Exosomes, PD-L1 and aGvHD: Perspectives for WJMSC-mediated Therapy

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Received date: March 08, 2021, Accepted date: March 29, 2021

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Commentary

Tumor-derived small extracellular vesicles or exosomes which carry the checkpoint PD-L1 are directly involved in immune evasion and uncontrolled tumor growth. We have recently reported that PD-L1 is also enriched on Wharton’s Jelly Mesenchymal Stromal Cell (WJMSC)-associated exosomes [1]. This biological property is likely associated with the physiologic role of WJMSCs at the maternal-fetal interface. These cells have been shown to protect the developing fetus from attack by the maternal immune system due to the foreign paternal antigens which the fetus express. In this manuscript, we review the important roles of exosome-enriched PD-L1 (exo-PD-L1) during WJMSC-mediated therapy for acute graft-versus-host disease (aGvHD). We focus on exo-PD-L1’s immunomodulation on T cell receptor (TCR) signaling in CD4+ T cells. In addition, we extend our discussions to other exosome-associated immune regulators, highlighting the potential to develop a cell-free, WJMSC-derived exosome therapy to treat complex immune diseases.

Exo-PD-L1 Underlies WJMSC-mediated aGvHD Therapy

Allogeneic hematopoietic stem cell transplantation (HSCT) provides a reliable treatment for various hematological malignancies [2]. Although the donor’s immune cells help eliminate the tumor cells (an effect of graft-versus leukemia, GVL), they also cause the serious complication of acute graft-versus-host disease (aGvHD) [3,4]. aGvHD occurs due to donor T cells recognizing and attacking the recipient’s non-malignant tissues, impacting the clinic application of HSCT for patients [2,5].

Immunomodulatory mesenchymal stem cells (MSCs) have been widely used to treat aGvHD and other immune diseases [6,7]. We recently reported the first investigator-initiated clinical trial investigating the safety and efficacy of WJMSC in steroid-refractory GvHD [8]. To date, there were at least 25 registered clinical trials for the exploration of the treatment of MSCs for aGvHD patients and 122 for other immunological diseases (http://clinicaltrials.gov/; accessed February 2021). One of the important molecular mechanisms lies in the immune regulatory checkpoint PD-L1. It is well known that checkpoint PD-L1 signal suppresses the T cell activation mediated by T cell receptors (TCRs) [9,10]. By way of in vivo studies, Kim et al. showed that transplantation of tonsil-derived-MSCs into imiquimod-induced psoriatic skin inflammation in mice significantly abrogated disease symptoms, mainly by blunting the Th17 response in a PD-L1-dependent manner [11]. Furthermore, Nie et al. demonstrated that human MSCs alleviated the pulmonary fibrosis by the PD-L1/PD1 signaling pathways [12]. In the bleomycin-induced pulmonary fibrosis model, abnormal PD1 expression in circulating T cells and lung tissues of patients with pulmonary fibrosis was observed [12].

Biologically, MSC-associated PD-L1 may interact with its receptor PD1 on the surface of T cells, leading to the exhaustion of T cells pathologically involved in the development of aGvHD. In aGvHD patients, Erkers et al. reported that decidual stromal cells have cell-cell
contact in order to suppress lymphocytes and blocking PD-L1 impaired their antiproliferative ability [13]. In liver transplant recipients diagnosed with aGVHD, higher PD1 expression was observed on donor CD4 and CD8 T cells. Blocking PD-L1 on the host-derived cells significantly enhanced alloreactivity by CD8 T cells in vivo [14]. This finding suggests that augmenting PD1/PD-L1 pathway may be a therapeutic strategy to control graft-versus-host-reactive T cells. In a mouse model of aGVHD, Tang et al. reported that systemic overexpression of PD-L1 inhibits the donor T cells activation, effector memory status, as well as Th1 and Th17 cells’ responses in vivo [15]. Furthermore, they demonstrated that PD-L1 Ig treatment led to decreased T cells proliferation, promoted apoptosis, and reduced pro-inflammatory cytokine expression by effector T cells in vivo, in the setting of anti-CD3/CD28 stimulation and allogeneic dendritic cell stimulation [15]. These studies suggest that MSCs provide the regulatory checkpoints necessary to target pathological CD4+ T cells during stem cell transplantation treatments.

Extracellular vesicles (EVs) include a heterogeneous group of lipid bilayer membranous structures released from their host cells including mesenchymal stem cells (MSCs). Small EVs (sEVs, also referred to as exosomes) usually have an average size between 30-150 nm [16]. It has been suggested that exosomes are effective at delivering many types of bioactive molecules, including mRNAs, miRNAs, lncRNAs, lipids, and proteins to a variety of target cells and that they can actively regulate the target cells even in organs distant from the cell of origin [17].

Meanwhile, more and more studies also suggest that tumor exosomal PD-L1 contributes to immunosuppression and is a valuable target for therapy and diagnosis. For instance, Cheng et al. reported that metastatic melanoma-derived exosomal PD-L1 is a rational predictor for patients and suppressed the function of CD8 T cells related to tumor growth [18]. In non-small cell lung cancer, Kim et al. reported that exosomal PD-L1 promotes tumor growth through immune escape [19]. Very similar observations were obtained in the studies of head and neck cancer [20] and gastric cancer [21]. Poggio et al. suggested that suppression of tumor exosomal PD-L1 may induce complex anti-tumor immunity and memory [22]. Therefore, both WJMSC-derived and tumor-associated exosomes which carry PD-L1 demonstrate the specific capability to target T cells through intervening with signaling pathways related to TCRs’ functions.

Like tumor-derived exosomes, recent studies have suggested that that MSC-derived exosomes can affect CD4+ and CD8+ T cells in the aGVHD patients. Lai et al. reported that MSC exosomes effectively prolonged the survival of chronic GVHD mice and diminished the clinical and pathological scores [23]. They observed that activated CD4 T cells and their infiltration into the lung were reduced in these animals. Wang et al. reported that WJMSC-EVs alleviated the in vivo manifestations of aGVHD and the associated histologic changes and significantly reduced the mortality of the recipient mice [21]. They also found that recipients treated with hUC-MSC-EVs had significantly lower frequencies and absolute numbers of CD3+CD8+ T cells. Similar findings were obtained in aGVHD mouse model. Fuji et al. reported that the systemic infusion of human bone marrow MSC EVs prolonged the survival of mice with aGVHD and reduced the pathologic damage in multiple GVHD-targeted organs [24]. They reported that both CD4+ and CD8+ T cells were suppressed in EV-treated GVHD mice. As shown in Figure 1, we have recently reported that WJMSC sEVs suppress activated CD4+ T cells that were stimulated with anti-CD3/CD28 beads [1]. This observation suggests that WJMSC sEVs intervened on the CD4+ T cell activation and we have shown that multiple inhibitory checkpoints exist on WJMSC sEVs.

We identified PD-L1 enriched on this WJMSC sEVs as one of the key regulators for suppressing T cells [1]. We observed that exo-PD-L1 from WJMSCs demonstrated some unique features. First, exo-PD-L1 is a membrane-bound protein even though exosomes themselves are very diffusible; second, PD-L1 is highly enriched on the sEVs and preserves its bioactive glycosylation as previously reported [25]; and third, exo-PD-L1 is inducible through licensing WJMSCs by proinflammatory cytokines, such as interferon-gamma (IFNγ). These features support that exosomes may provide a functional form of PD-L1 that can be isolated to create a cell-free therapy. Exosomal PD-L1 is also likely superior to a recombinant PD-L1 for its ability to persist in vivo, partially due to co-expression of CD47 on sEVs [26, 27], and home to various inflammatory sites, although the mechanism behind this ability is poorly characterized [26]. Consistent with the above findings from tumor exosomes, we found that WJMSC-derived sEVs also rely on PD-L1 to enhance the exhaustion of activated CD4+ T cells. Both pharmacic blocking of PD-L1 protein and genetic disrupting of the PD-L1 gene can dramatically impair WJMSC sEV-mediated inhibition of CD4+ T cells. More importantly, WJMSC exo-PD-L1 seems more efficient to block the activation of CD4+ T cells than both soluble PD-L1 or cell surface PD-L1. These observations support the concept that therapeutic WJMSCs provide other exosome-derived checkpoint signals which synergize with the exo-PD-L1 to regulate the immune activities in aGVHD patients.

Kordelas et al. reported that MSC EVs reduced the proinflammatory cytokine response of patient’s PBMCs and the clinical GVHD symptoms during the course of MSC EVs therapy [28]. In support, we observed a rapid increase of peripheral plasma exo-PD-L1 in aGVHD patients shortly after infusing with therapeutic WJMSCs [1]. These
findings suggest that increased levels of exo-PD-L1 are correlated with the therapeutic infusion of WJMSCs. In aGvHD patients, we hypothesize that licensing WJMSCs is a necessary step to induce high levels of exo-PD-L1, further contributing to the “pool” of exo-PD-L1 in patient blood (Figure 1). However, we also noticed that the distribution of exo-PD-L1 in patients’ circulation system is completely dependent on the subsequent cell uptake from other blood cells. Thus, our group’s study as well as other groups’ studies provide strong evidence to support that exo-PD-L1 is essential for WJMSC-mediated therapy of aGvHD in acute AML patients after HSCT.

**Perspectives: Other Exo-regulators underlay WJMSC-mediated Therapy**

Beside checkpoints (PD-L1, PD-L2 and CD276), exosomal markers (CD9, CD63, CD81, and HSP70), stem cell markers (CD73, CD105 and CD90) and other antigens (CD24, CD44, CD151 and CD248) are enriched on WJMSC exosomes [1,29]. Their potential roles remain to be further determined. For instance, antigen CD73, recognized as a stem cell marker owns the enzyme activity of 5'-nucleotidase. Clayton et al. reported that exosomes from diverse cancer cell types exhibited potent ATP- and 5' AMP-phosphohydrolytic activity [30]. They found that these exosomes can perform hydrolytic steps sequentially to form adenosine from ATP, triggering a cAMP response in adenosine A (2A) receptor-positive but not A (2A) receptor-negative cells [30]. Ludwig et al. reported that tumor exosomes carried enzymatically active CD39/CD73 and adenosine [31]. They found that tumor exosomes promoted A2BR-mediated polarization of macrophages toward an M2-like phenotype and enhanced their secretion of angiogenic factors [31]. Theodoraki et al. reported the highest level of CD39/CD73 ectoenzymes of adenosine...
Li M, Abdelhakim H, Braun MW, Godwin AK. Exosomes, PD-L1 and aGVHD: Perspectives for WJMSC-mediated Therapy. J Cancer Immunol. 2021; 3(1): 98-103.

production was found in CD3 (-) exosomes in patients with advanced-stage (III/IV) head and neck squamous cell carcinoma. Also, the production of 5’-AMP and purines was significantly higher inTreg co-incubated with CD3 (-) than CD3 (+) exosomes [32].

WJSMC exosomes were defined as positive for CD9, CD63, and CD73 [33]. Cytokine TNF-α stimulation not only increased the amount of exosome secreted from gingiva-derived MSCs but also enhanced the exosomal expression of CD73 [34]. Bone marrow-derived MSC recipients had increased serum CD73 expressing exosomes that promoted adenosine accumulation ex vivo [18]. Using exosomes that were isolated from HPV-16 E6/E7 transformed human bone marrow stromal cells, Hettich et al. found that both CD73 and constitutional lipids on these exosomes are identified as key bioactive components promoting the exosome-driven acceleration of processes required for wound repair [35]. Using chondrocyte cultures, Zhang et al. reported the rapid cellular proliferation and infiltration during exosome-mediated cartilage repair is due to exosomal CD73-mediated adenosine activation of AKT and ERK signaling [36]. Chew et al. found that MSC exosomes could increase bone and periodontal ligament cell migration and proliferation through CD73-mediated adenosine receptor activation of pro-survival AKT and ERK signaling [37]. Accordingly, WJMSC exo-CD73 may inhibit T cells through adenosine signal pathways. However, the detailed mechanism needs to be further characterized by either molecular or pharmaceutical methods.

In summary, exosome-associated proteins derived from therapeutic WJMSCs can provide important regulatory signals to impact adaptive immune activities. Further, identifying and characterizing these exosomal immune regulators will help us gain deeper insights into the therapeutic value of WJMSCs and WJMSC-derived exosomes in the treatment of complex human immune diseases. Finding the balance between inhibiting alloreactive T-cells mediating aGVHD without impairing the GvL effect and tumor elimination is a complex issue when it comes to leveraging immunosuppressive treatments after HSCT. However, we believe it is possible to find this balance through the proper timing and dosing of the WJMSC-associated seVs. Critically, these small nano-size particles may be very helpful in developing novel cell-free therapies for many patient populations.

References

1. Li M, Soder R, Abhyankar S, Abdelhakim H, Braun MW, Trinidad CV, Pathak HB, Pessetto Z, Deighan C, Ganguly S, Dawn B. WJMSC-derived small extracellular vesicle enhance T cell suppression through PD-L1. Journal of Extracellular Vesicles. 2021 Feb;10(4):e12067.

2. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nature Reviews Immunology. 2012 Jun;12(6):443-58.

3. Zeiser R, Blazar BR. Acute graft-versus-host disease—biologic process, prevention, and therapy. New England Journal of Medicine. 2017 Nov 30;377(22):2167-79.

4. Kolb HJ, Mittermüller J, Clemm CH, Holler E, Ledderose G, Brehm G, et al. Donor leukocyte transusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood. 1990 Dec 15;76(12):2462-5.

5. Ratanatharthorn V, Ayash L, Lazarus HM, Fu J, Uberti JP. Chronic graft-versus-host disease: clinical manifestation and therapy. Bone Marrow Transplantation. 2001 Jul;28(2):121-9.

6. Kebrlaiai P, Hayes J, Daly A, Uberti J, Marks DI, Soiffer R, et al. A phase 3 randomized study of remestemcel-L versus placebo added to second-line therapy in patients with steroid-refractory acute graft-versus-host disease. Biology of Blood and Marrow Transplantation. 2020 May 1;26(5):835-44.

7. Kurtzberg J, Prockop S, Teira P, Bittencourt H, Lewis V, Chan KW, et al. Allogeneic human mesenchymal stem cell therapy (remestemcel-L, Prochymal) as a rescue agent for severe refractory acute graft-versus-host disease in pediatric patients. Biology of Blood and Marrow Transplantation. 2014 Feb 1;20(2):229-35.

8. Soder RP, Dawn B, Weiss ML, Dunavin N, Weir S, Mitchell J, et al. A phase I study to evaluate two doses of Wharton’s jelly-derived mesenchymal stromal cells for the treatment of de novo high-risk or steroid-refractory acute graft versus host disease. Stem Cell Reviews and Reports. 2020 Oct;16(5):979-91.

9. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity. 2007 Jul 27;27(1):111-22.

10. Keir ME, Freeman GJ, Sharpe AH. PD-1 regulates self-reactive CD8+ T cell responses to antigen in lymph nodes and tissues. The Journal of Immunology. 2007 Oct 15;179(8):5064-70.

11. Kim JY, Park M, Kim YH, Ryu KH, Lee KH, Cho KA, et al. Tonsil-derived mesenchymal stem cells (T-MSCs) prevent Th17-mediated autoimmune response via regulation of the programmed death-1/programmed death ligand-1 (PD-1/PD-L1) pathway. Journal of tissue engineering and regenerative medicine. 2018 Feb;12(2):e1022-33.

12. Ni K, Liu M, Zheng J, Wen L, Chen Q, Xiang Z, et al. PD-1/PD-L1 pathway mediates the alleviation of
pulmonary fibrosis by human mesenchymal stem cells in humanized mice. American Journal of Respiratory Cell and Molecular Biology. 2018 Jun;58(6):684-95.

13. Erkers T, Nava S, Yosef J, Ringdén O, Kaipe H. Decidual stromal cells promote regulatory T cells and suppress alloreactivity in a cell contact-dependent manner. Stem Cells and Development. 2013 Oct 1;22(19):2596-605.

14. Schuchmann M, Meyer RG, Distler E, Von Stebut E, Kuball J, Schnürer E, et al. The programmed death (PD)-1/PD-ligand 1 pathway regulates graft-versus-host-reactive CD8 T cells after liver transplantation. American Journal of Transplantation. 2008 Nov;8(11):2434-44.

15. Tang L, Ma S, Gong H, Wang J, Xu Y, Wu D, et al. PD-L1 ameliorates murine acute graft-versus-host disease by suppressing effector but not regulatory T cells function. Archivum Immunologiae et Therapiae Experimentalis. 2019 Jun;67(3):179-87.

16. Amarnath S, Foley JE, Farthing DE, Gress RE, Laurence A, Eckhaus MA, et al. Bone Marrow-Derived Mesenchymal Stromal Cells Harness Purinergic Signaling to Tolerize Human T h1 Cells In Vivo. Stem Cells. 2015 Apr;33(4):1200-12.

17. Kim DH, Kim H, Choi YJ, Kim SY, Lee JE, Sung KJ, et al. Exosomal PD-L1 promotes tumor growth through immune escape in non-small cell lung cancer. Experimental & Molecular Medicine. 2019 Aug;51(8):1-3.

18. Theodoraki MN, Yerneni SS, Hoffmann TK, Gooding WE, Whiteside TL. Clinical significance of PD-L1+ exosomes in plasma of head and neck cancer patients. Clinical Cancer Research. 2018 Feb 15;24(4):896-905.

19. Wang L, Gu Z, Zhao X, Yang N, Wang F, Deng A, et al. Extracellular vesicles released from human umbilical cord-derived mesenchymal stromal cells prevent life-threatening acute graft-versus-host disease in a mouse model of allogeneic hematopoietic stem cell transplantation. Stem Cells and Development. 2016 Dec 15;25(24):1874-83.

20. Poggio M, Hu T, Pai CC, Chu B, Belair CD, Chang A, et al. Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. Cell. 2019 Apr 4;177(2):414-27.

21. Lai P, Chen X, Guo L, Wang Y, Liu X, Liu Y, et al. A potent immunomodulatory role of exosomes derived from mesenchymal stromal cells in preventing cGVHD. Journal of Hematology & Oncology. 2018 Dec;11(1):1-5.

22. Fujii S, Miura Y, Fujishiro A, Shindo T, Shimazu Y, Hirai H, et al. Graft-versus-host disease amelioration by human bone marrow mesenchymal stromal/stem cell-derived extracellular vesicles is associated with peripheral preservation of naive T cell populations. Stem Cells. 2018 Mar;36(3):434-45.

23. Strauch V, Saul D, Berisha M, Mackensen A, Mougiakakos D, Jitschin R. N-glycosylation controls inflammatory licensing-triggered PD-L1 upregulation in human mesenchymal stromal cells. Stem Cells. 2020 Aug;38(8):986-93.

24. Kaur S, Singh SP, El Khaliloung AG, Wu W, Abu-Asab MS, Roberts DD. CD47-dependent immunomodulatory and angiogenic activities of extracellular vesicles produced by T cells. Matrix Biology. 2014 Jul 1;37:49-59.

25. Lian S, Xie X, Lu Y, Jia L. Checkpoint CD47 function on tumor metastasis and immune therapy. OncoTargets and Therapy. 2019;12:9105.

26. Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doepnner TR, et al. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. Leukemia. 2014 Apr;28(4):970-3.

27. Gomzikova MO, James V, Rizvanov AA. Therapeutic application of mesenchymal stem cells derived extracellular vesicles for immunomodulation. Frontiers in Immunology. 2019 Nov 15;10:2663.

28. Clayton A, Al-Taei S, Webber J, Mason MD, Tabi Z. Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production. The Journal of Immunology. 2011 Jul 15;187(2):676-83.

29. Ludwig N, Yerneni SS, Azambuja JH, Gillespie DG, Menshikova EV, Jackson EK, et al. Tumor-derived exosomes promote angiogenesis via adenosine A 2B receptor signaling. Angiogenesis. 2020 Nov 1:1-2.

30. Theodoraki MN, Hoffmann TK, Jackson EK, Whiteside TL. Exosomes in HNSCC plasma as surrogate markers of tumour progression and immune competence. Clinical & Experimental Immunology. 2018 Oct;194(1):67-78.

31. Monguió-Tortajada M, Roura S, Gálvez-Montón C, Pujal JM, Aran G, Sanjurjo L. Nanosized UCMSC-derived extracellular vesicles but not conditioned medium exclusively inhibit the inflammatory response of stimulated T cells: implications for nanomedicine. Theranostics. 2017;7(2):270.

32. Nakao Y, Fukuda T, Zhang Q, Sanui T, Shinjo T, Kout X, et al. Exosomes from TNFα-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss. Acta Biomaterialia. 2021 Mar 1;122:306-24.
33. Hettich BF, Ben-Yehuda Greenwald M, Werner S, Leroux JC. Exosomes for Wound Healing: Purification Optimization and Identification of Bioactive Components. Advanced Science. 2020 Dec;7(23):2002596.

34. Zhang S, Chuah SJ, Lai RC, Hui JH, Lim SK, Toh WS. MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity. Biomaterials. 2018 Feb 1;156:16-27.

35. Chew JR, Chuah SJ, Teo KY, Zhang S, Lai RC, Fu JH, et al. Mesenchymal stem cell exosomes enhance periodontal ligament cell functions and promote periodontal regeneration. Acta Biomaterialia. 2019 Apr 15;89:252-64.