reconstruct the hematopoietic system and maintain the stability of hematological system [1, 9]. GVHD is a major cause of failure after HSCs transplantation and may cause some adverse effects (such as fever, rash and liver dysfunction) or even death if it is severe enough [13]. Transplantation of immunosuppressive cells and HSCs can significantly the incidence of GVHD and reduce the dose of immunosuppressants used for rejection prevention. Previous studies revealed that combined transplantation of mesenchymal stem cells and HSCs promoted the reconstruction of hematopoietic system and maintained the stemness of HSCs. Treg cells can induce immune tolerance to grafts and inhibit Allogeneic rejection. In addition, they can secret some immunosuppressive factors to indirectly inhibit the immune rejection. Treg cells have the expression of some immunosuppressive molecules on their surface (such as HLA-G and CTLA-4), which may directly induce the immune tolerance [14, 15]. These results showed Treg cells could also induce the proliferation of HSCs and promote the reconstruction of hematopoietic system, which provides theoretical evidence for the treatment of GVHD with Treg cells.

Recent studies indicate that Treg cells can be further divided into CD150+Treg cells and CD150-Treg cells. CD150+Treg cells are crucial for the maintenance of quiescent status of HSCs [11]. This study showed both subsets of Treg cells could directly or indirectly induce the immune tolerance, but CD150+Treg cells could also promote HSCs proliferation and inhibit their differentiation. Stem cell proliferation is important for the increase in the number of HSCs in vitro, exerting better therapeutic effects. The inhibited differentiation of HSCs may maintain the proliferative status of HSCs for a longer time and maximize the number of stem cells, which leads to the reconstruction of immune system and hematopoiesis, exerting therapeutic effects [16, 17].

Adenosine is an end product of deoxynucleotides and has been widely used in clinical practice to treat diseases such as myocardial ischemia. Adenosine can also effectively induce anngiogenesis and promote the long-term patency of tissue engineering blood vessels

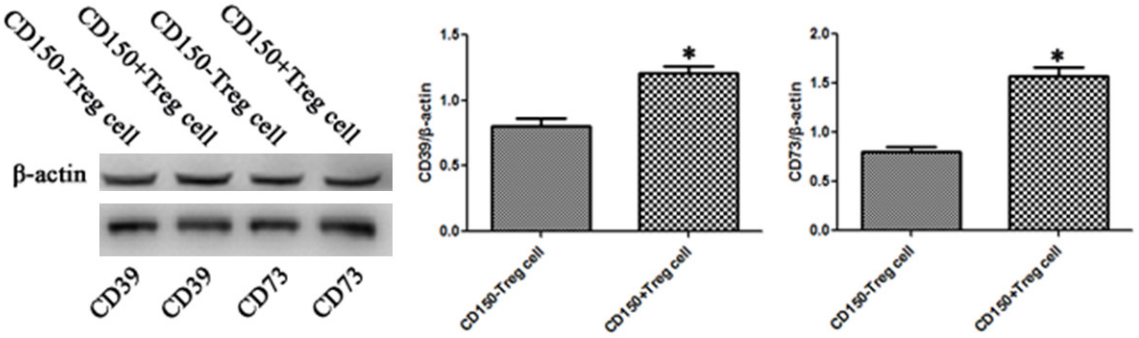

Figure 8. CD39 and CD73 protein expression. CD39 and CD73 protein expression in CD150+Treg cell was significantly higher than CD150-Treg cell, which confirmed the above results.

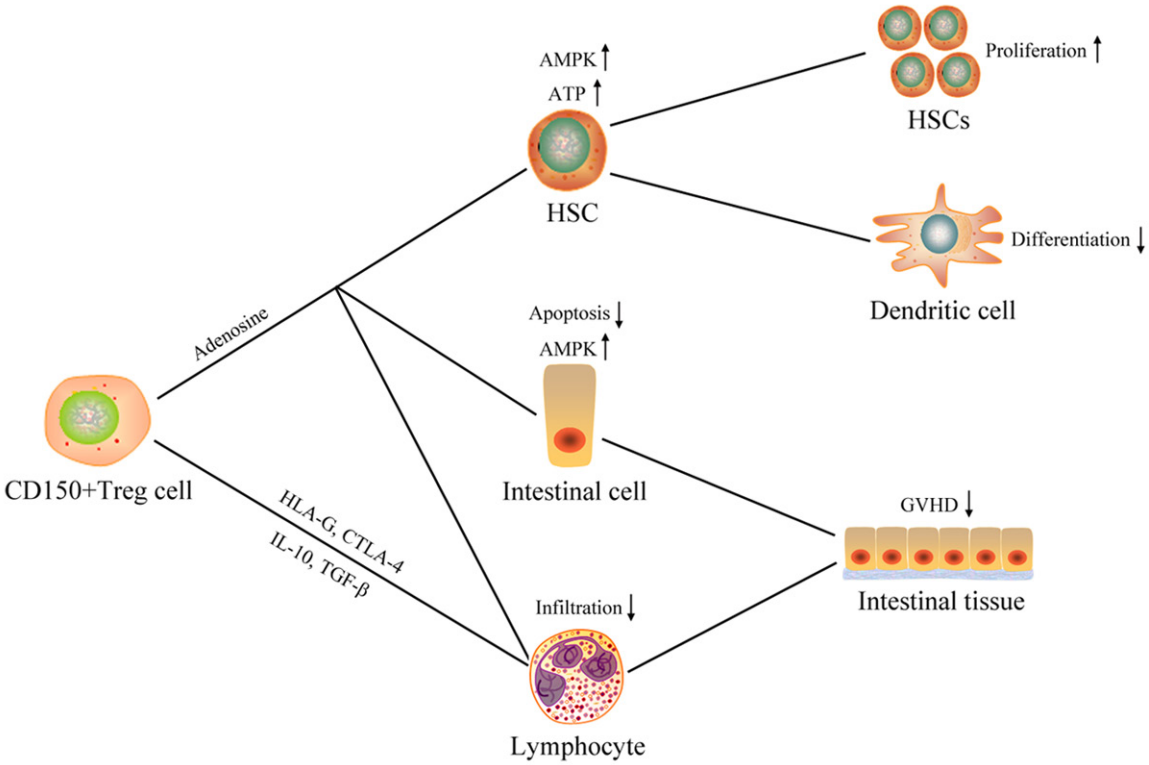

Figure 9. CD150^{high}Treg cell transplantation attenuates graft versus host disease after hematopoietic stem cell transplantation. CD150+Treg cells can regulate energy metabolism to inhibit the GVHD and intestinal cell apoptosis secondary to HSCs transplantation.

[18]. In addition, adenosine plays a key role in the maintenance of quiescent status of HSCs and it can regulate energy metabolism to maintain the functions of stem cells [7]. These results indicated that adenosine mediated the CD150+Treg related inhibition of GVHD. CD150+Treg cells could secret adenosine to activate AMPK expression and regulate energy metabolism, which promotes the proliferation of HSCs and maintain their stemness. Moreover, adenosine may protect cells against injury and inhibit inflammation. These findings revealed CD150+Treg cells could secret adenosine to inhibit the intestinal cell apoptosis secondary to HSCs transplantation, in which AMPK played an important role.

Taken together, CD150+Treg cells can regulate energy metabolism to inhibit the GVHD and intestinal cell apoptosis secondary to HSCs

transplantation (**Figure 9**). These findings provide theoretical evidence for the feasibility of combined transplantation with Treg cells and HSCs.

Disclosure of conflict of interest

None.

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References

[1] Cutler CS, Koreth J and Ritz J. Mechanistic approaches for the prevention and treatment of chronic GVHD. Blood 2017; 129: 22-29.

[2] Lu LF, Boldin MP, Chaudhry A, Lin LL, Taganov KD, Hanada T, Yoshimura A, Baltimore D and Rudensky AY. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. Cell 2010; 142: 914-929.

[3] Oldenhove G, Bouladoux N, Wohlfert EA, Hall JA, Chou D, Dos Santos L, O'Brien S, Blank R, Lamb E, Natarajan S, Kastenmayer R, Hunter C, Grigg ME and Belkaid Y. Decrease of Foxp3+ Treg cell number and acquisition of effector cell phenotype during lethal infection. Immunity 2009; 31: 772-786.

[4] Chopra M, Biehl M, Steinfatt T, Brandl A, Kums J, Amich J, Vaeth M, Kuen J, Holtappels R, Podlech J, Mottok A, Kraus S, Jordan-Garrote AL, Bauerlein CA, Brede C, Ribechini E, Fick A, Seher A, Polz J, Ottmuller KJ, Baker J, Nishikii H, Ritz M, Mattenheimer K, Schwinn S, Winter T, Schafer V, Krappmann S, Einsele H, Muller TD, Reddehase MJ, Lutz MB, Mannel DN, Berberich-Siebelt F, Wajant H and Beilhack A. Exogenous TNFR2 activation protects from acute GvHD via host T reg cell expansion. J Exp Med 2016; 213: 1881-1900.

[5] Kim N, Jeon YW, Nam YS, Lim JY, Im KI, Lee ES and Cho SG. Therapeutic potential of low-dose IL-2 in a chronic GVHD patient by in vivo expansion of regulatory T cells. Cytokine 2016; 78: 22-26.

[6] Leclerc M, Naserian S, Pilon C, Thiolat A, Martin GH, Pouchy C, Dominique C, Belkacemi Y, Charlotte F, Maury S, Salomon BL and Cohen JL. Control of GVHD by regulatory T cells depends on TNF produced by T cells and TNFR2 expressed by regulatory T cells. Blood 2016; 128: 1651-1659.

[7] Jing L, Tamplin OJ, Chen MJ, Deng Q, Patterson S, Kim PG, Durand EM, McNeil A, Green JM, Matsuura S, Ablain J, Brandt MK, Schlaeger TM, Huttenlocher A, Daley GQ, Ravid K and Zon LI. Adenosine signaling promotes hematopoietic stem and progenitor cell emergence. J Exp Med 2015; 212: 649-663.

[8] Yamazaki S, Ema H, Karlsson G, Yamaguchi T, Miyoshi H, Shioda S, Taketo MM, Karlsson S, Iwama A and Nakauchi H. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell 2011; 147: 1146-1158.

[9] Alho AC, Kim HT, Chammas MJ, Reynolds CG, Matos TR, Forcade E, Whangbo J, Nikiforow S, Cutler CS, Koreth J, Ho VT, Armand P, Antin JH, Alyea EP, Lacerda JF, Soiffer RJ and Ritz J. Unbalanced recovery of regulatory and effector T cells after allogeneic stem cell transplantation contributes to chronic GVHD. Blood 2016; 127: 646-657.

[10] Mielcarek M, Martin PJ, Leisenring W, Flowers ME, Maloney DG, Sandmaier BM, Maris MB and Storb R. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. Blood 2003; 102: 756-762.

[11] Hirata Y, Furuhashi K, Ishii H, Li HW, Pinho S, Ding L, Robson SC, Frenette PS and Fujisaki J. CD150(high) bone marrow tregs maintain hematopoietic stem cell quiescence and immune privilege via adenosine. Cell Stem Cell 2018; 22: 445-453, e445.

[12] Chopra M, Brandl A, Siegmund D, Mottok A, Schafer V, Biehl M, Kraus S, Bauerlein CA, Ritz M, Mattenheimer K, Schwinn S, Seher A, Grabinger T, Einsele H, Rosenwald A, Brunner T, Beilhack A and Wajant H. Blocking TWEAK-Fn14 interaction inhibits hematopoietic stem cell transplantation-induced intestinal cell death and reduces GVHD. Blood 2015; 126: 437-444.

[13] Carapito R, Jung N, Kwemou M, Untrau M, Michel S, Pichot A, Giacometti G, Macquin C, Ilias W, Morlon A, Kotova I, Apostolova P, Schmitt-Graeff A, Cesbron A, Gagne K, Oudshoorn M, van der Holt B, Labalette M, Spierings E, Picard C, Loiseau P, Tamouza R, Toubert A, Parissiadis A, Dubois V, Lafarge X, Maumy-Bertrand M, Bertrand F, Vago L, Ciceri F, Paillard C, Querol S, Sierra J, Fleischhauer K, Nagler A, Labopin M, Inoko H, von dem Borne PA, Kuball J, Ota M, Katsuyama Y, Michallet M, Lioure B, Peffault de Latour R, Blaise D, Cornelissen JJ, Yakoub-Agha I, Claas F, Moreau P, Milpied N, Charron D, Mohty M, Zeiser R, Socie G and Bahram S. Matching for the non-conventional MHC-I MICA gene significantly reduces the incidence of acute and chronic GVHD. Blood 2016; 128: 1979-1986.

[14] Lu Y, Hippen KL, Lemire AL, Gu J, Wang W, Ni X, Ranganathan P, Levine BL, Riley JL, June CH,

Turka LA, Munn DH, Garzon R, Lu L and Blazar BR. miR-146b antagomir-treated human Tregs acquire increased GVHD inhibitory potency. Blood 2016; 128: 1424-1435.

[15] McDonald-Hyman C, Flynn R, Panoskaltsis-Mortari A, Peterson N, MacDonald KP, Hill GR, Luznik L, Serody JS, Murphy WJ, Maillard I, Munn DH, Turka LA, Koreth J, Cutler CS, Soiffer RJ, Antin JH, Ritz J and Blazar BR. Therapeutic regulatory T-cell adoptive transfer ameliorates established murine chronic GVHD in a CXCR5-dependent manner. Blood 2016; 128: 1013-1017.

[16] Curtis RE, Metayer C, Rizzo JD, Socie G, Sobocinski KA, Flowers ME, Travis WD, Travis LB, Horowitz MM and Deeg HJ. Impact of chronic GVHD therapy on the development of squamous-cell cancers after hematopoietic stem-cell transplantation: an international case-control study. Blood 2005; 105: 3802-3811.

[17] Li Pira G, Di Cecca S, Montanari M, Moretta L and Manca F. Specific removal of alloreactive T-cells to prevent GvHD in hemopoietic stem cell transplantation: rationale, strategies and perspectives. Blood Rev 2016; 30: 297-307.

[18] Chen W, Wu Y, Li L, Yang M, Shen L, Liu G, Tan J, Zeng W and Zhu C. Adenosine accelerates the healing of diabetic ischemic ulcers by improving autophagy of endothelial progenitor cells grown on a biomaterial. Sci Rep 2015; 5: 11594.