Molecular Mechanisms of Immunoregulatory Function of DPSCs

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Contributor: Qi Min , Liqiong Yang , Hua Tian , Lu Tang , Zhangang Xiao , Jing Shen

Dental pulp stem cells (DPSCs) are mesenchymal stem cells (MSCs) derived from dental pulp tissue, which have high self-renewal ability and multi-lineage differentiation potential. With the discovery of the immunoregulatory ability of stem cells, DPSCs have attracted much attention because they have similar or even better immunomodulatory effects than MSCs from other sources. DPSCs and their exosomes can exert an immunomodulatory ability by acting on target immune cells to regulate cytokines. DPSCs can also migrate to the lesion site to differentiate into target cells to repair the injured tissue, and play an important role in tissue regeneration.

dental pulp stem cells immunoregulatory mechanisms immune-mediated diseases

1. Soluble Factors Secreted by DPSCs

1.1. Transforming Growth Factor Beta (TGF-β) Is a Major Soluble Factor Mediating Immune Tolerance by Dental pulp stem cells (DPSCs)

In a study by Ding et al., through examining the key soluble factors mediating the immunosuppressive function of DPSCs, it was found that TGF-β1 was significantly up-regulated after DPSCs were co-cultured with peripheral blood mononuclear cells (PBMCs) and phytohemagglutinin (PHA) [1]. Moreover, the anti-TGF-β1 monoclonal antibody could restore the proliferation of T cells inhibited by DPSCs, indicating that TGF-β1 is essential in the process of DPSC-mediated immune regulation. Its down-regulation may lead to the inhibition of the immunoregulatory function of DPSCs [1]. This finding was also supported by an investigation by Tomic et al. [2]. In later study, it was found that TGF-β secreted by DPSCs was a major contributor to immunosuppression induced by DPSCs in acute allogeneic immune responses [3]. TGF-β completely abrogated the production of IgM and IgG by allogeneic activation of responder B lymphocytes. Numerous studies have shown that TGF-β secreted by DPSCs can increase $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) [4] and inhibit the proliferation of allogeneic lymphocytes [5].

1.2. Other Soluble Factors

Mesenchymal stem cells secrete many soluble molecules with immunomodulatory effects and act on immune cells. Following contents summarized the immunomodulatory soluble factors secreted by DPSCs from a large number of studies, including interleukin (IL)-6, IL-10, IL-13, IL-29, tumor necrosis factor (TNF-α), macrophage colony

stimulating factor (M-CSF), HLA-G, intercellular cell adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM-1), insulin growth factor (IGF)-1, granulocyte macrophage colony stimulating factor (GM-CSF), adiponectin, keratinocyte growth factor, hepatocyte growth factor (HGF), stem cell factor (SCF), vascular endothelial growth factor (VEGF), nitric oxide (NO), and prostaglandin E2 (PGE2) [5][6][7][8][9][10]. These soluble factors are involved in the differentiation and recruitment of lymphocytes and macrophages to varying degrees, as well as the regulation of other related cells, and have made a huge contribution to the immunomodulatory capacity of DPSCs (**Table 1**).

Table 1. Effects of soluble factors secreted by DPSCs on target cells.

Soluble Factors	Target Cells	Mediated Effects	References
TGF-β1, IL-6, IL-10, IL-29, IL-13, HLA-G, IDO, ICAM-1, VCAM-1, HGF, VEGF, NO, PGE2	Lymphocyte	Inhibit lymphocyte proliferation	[5][6][8]
TGF-β1, IL-29, TNF-α, HGF, IDO	Macrophage	Inhibit macrophage function	[8][11][12]
IL-6, IL-10, IL-13, M-CSF, IGF-1, GM-CSF, PGE2	Macrophage	Induce m1 differentiation into m2	[8][9][13]
HGF	Hepatocytes	Promotes hepatocyte regeneration	[14]
SCF, VEGF	Endothelial cells	Promote neo-vascularization	[9][15]

TGF-β1: transforming growth factor beta 1; IL: interleukin; HLA-G: human leukocyte antigen; IDO: indoleamine-2,3-dioxygenase; ICAM-1: intercellular cell adhesion molecule; VCAM-1: vascular cell adhesion molecule; HGF: hepatocyte growth factor; VEGF: vascular endothelial growth factor; NO, nitric oxide; PGE2: prostaglandin E2; TNF-α: tumor necrosis factor; M-CSF: macrophage-colony stimulating factor; IGF-1: insulin growth factor-1; GM-CSF: granulocyte macrophage colony-stimulating factor; SCF: stem cell factor.

2. Role of Indoleamine 2,3-Dioxygenase (IDO)

IDO is a heme-containing cytosolic enzyme that acts as a rate-limiting catalyst in the metabolism of an essential amino acid (tryptophan) of the canine uric acid pathway [11]. IDO degrades the essential amino acid tryptophan into kynurenine, which leads to tryptophan depletion, resulting in the suppression of T-cell proliferation or induction of apoptosis in activated T cells and consequently the induction of tolerance [16]. It has already been reported that the immunosuppressive activity of DPSCs is abolished after the inhibition of IDO-1 expression [2]. In another report, IDO was expressed in DPSCs co-cultured with allogeneic PBMCs and inhibited the proliferation of allogeneic PBMCs for immune regulation [5]. In addition, IDO has also been demonstrated to mediate the inhibitory effect of DPSCs on macrophages. Lee et al. showed that the IDO expression level in DPSCs increased with lipopolysaccharide (LPS) or TNF-α stimulation in a time-dependent manner. The results of co-culture experiments showed that DPSCs can inhibit TNF-α secreted by LPS-triggered macrophages via an IDO-dependent mechanism for immune regulation [11].

3. Role of Fas/FasL

Activation of the Fas/FasL pathway typically occurs following exposure to an inflammatory microenvironment and induces T cell apoptosis [17]. Mechanistically, upon the binding of FasL to the Fas receptor (CD95), the extrinsic apoptotic pathway is activated, with Pro-Caspase 8 and Fas-associated death domain (FADD) being recruited to form the death-inducing signaling complex (DISC), in which Pro-Caspase 8 undergoes activation. Then, Caspase 8 leaves the DISC, activates caspase 3/7 and induces apoptosis. Alternatively, c-FLIP, a protease-deficient caspase homolog, can interact with FADD and act as an apoptosis inhibitor [18]. MSC-mediated immunotherapy has been found to be associated with FasL expression, and FasL-expressing MSCs are able to induce apoptosis to trigger immune tolerance [19]. Similarly, DPSCs can suppress the activation of allogeneic T lymphocytes by Fas/Fas ligand (Fas/FasL) interaction and the up-regulation of Tregs [20]. In addition, DPSCs improve disease symptoms by expressing FasL in a variety of diseases; for example, knockdown of FasL in DPSCs leads to a reduced ability to improve the colitis phenotype, indicating that FasL is required for DPSC-mediated immune regulation [20]. In another study, DPSCs were able to modulate $CD4^+$ T lymphocyte responses in monocytes from patients with primary Sjögren's syndrome (pSS) by increasing Fas ligand expression [21].

4. Role of Programmed Cell Death(PD)-1/PD-L1

The PD-1 pathway plays an important role in maintaining central and peripheral immune tolerance [22]. PD-L1 binds to the receptor PD-1 on activated T cells and suppresses antitumor immunity by counteracting T cell-activating signals. Currently, a blockade of PD-1 has been identified as a promising immunotherapeutic approach for cancer and chronic infectious diseases [23]. Previously, it has been found that not only DPSCs express PD-L1 and PD-1, but also PD-1 is important to maintain stem cell properties in hDPSCs [24]. DPSCs can modulate the inflammatory microenvironment by activating PD-1/PD-L1 immunomodulation [25]. When exposed to CD3/CD28-costimulated PBMCs, DPSCs were able to up-regulate PD-L1 through both direct and indirect interaction-dependent mechanisms to suppress immune responses [25]. Pignatti et al. showed that PD-L1 expression was increased in DPSCs after stimulation with activated PBMCs and was involved in the regulation of the immune response, resulting in the increased expression of cleaved caspase3 and decreased expression of IL-2 in PBMCs [26]. The PD-1/PD-L1 signaling pathway is widely involved in a series of processes such as the activation, proliferation, and apoptosis of T cells and inhibits T cell-mediated cellular immune responses [27][28][29]. Further study of the PD-1/PD-L1 signaling pathway may contribute to the clinical application of DPSCs in immune-mediated diseases.

5. Role of Toll-Like Receptor (TLR) 4

TLR4 is expressed in the odontoblastic cell layer and in areas that associate with blood vessels. When TLR4 is activated, it can regulate the proliferation and migration of DPSCs in deep caries, and it is believed that TLR4 may play an important role in the immune response of DPSCs [30]. The immunomodulatory properties of DPSCs are mentioned to be susceptible to TLR receptor activation, according to a study by Tomic et al. [2]. For example, when DPSCs were exposed to LPS, DPSCs showed enhanced TLR4 expression, mediated increased expression of the anti-inflammatory factor IL-8 through the TLR4 pathway [31], and promoted Wnt5a expression through the TLR4/MyD88/PI3-kinase/AKT pathway [32]. A recent study reported that dental pulp stem cells-derived exosomes

(DPSC-exo) could ameliorate cerebral ischemia-reperfusion-induced brain injury in mice, and its anti-inflammatory mechanism may be related to the inhibition of the HMGB1/TLR4/MyD88/NF-κB pathway [33].

6. Role of PGE2

PGE2 is one of the major effectors of MSC-mediated immunosuppression [34]. PGE2 is a catabolite of arachidonic acid, and during inflammation, PGE2 is thought to act as an anti-inflammatory agent, modulating the inflammatory response and helping to restore tissue homeostasis [35]. PGE2 is an important regulatory molecule that normally synergizes with other immunomodulatory factors such as inducible nitric oxide synthase (iNOS) or IDO to inhibit the proliferation of immune cells and the production of inflammatory factors [36]. DPSCs inhibit the proliferation of allogeneic PBMCs by secreting cytokines such as PGE2 and IL-6 [5]. When stimulated by TNF-α or IL-1β, MSCs secreted significantly more PGE2 [34]. Furthermore, PGE2 has an inhibitory effect on the proliferation of T and NK cells, causes an increase in the pool of Treg, reprograms macrophages to produce the anti-inflammatory cytokine IL-10, and prevents the differentiation of monocytes into DCs [34]. Immunosuppressive factors have been reported to cooperate to exert their regulatory role within inflamed synovium, and PGE2 co-exerts regulatory activity by up-regulating IL-6 [37], reducing local inflammation. It is suggested that PGE2 may play an important role in the immune dysregulation and bone remodeling in the treatment of osteoarthritis by DPSCs [5].

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