Overexpression of PD-L1 in mesenchymal stromal/ stem cells and their derived exosomes improve the immunomodulatory properties and therapeutic effects in nonobese diabetic mice

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Background

Type 1 diabetes (T1D): is an autoimmune disease, results from the destruction of insulin-producing β cells targeted by autoreactive T cells.

Mesenchymal stromal/ stem cells (MSCs): MSCs are adult multipotent stem cells that can be isolated from different tissues such as bone marrow adipose tissue, and umbilical cord. MSCs and their secreted exosomes (exo) have been reported to regulate the immune response in many diseases.

PD-L1: is a protein that plays a crucial role in regulating the immune response. The interaction between PD-L1 and PD-1 serves as a regulatory mechanism to prevent excessive immune responses and immune-mediated damage to healthy tissues.

Hypothesis

We hypothesize that overexpression of PD-L1 on MSCs and their exosomes will increase their immunomodulatory effect and can maximize the therapeutic effect of MSCs against Type 1 diabetes.

Objectives

Evaluate the immunomodulatory properties of PD-L1 overexpressed MSC (**PDL1-MSC**) and their derived exosomes (**Pexo**) in vitro and in vivo against nonobese diabetic mice (NOD) as a model for T1D.

Study Design

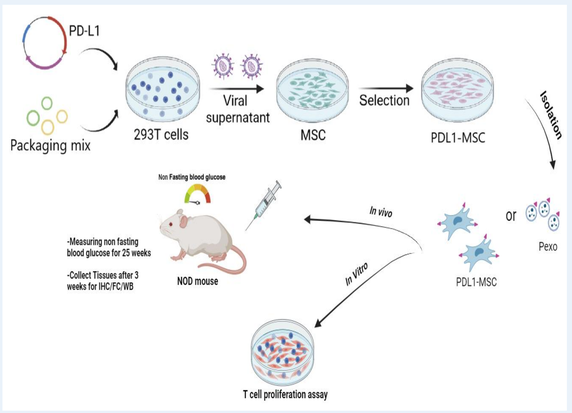


Fig 1. Illustration of the study design starting with the generation of PDL1-MSCs and Pexo, then the isolation and characterization steps, then the in vivo injection in NOD mice, and finally the T cell proliferation assay and coculture in vitro.

Results

Generation of PDL1-MSCs by lentiviral transduction

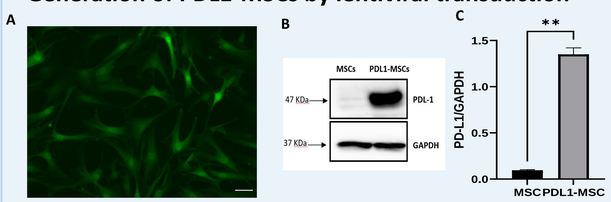
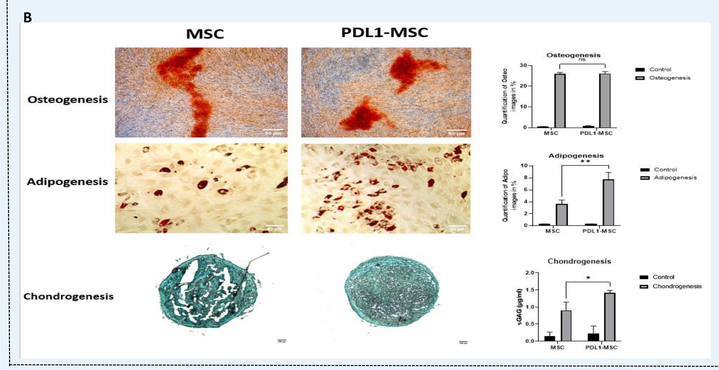
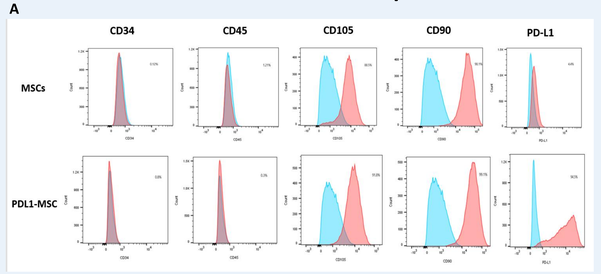


Fig 2. Generation of PDL1-MSCs. (A) Fluorescent image of PDL1-MSCs tagged with GFP, scale bar 100 um. (B) Western blot analysis of PD-L1 in normal MSCs and PDL-MSCs. (C) Quantification of B.



Characterization of PDL1-MSCs/Pexo



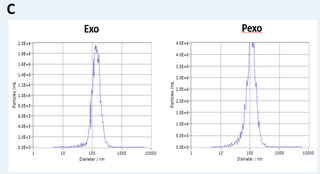
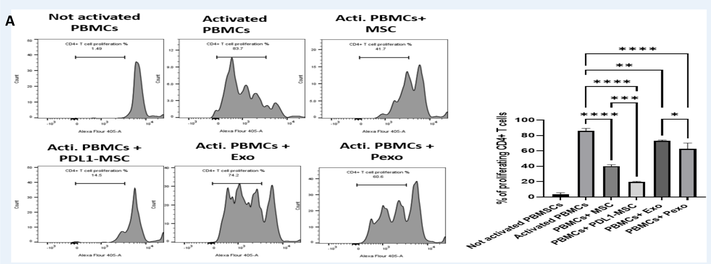
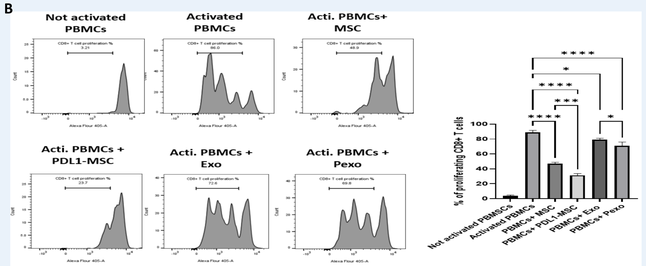


Fig 3. Characterization of PDL1-MSC and Pexo. (A) Immunophenotypic characterization by flow cytometry for both MSC and PDL1-MSC. (B) Differentiation capability of both MSC and PDL1-MSC for osteogenesis, adipogenesis, and chondrogenesis stained by alizarin red, oil red o, and alcian blue respectively. Scale bar 50 um (n = 3). (C) Characterization of Exo and Pexo by Nanoparticle Tracking Analysis (NTA). (D) MTT proliferation assay to analyze the MSC and PDL1-MSC proliferation rate (n = 3). Statistical analysis was performed using two-way ANOVA (**P < .01, ***P < .001, ****P <)

PDL1-MSC and Pexo suppress CD4+/CD8+ T cell and increase the frequency of Treg cells





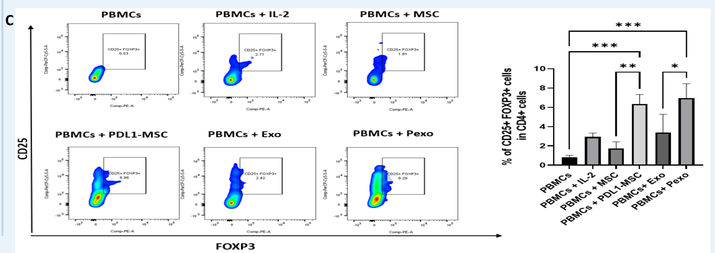
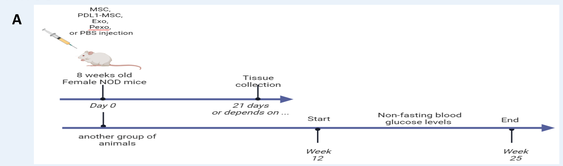
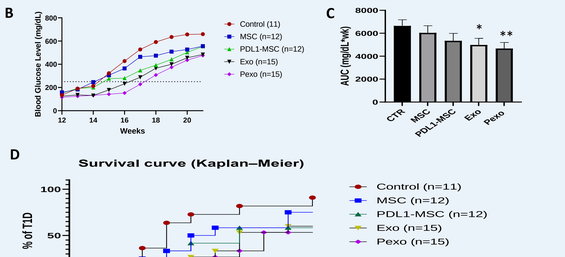


Fig 4. In vitro T cell proliferation assay and PBMC/MSC coculture. (A) In vitro CD4+ T cell proliferation as measured by Cell Trace dilution followed by Quantitative analysis of proliferated CD4+ T cell percentage shown in (n = 3). (B) In vitro CD8+ T cell proliferation as measured by Cell Trace dilution followed by Quantitative analysis of proliferated CD8+ T cell percentage shown in (n = 3). (C) PDL1-MSC/Pexo + PBMC cocultures increase the percentage of Treg cells after 7 days (n = 3). The MSC/PDL1-MSC used in all in vitro assays is 1:5 PBMC. The Exo/Pexo concentration is 160ug/ml. Statistical analysis was performed using two-way ANOVA (**P < .01, ***P < .001, ****P <)

Results

PDL1-MSC and Pexo delayed the onset of T1D in NOD mice.





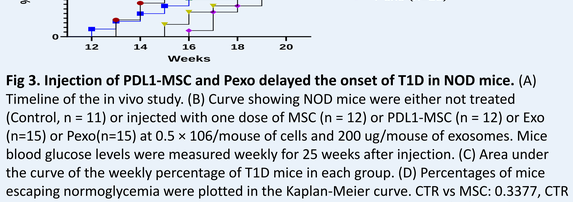
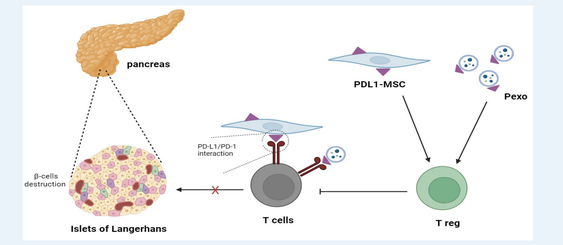


Fig 3. Injection of PDL1-MSC and Pexo delayed the onset of T1D in NOD mice. (A) Timeline of the in vivo study. (B) Curve showing NOD mice were either not treated (Control, n = 11) or injected with one dose of MSC (n = 12) or PDL1-MSC (n = 12) or Exo (n=15) or Pexo(n=15) at 0.5 × 106/mouse of cells and 200 ug/mouse of exosomes. Mice blood glucose levels were measured weekly for 25 weeks after injection. (C) Area under the curve of the weekly percentage of T1D mice in each group. (D) Percentages of mice escaping normoglycemia were plotted in the Kaplan-Meier curve. CTR vs MSC: 0.3377, CTR vs PDL1-MSC: P = 0.1710, CTR vs Exo: P = 0.0261, CTR vs Pex: P= 0.0052 by logrank test.

Summary



-PDL1-MSCs showed improved proliferation and differentiation ability compared to MSCs.
-PDL1-MSC/Pexo inhibit activated CD4+/CD8+ T cells proliferation.
-PDL1-MSC/Pexo increase the frequency of Treg.
-PDL1-MSC/Pexo delay the onset of T1D in NOD mice.

Acknowledgment

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