SUMMARY (cutting edge) 6/20/25 ciT1zen science

Pluripotent stem cell–derived extracellular vesicles for systemic immune modulation in diabetes therapy ciT1zen science summary

The study highlights the superior immunomodulatory capabilities of extracellular vesicles (EVs) derived from pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), compared to those from mesenchymal stem cells (MSCs). PSC EVs demonstrate a unique molecular profile, including distinct lipid compositions and the presence of pluripotency-associated proteins (ROR1, CD133, SSEA-4) and microRNAs (miR-302 family), which distinguish them from MSC EVs. Critically, PSC EVs exhibit enhanced *in vitro* immunosuppressive effects, effectively suppressing pro-inflammatory cytokines, inhibiting T-cell proliferation, and promoting regulatory T-cell (Treg) formation through mechanisms such as CDK8 downregulation.
The research also introduces a novel hyaluronic acid–Pluronic F127 hydrogel for controlled and prolonged delivery of iPSC EVs *in vivo*. In an antigen-specific type 1 diabetes (T1D) mouse model, hydrogel-encapsulated iPSC EVs significantly improved diabetes-free survival, increased Treg populations in lymphoid organs, and preserved pancreatic β-cell mass. These findings position PSC EVs as a highly promising "compelling platform for next-generation immunotherapies and cell-based treatment strategies" for autoimmune diseases and for promoting immune tolerance in various therapeutic applications. The scalability and reproducibility of PSC EV production via dynamic suspension culture further enhance their clinical and industrial potential.

Main Themes and Key Ideas/Facts

1. Superior Immunomodulatory Properties of PSC EVs Compared to MSC EVs

- **Enhanced Anti-inflammatory Potential:** PSC EVs (from ESCs and iPSCs) significantly outperform MSC EVs in modulating immune responses.
- "PSC EVs... markedly outperform mesenchymal stem cell (MSC)–derived EVs in suppressing pro-inflammatory cytokine secretion, inhibiting activated T-cell proliferation, and inducing regulatory T-cell (Treg) formation through CDK8 downregulation."
- While all tested EVs (MSC, ESC, iPSC) decreased TNF-α secretion by macrophages, "ESC and iPSC EVs induced significantly higher IL-10 secretion than MSC EVs, suggesting stronger anti-inflammatory potential."
- PSC EVs, unlike MSC EVs, reduced IFN-γ secretion by mouse T cells and significantly increased IL-10 secretion.
- **Modulation of T-Cell Activation and Treg Induction:**ESC and iPSC EVs "significantly reduced the activation of T cells, as indicated by the lower mean fluorescence intensity (MFI) of the CD44 activation marker compared to that of MSC EVs and the control group."
- They also "promoted the generation of Tregs at significantly higher levels than MSC EVs and the control group, indicating a strong capability to induce an immunoregulatory phenotype."
- In human T-cell cultures, PSC EVs suppressed T-cell proliferation and "significantly increased the proportion of Tregs among human CD4+ T cells, further supporting their immunoregulatory potential."

2. Unique Molecular Profile of PSC EVs

- **Distinct Molecular Fingerprints:** Nuclear Magnetic Resonance (NMR) analysis revealed "distinct molecular fingerprints of PSC EVs compared to those of MSC EVs."
- Hierarchical clustering showed iPSC- and ESC-derived EVs clustering together, separate from MSC EVs.
- "PSC EVs are enriched in cholesterol and polyunsaturated fatty acids (PUFAs), including omega-3, whereas MSC EVs contain higher levels of phosphatidylcholine (PC) and phosphatidylethanolamine (PE)." This unique lipid composition is a primary contributor to their distinct NMR fingerprint.
- **Unique Protein Cargo:** Proteomic profiling identified distinct protein compositions.
- "891 proteins were shared exclusively between iPSC- and ESC-derived EVs and were absent in MSC EVs, underscoring the difference between PSC EVs and MSC EVs."
- Key pluripotency-associated proteins uniquely identified in PSC EVs include "ROR1 (receptor tyrosine kinase–like orphan receptor 1) and CD133." ROR1 is largely absent in adult tissues.
- Flow cytometry validated "significantly higher expression of all three markers [ROR1, CD133, and SSEA-4] compared to MSC EVs, while EVs from differentiating cells showed reduced expression of these markers." These markers can serve as "quality control" indicators.
- **Unique MicroRNA Cargo:** MicroRNA sequencing revealed specific miRNA enrichment.
- PSC EVs contain unique microRNAs, "such as the pluripotency-associated proteins ROR1 and CD133 and members of the miR-302 family, which are not found in MSC EVs."
- "miR-302a-3p and miR-302d-3p being the most highly expressed" in PSC EVs. These miRNAs are "crucial role in maintaining pluripotency and promoting cellular reprogramming."

3. Mechanism of Immunomodulation: CDK8/19 Downregulation

- **Localizes to Draining Lymph Nodes:** *In vivo* tracking showed "EV uptake was predominantly localized to the draining lymph nodes, with significantly fewer EV-positive cells detected in the spleen and liver."
- **Transcriptional Reprogramming of Immune Cells:** Single-cell RNA sequencing (scRNA-seq) of draining lymph nodes revealed "broad transcriptional shifts across several immune cell types following iPSC EV treatment."
- "Both CD4+ and CD8+ T cells exhibited significant changes in gene expression, including marked downregulation of cyclin-dependent kinase 8 (CDK8)."
- CDK8, along with its paralog CDK19, "functions as a physiological suppressor of Foxp3 expression in conventional T cells (Tconv). Their inhibition is known to enhance STAT5 activation, thereby promoting Treg conversion even under inflammatory conditions."
- This suggests "iPSC EVs may facilitate a regulatory T cell phenotype by suppressing CDK8 expression."
- **Role of miR-302:** *In vitro* validation in human CD4+ T cells confirmed downregulation of *CDK8* and *CDK19* by iPSC EVs. "Treatment with miR-302 mimics similarly reduced *CDK8* and *CDK19* expression, suggesting that this miRNA may contribute to the immunomodulatory effects of iPSC EVs."

4. Scalable Production and Hydrogel-Based Delivery System

- **Enhanced EV Yield with Dynamic Culture:** "The dynamic suspension culture of PSC aggregates significantly increases EV yield, offering a scalable and reproducible source superior to other cell sources."
- Dynamic culture supported increased spheroid size and maintained high cell viability and pluripotency markers (Oct4, Sox2, Tra-1-60, SSEA-4).
- EV production in dynamic culture was "statistically significant 3.4-fold increase in production per million cells during 24 hours of dynamic culture compared to static culture."
- This addresses "limitations [of MSC EVs] that hinder the scalability and reproducibility of MSC EVs and present significant obstacles to their clinical application."
- **Controlled and Prolonged Release with Hydrogel:** An "injectable hydrogel composed of hyaluronic acid (HA) and Pluronic F127" was developed to "enhance their therapeutic potential by achieving controlled and prolonged release *in vivo*."
- The hydrogel transitions from solution to gel at body temperature, and its properties (EV release kinetics, stiffness) can be tuned by varying Pluronic F127 and HA concentrations.
- *In vivo* imaging confirmed that "EVs embedded in the hydrogel remained at the injection site for at least 48 hours, whereas soluble EVs were rapidly cleared within 3 hours."
- Cytotoxicity testing showed the hydrogel and iPSC EVs were "non-toxic and biocompatible, supporting their safe use for prolonged local delivery of EVs in therapeutic applications."

5. Therapeutic Efficacy in Type 1 Diabetes Model

- **Improved Diabetes-Free Survival:** In an antigen-specific adoptive transfer T1D mouse model, "two local injections of iPSC EVs, particularly when delivered via a biomaterial scaffold, significantly enhanced diabetes-free survival."
- "Mice treated with iPSC EVs encapsulated in hydrogel displayed the highest percentage of normoglycemic survival compared to that of the control group, hydrogel alone, or iPSC EVs alone."
- Repeated dosing (day 0 and day 7) further improved outcomes, with "55% of mice remaining diabetes-free at the end of the 90-day experiment" in the hydrogel-EV group.
- **Increased Systemic Treg Populations:** "Treatment with iPSC EVs encapsulated in hydrogel significantly increased Treg populations, particularly in the draining lymph nodes, pancreatic lymph nodes, and spleen, compared to all other groups."
- This suggests "the combination of iPSC EVs and hydrogel induces both localized and systemic immunosuppressive effects, which is critical for mitigating autoimmune responses."
- **Preservation of β-Cell Mass:** "Mice in the group where iPSC EVs were encapsulated in hydrogel retained a significantly greater beta-cell mass compared to the control and hydrogel groups."
- Histological analysis "revealed substantial preservation of insulin-producing beta cell clusters in the group in which iPSC EVs were encapsulated in hydrogel, whereas the control and hydrogel groups exhibited almost complete beta cell destruction."

Future Implications and Significance

- **Next-Generation Immunotherapies:** PSC EVs offer a "promising avenue for scalable immunotherapeutic and regenerative applications" due to their superior properties and scalability.
- **Broad Applications in Autoimmune Diseases:** The immunomodulatory capability and Treg-inducing potential of PSC EVs "suggest broad applications in treating autoimmune diseases"

such as rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease, where Treg dysfunction is central.

- **Transplant Tolerance:** PSC EVs "could aid in promoting transplant tolerance by mitigating alloimmune responses."
- **Advanced Characterization and Quality Control:** The use of NMR spectroscopy for "rapid phenotyping, distinguishing PSC EVs from other EV types and ensuring batch-to-batch quality control" is a significant advancement in EV research.
- **Clinical Translation:** The combination of scalable production methods (dynamic suspension culture), unique therapeutic cargo, and sustained delivery systems (hydrogels) "position PSC EVs as a compelling platform for next-generation immunotherapies and cell-based treatment strategies."