

World Journal of *Stem Cells*

World J Stem Cells 2024 May 26; 16(5): 462-614



EDITORIAL

- 462 Single-cell sequencing technology in diabetic wound healing: New insights into the progenitors-based repair strategies
Xiang Z, Cai RP, Xiao Y, Huang YC
- 467 Mesenchymal stem cells' "garbage bags" at work: Treating radial nerve injury with mesenchymal stem cell-derived exosomes
Mushtaq M, Zineldeen DH, Mateen MA, Haider KH
- 479 Deer antler stem cell niche: An interesting perspective
Cavallini C, Olivi E, Tassinari R, Zannini C, Ragazzini G, Marcuzzi M, Taglioli V, Ventura C

ORIGINAL ARTICLE**Basic Study**

- 486 Sinomenine increases osteogenesis in mice with ovariectomy-induced bone loss by modulating autophagy
Xiao HX, Yu L, Xia Y, Chen K, Li WM, Ge GR, Zhang W, Zhang Q, Zhang HT, Geng DC
- 499 Hydrogel loaded with bone marrow stromal cell-derived exosomes promotes bone regeneration by inhibiting inflammatory responses and angiogenesis
Zhang S, Lu C, Zheng S, Hong G
- 512 Patient-derived induced pluripotent stem cells with a *MERTK* mutation exhibit cell junction abnormalities and aberrant cellular differentiation potential
Zhang H, Wu LZ, Liu ZY, Jin ZB
- 525 Therapeutic potential of urine-derived stem cells in renal regeneration following acute kidney injury: A comparative analysis with mesenchymal stem cells
Li F, Zhao B, Zhang L, Chen GQ, Zhu L, Feng XL, Gong MJ, Hu CC, Zhang YY, Li M, Liu YQ
- 538 GATA binding protein 2 mediated ankyrin repeat domain containing 26 high expression in myeloid-derived cell lines
Jiang YZ, Hu LY, Chen MS, Wang XJ, Tan CN, Xue PP, Yu T, He XY, Xiang LX, Xiao YN, Li XL, Ran Q, Li ZJ, Chen L
- 551 Cardiac differentiation is modulated by anti-apoptotic signals in murine embryonic stem cells
Yehya A, Azar J, Al-Fares M, Boeuf H, Abou-Kheir W, Zeineddine D, Hadadeh O
- 560 Effects of interleukin-10 treated macrophages on bone marrow mesenchymal stem cells *via* signal transducer and activator of transcription 3 pathway
Lyu MH, Bian C, Dou YP, Gao K, Xu JJ, Ma P
- 575 Hepatocyte growth factor enhances the ability of dental pulp stem cells to ameliorate atherosclerosis in apolipoprotein E-knockout mice
Duan H, Tao N, Lv L, Yan KX, You YG, Mao Z, Wang CY, Li X, Jin JY, Wu CT, Wang H

- 591** Effect of ginsenoside Rg1 on hematopoietic stem cells in treating aplastic anemia in mice *via* MAPK pathway

Wang JB, Du MW, Zheng Y

SYSTEMATIC REVIEWS

- 604** Role of glioma stem cells in promoting tumor chemo- and radioresistance: A systematic review of potential targeted treatments

Agosti E, Zeppieri M, Ghidoni M, Ius T, Tel A, Fontanella MM, Panciani PP

ABOUT COVER

Editorial Board Member of *World Journal of Stem Cells*, Umberto Galderisi, PhD, Full Professor, Department of Experimental Medicine, Molecular Biology at the School of Medicine, University of Campania "Luigi Vanvitelli", Via Luigi De Crecchio 7, Naples I-80138, Italy. mberto.galderisi@unicampania.it

AIMS AND SCOPE

The primary aim of *World Journal of Stem Cells (WJSC, World J Stem Cells)* is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, *etc.*

INDEXING/ABSTRACTING

The *WJSC* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Journal Citation Reports/Science Edition, PubMed, PubMed Central, Scopus, Biological Abstracts, BIOSIS Previews, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJSC* as 4.1; IF without journal self cites: 3.9; 5-year IF: 4.5; Journal Citation Indicator: 0.53; Ranking: 15 among 29 journals in cell and tissue engineering; Quartile category: Q3; Ranking: 99 among 191 journals in cell biology; and Quartile category: Q3. The *WJSC*'s CiteScore for 2022 is 8.0 and Scopus CiteScore rank 2022: Histology is 9/57; Genetics is 68/325; Genetics (clinical) is 19/90; Molecular Biology is 119/380; Cell Biology is 95/274.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiao-Mei Zheng*; Production Department Director: *Xu Guo*; Cover Editor: *Jia-Ru Fan*.

NAME OF JOURNAL

World Journal of Stem Cells

ISSN

ISSN 1948-0210 (online)

LAUNCH DATE

December 31, 2009

FREQUENCY

Monthly

EDITORS-IN-CHIEF

Shengwen Calvin Li, Carlo Ventura

EDITORIAL BOARD MEMBERS

<https://www.wjgnet.com/1948-0210/editorialboard.htm>

PUBLICATION DATE

May 26, 2024

COPYRIGHT

© 2024 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS

<https://www.wjgnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjgnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjgnet.com/bpg/gerinfo/240>

PUBLICATION ETHICS

<https://www.wjgnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

<https://www.f6publishing.com>

Basic Study

Patient-derived induced pluripotent stem cells with a *MERTK* mutation exhibit cell junction abnormalities and aberrant cellular differentiation potential

Hang Zhang, Ling-Zi Wu, Zhen-Yu Liu, Zi-Bing Jin

Specialty type: Cell and tissue engineering**Provenance and peer review:** Unsolicited article; Externally peer reviewed.**Peer-review model:** Single blind**Peer-review report's scientific quality classification**Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0**P-Reviewer:** Li SC, United States; Zhang H, China**Received:** December 13, 2023**Peer-review started:** December 13, 2023**First decision:** January 15, 2024**Revised:** January 29, 2024**Accepted:** April 1, 2024**Article in press:** April 1, 2024**Published online:** May 26, 2024**Hang Zhang, Ling-Zi Wu, Zhen-Yu Liu, Zi-Bing Jin**, Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China**Co-first authors:** Hang Zhang and Ling-Zi Wu.**Corresponding author:** Zi-Bing Jin, MD, PhD, Research Scientist, Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, No. 1 Dongjiaomin Lane, Dongcheng District, Beijing 100730, China.jinzb502@ccmu.edu.cn

Abstract

BACKGROUND

Human induced pluripotent stem cell (hiPSC) technology is a valuable tool for generating patient-specific stem cells, facilitating disease modeling, and investigating disease mechanisms. However, iPSCs carrying specific mutations may limit their clinical applications due to certain inherent characteristics.

AIM

To investigate the impact of *MERTK* mutations on hiPSCs and determine whether hiPSC-derived extracellular vesicles (EVs) influence anomalous cell junction and differentiation potential.

METHODS

We employed a non-integrating reprogramming technique to generate peripheral blood-derived hiPSCs with and without a *MERTK* mutation. Chromosomal karyotype analysis, flow cytometry, and immunofluorescent staining were utilized for hiPSC identification. Transcriptomics and proteomics were employed to elucidate the expression patterns associated with cell junction abnormalities and cellular differentiation potential. Additionally, EVs were isolated from the supernatant, and their RNA and protein cargos were examined to investigate the involvement of hiPSC-derived EVs in stem cell junction and differentiation.

RESULTS

The generated hiPSCs, both with and without a *MERTK* mutation, exhibited normal karyotype and expressed pluripotency markers; however, hiPSCs with a

MERTK mutation demonstrated anomalous adhesion capability and differentiation potential, as confirmed by transcriptomic and proteomic profiling. Furthermore, hiPSC-derived EVs were involved in various biological processes, including cell junction and differentiation.

CONCLUSION

HiPSCs with a *MERTK* mutation displayed altered junction characteristics and aberrant differentiation potential. Furthermore, hiPSC-derived EVs played a regulatory role in various biological processes, including cell junction and differentiation.

Key Words: Cell junction; Cellular differentiation; Extracellular vesicle; Human induced pluripotent stem cells; Transcriptomics; Proteomics

©The Author(s) 2024. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Patient-specific human induced pluripotent stem cell (hiPSC) technology is a valuable tool for disease modeling and the investigation of disease mechanisms, but altered biological properties caused by pathogenic genes may limit hiPSC applications. Through transcriptomics and proteomics, this study revealed cell junction abnormalities and aberrant cellular differentiation potential in hiPSCs with a *MERTK* mutation. Furthermore, the profiles of hiPSC-derived extracellular vesicles collected for transcriptomic and proteomic analysis indicated their involvement in the changes of biological characteristics occurring in hiPSCs.

Citation: Zhang H, Wu LZ, Liu ZY, Jin ZB. Patient-derived induced pluripotent stem cells with a *MERTK* mutation exhibit cell junction abnormalities and aberrant cellular differentiation potential. *World J Stem Cells* 2024; 16(5): 512-524

URL: <https://www.wjgnet.com/1948-0210/full/v16/i5/512.htm>

DOI: <https://dx.doi.org/10.4252/wjsc.v16.i5.512>

INTRODUCTION

Induced pluripotent stem cells (iPSCs) are a type of stem cells that are generated from adult somatic cells, typically peripheral blood or fibroblasts, through different reprogramming processes. iPSCs possess similar potential for division and differentiation as embryonic stem cells (ESCs) and can be induced to differentiate into various cell types, tissues, and even organs[1,2]. The groundbreaking research on human iPSCs that has significant implications in the fields of regenerative medicine and stem cell research was first demonstrated by Takahashi *et al*[3] in 2007. Since then, numerous research teams have aimed to develop iPSC-derived products for the treatment of various diseases, with some already undergoing clinical trials[4]. Currently, there are high expectations for multiple applications of iPSCs, although there remain many challenges to overcome.

Autologous iPSC-based therapy offers several advantages, such as personalized drug discovery *via* patient-derived iPSC models that share the same genetic characteristics as the patient, thereby avoiding the need for immunosuppressants and reducing transplantation-related immune risks. Biologically, patient-specific iPSCs are derived from the patient's own cells, thereby avoiding ethical controversies and legal constraints[5]. However, for patients with specific gene mutations, iPSCs derived from somatic cell reprogramming usually carry the same gene mutations[6]. Although these iPSCs or ESCs with mutations may appear to be similar to healthy controls, with unlimited proliferative potential, they may differ in various aspects, such as cell apoptosis, metabolism, proliferation, and directed differentiation potential, compared to healthy PSCs[7,8].

Extracellular vesicles (EVs) are tiny membrane-wrapped structures that are released by cells into the extracellular environment[9]. EVs contain various molecules, including nucleic acids, proteins, and metabolites, which allow them to function in signal transduction and cell-cell communication[10-12]. Recipient cells can take up the EVs that donor cells have released to achieve intracellular signal transmission and self-regulation[13]. Autocrine EVs can carry information required for cell signaling pathway activation or cell self-regulation, which contributes to the cell's adjustment of its intrinsic state and functionality as it adapts to different environmental and physiological demands. Furthermore, EVs can be released by neighboring cells and taken up by nearby target cells, thereby enabling intercellular signal transduction. Paracrine EVs play a crucial role in regulating cellular differentiation, apoptosis, angiogenesis, immune response, cellular migration, and tissue repair[10,13].

Although mesenchymal stem cells (MSCs) have been studied extensively in the context of EVs, the composition and functions of iPSC-derived EVs remain largely unknown[14,15]. In this study, we proposed that iPSCs with specific gene mutations will exhibit differences in their transcription and protein levels, as well as their ability to regulate their own and neighboring cells' proliferation and differentiation capacities through the secretion of EVs. Investigating iPSCs and their secreted EVs is crucial for achieving a comprehensive understanding and effective utilization of iPSCs.

Mutations in the *MERTK* gene, which is responsible for encoding a receptor tyrosine kinase that is involved in cellular signal transduction, particularly in immune regulation, apoptotic debris clearance, cell survival, proliferation, and migration, have become a particular focus of interest[16-18]. Although *MERTK* receptors are typically expressed in immune cells[19], our observations during cultivation revealed that iPSCs carrying *MERTK* gene mutations exhibited altered cell junctions compared to the control iPSCs. Furthermore, they exhibited lower efficiency in directed cellular differentiation induced by small molecular compounds compared to that of the control iPSCs.

A recent study also reported altered bioenergetics and aberrant differentiation potential occurring in patient-specific iPSCs carrying mitochondrial mutations[8]. Similar to MSC-derived EVs, iPSC-derived EVs contain abundant components, including microRNA (miRNA) and proteins, that play a role in regulating various biological functions[15]. Therefore, we aimed to investigate the transcriptomic and proteomic profiles of iPSCs carrying a *MERTK* gene mutation and their secreted EVs to elucidate the biological characteristics of iPSCs.

MATERIALS AND METHODS

Establishment and maintenance of iPSC lines

Peripheral blood mononuclear cells (PBMCs) in 6 mL peripheral blood were collected from healthy donors or patients who provided written informed consent, with approval from the Institutional Ethics Committee. PBMCs were cultured in StemSpan™ Serum-Free Expansion Medium II supplemented with StemSpan™ Erythroid Expansion Supplement (STEMCELL Technologies) for 7 d to expand the erythroid cells. A total of 1×10^6 erythroid progenitors were nucleofected with episomal plasmids encoding reprogramming factors Oct4, Sox2, Myc, Nanog, Lin28, and Klf4[20]. Nucleofection was executed using the Amaxa P3 Primary Cell 4D Nucleofector X Kit and program EA-100 (LONZA). Transfected erythroid progenitors were cultured in ReproTeSR™ xeno-free reprogramming medium and later transitioned to Essential 8 hPSC medium (STEMCELL Technologies), and iPSC colonies appeared after approximately 20 d of culturing. Five iPSC clones were carefully selected, purified, and expanded until passage 5, following previously established methods[21]. The purified iPSCs were maintained in ncEpic hPSC medium (Nuwacell Biotechnologies) and were passaged using 0.5 mmol/L ethylenediaminetetraacetic acid solution (Thermo Fisher Scientific). The cells were cultured in a 37 °C incubator with 5% CO₂.

Mutation sequencing

Genomic DNA was extracted from iPSC cell lines using a DNA extraction kit (QIAGEN) and amplified through a polymerase chain reaction (PCR). *MERTK* gene mutation was verified *via* Sanger sequencing using forward primer GGAAGACCACATACAGGAA and reverse primer TGAAGGAAGCGATTATTGC.

Karyotype analysis

Human iPSCs (hiPSCs) were treated for 2 h with colchicine to arrest cells in mitotic metaphase, followed by cell harvesting. Cells were then swollen *via* incubation in a pre-warmed 75 mmol/L KCl hypotonic solution at 37 °C for 15 min. Following cell swelling, cells were fixed using a freshly prepared methanol/acetic acid fixative solution. Cell suspensions were dropped and evenly spread onto pre-chilled glass slides. After air-drying the slides, they were stained with Giemsa working solution for 20-30 min, followed by rinsing and drying. Chromosome analysis was performed using a Zeiss Metafer chromosome automated scanning and imaging system.

Differentiation of three germ layers

For *in vitro* differentiation of three germ layers, iPSC aggregates were cultured in suspension within DMEM/F12 medium (Gibco) with 20% KSR (Gibco), 0.1 mM 2-mercaptoethanol (Sigma), 0.1 mM non-essential amino acids (Sigma), 2 mM GlutaMAX (Life Technologies), 10 mM Y-27632 (Selleck), 100 U/mL penicillin, and 100 mg/mL streptomycin (Gibco) supplement for 8 d to form embryoid bodies. Then the embryoid bodies were transferred into DMEM/F12 medium with 10% foetal bovine serum (Gibco), 0.1 mM 2-mercaptoethanol, 0.1 mM non-essential amino acids, 2 mM GlutaMAX 100 U/mL penicillin and 100 mg/mL streptomycin supplement, and attached to glass slides coated with 0.1% gelatin (Millipore) for 10 d.

Flow cytometry

The generated hiPSCs were fixed with 4% paraformaldehyde after digestion into single cells. Following a 5-min incubation with 0.1% Triton X-100, the cells were blocked with a 3% fetal bovine serum solution for 30 min. Cells were then incubated with mouse monoclonal antibodies against human SSEA4 (Abcam, ab16287) or TRA-1-81 (MilliporeSigma, MAB4381) at room temperature for 1 h, followed by a 30-min incubation with secondary antibodies (Invitrogen, 1:400 dilution) at room temperature in the dark. After washing with Dulbecco's phosphate-buffered saline (DPBS), flow cytometry analysis was performed on a BD Accuri™ C6 Plus Flow Cytometer using excitation wavelengths of 488 or 594 nm. Data analysis was carried out using FlowJo™ software (version 10.4).

EVs obtained through the EXODUS system were incubated with fluorescently labeled antibodies in the dark for 30 min. After resuspension in DPBS, the samples were centrifuged at 4 °C, 110000 g for 70 min twice to remove excess dye. The supernatant was carefully removed, and the samples were resuspended in PBS for analysis using a N30E Nanoparticle Flow Cytometer (NanoFCM, China). Fluorescein isothiocyanate-conjugated mouse anti-human CD9, CD63, and CD81 antibodies obtained from BD were utilized (Supplementary Figure 1A).

Immunofluorescence staining

Cells were seeded on glass coverslips at an appropriate density, fixed in 4% PFA for 15 min, and washed with PBS. Permeabilized and blocked cells with 0.5% Triton X-100 and 4% bovine serum albumin for 1 h. Cells were incubated overnight at 4 °C with primary antibody, then incubated with secondary antibody and DAPI solution for 1 h at room temperature. Following primary antibodies were used: OCT4 (Cat. #ab18976; Abcam), SOX2 (Cat. #sc-365823; Santa Cruz), NANOG (Cat. #ab80892; Abcam), SSEA4 (Cat. #ab16287; Abcam), GFAP (Cat. #HPA056030; Sigma), α -SMA (Cat. #A5228; Sigma), AFP (Cat. #MAB1368; R&D System). Images were acquired using an Olympus confocal system (SpinSR10, Japan).

EVs isolation

Cells were seeded on plates at a density of 5.0×10^4 cells/cm², and the supernatant was collected until the cells reached approximately 90% confluence. The supernatant was centrifuged at 4 °C 300 g for 10 min, followed by centrifugation at 4 °C, 3000 g for 20 min, and temporary storage at -80 °C. Prior to EV separation, the supernatant was thawed at 4 °C, centrifuged at 4 °C 12000 g for 30 min, then filtered through a 0.22- μ m filter. The EXODUS platform was employed for EV isolation, and after the program was run, the EVs were resuspended in DPBS and restored at -80 °C for subsequent steps.

Nanoparticle tracking analysis

Nanoparticle tracking analysis was employed to assess the size distribution and concentration of EVs using a NanoSight NS300 instrument (Malvern). The analysis settings were optimized, and each sample was diluted 1000- to 2000-fold with DPBS before measurement. Each sample was diluted in triplicate, and each diluted sample was measured three times with a capture time of 30 s to ensure measurement accuracy (Supplementary Figure 1B).

Transmission electron microscopy

EVs were diluted to an appropriate concentration and then dripped on carbon-coated copper grids for 90 s. Subsequently, negative staining was performed with uranyl acetate dye for 30 s; excess dye was removed; and the sample was dried. Images were captured using a Tecnai G2 12 transmission electron microscope (FEI, United States) (Supplementary Figure 1C).

RNA sequencing

Library preparation and sequencing of iPSCs were executed as previously described[22]. Briefly, total RNA from lysed samples was extracted using TRIzol™ Reagent, while the total RNA from EVs was extracted using the exoRNeasy Serum/Plasma Maxi Kit (Qiagen). The purity and integrity of RNA in the samples were assessed, and mycoplasma contamination detection was performed. Libraries were constructed using the NEBNext® Ultra™ II RNA Library Prep Kit and NEBNext Multiplex Small RNA Library Prep Set (NEB, United States). High-throughput sequencing was conducted on the Illumina PE150 and SE50 platform. Data were aligned to the human genome (version hg38), and miRNA and target genes were predicted using TargetScan and miRanda databases. Additionally, long non-coding RNA (lncRNA) and co-expression target gene correlations were calculated using cor.test R (version 4.3.0) for data analysis and visualization. Library construction and sequencing were conducted by Novogene Bioinformatics Technology Co., Ltd.

Proteomics

The total protein was extracted from the samples, and the protein concentration was determined using the Bradford method. Specimen separation was achieved using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by 4D label-free detection and quantification analysis using the timsTOF pro2 platform (Novogene Bioinformatics Technology Co., Ltd). The obtained data were analyzed using R, with differentially expressed proteins (DEPs) defined as those with a fold change > 2 and *P*-value < 0.05.

Cell counting kit-8 analysis

Cell counting kit-8 (CCK-8) analysis was conducted using previously published protocol[23]. Briefly, 2000 cells per well were seeded on a 96-well plate. After 12-h culture, 10- μ L CCK-8 solution was added to each well. After incubation in the dark for 1 h, the absorbance value at 450 nm was detected by a microplate reader (Molecular Devices).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.3.0 and R Studio software (version 4.3). All data are presented as the mean \pm standard error. A two-tailed unpaired Student's *t*-test was used to compare the two groups.

RESULTS

Generation of patient-specific iPSCs with a homozygous MERTK mutation

A young proband presenting with night blindness and progressive vision loss was diagnosed with retinitis pigmentosa (RP), while neither parent exhibited similar symptoms (Figure 1A). Exon sequencing targeting 164 recognized genes associated with retinal diseases was executed[24], revealing a homozygous MERTK gene mutation (c.296_297delCA). Sanger sequencing confirmed the mutation (Figure 1B). This deletion caused a frameshift mutation, resulting in the substitution of the 99th threonine with serine and the appearance of a new reading frame. Translation terminated at the 8th

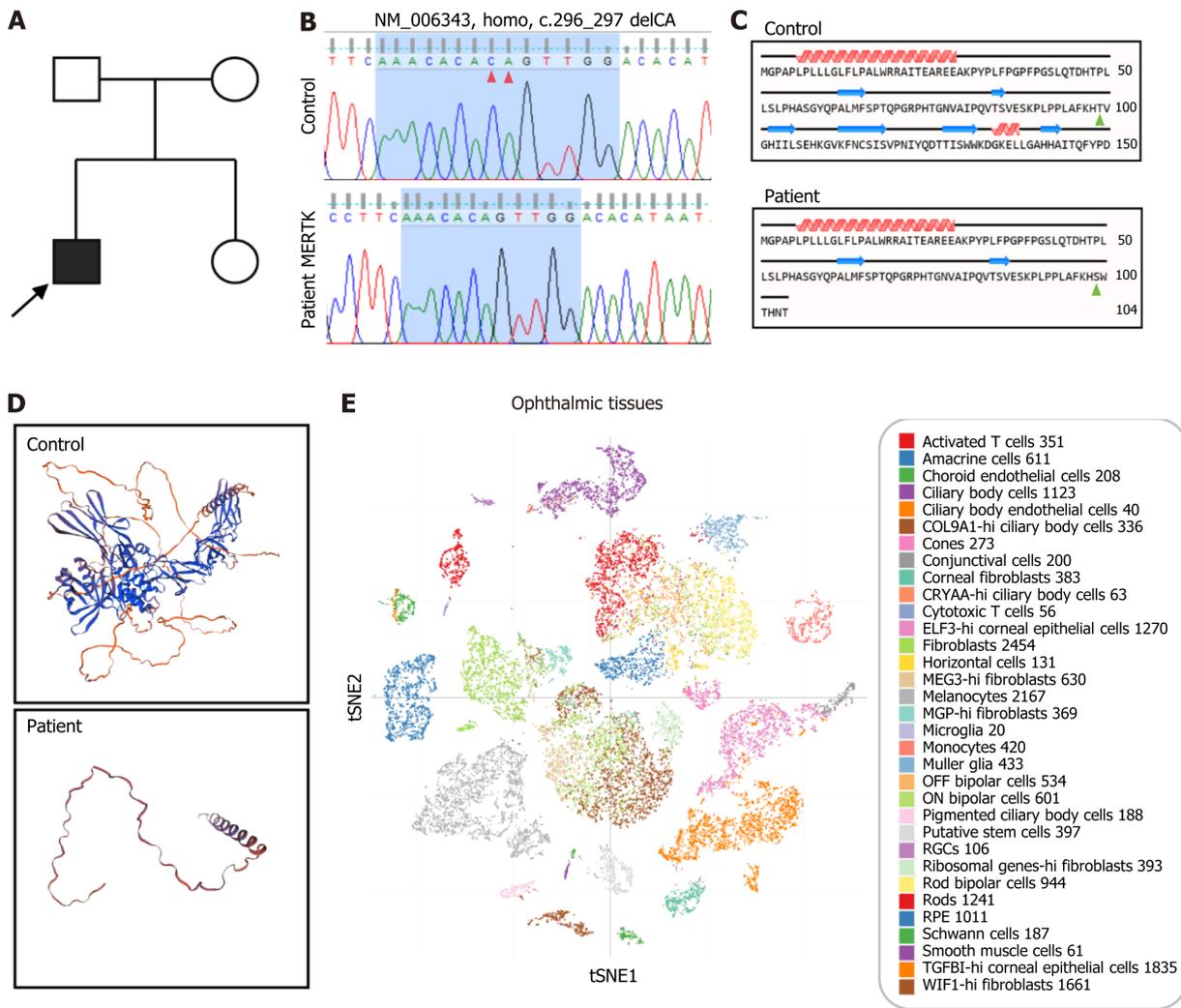


Figure 1 Identification of the *MERTK* mutation in the retinitis pigmentosa patient. A: Family pedigree; B: Sanger sequencing of the healthy control and the retinitis pigmentosa (RP) patient. Two-base deletion of CA in the RP patient (red arrows); C: Protein translation of the healthy control and RP patient. The 99th threonine was replaced by serine in the RP patient (green arrows), resulting in the emergence of a new reading frame, leading to translation termination at the 8th codon downstream; D: *MERTK* protein structure prediction of the healthy control and the RP patient; E: *MERTK* gene expression in ophthalmic tissue.

codon downstream (Figure 1C)[25]. Protein tertiary structure prediction showed that the normal *MERTK* protein exhibited complex protein domains, while the mutant *MERTK* protein retained only a short peptide chain (Figure 1D). The Single Cell Portal database indicated that the *MERTK* gene was expressed in various cell types found in ophthalmic tissue, such as rods, cones, bipolar cells, microglia, retinal pigment epithelium, *etc.* (Figure 1E).

Fresh peripheral blood obtained from the *MERTK* patient and healthy individuals was used to generate iPSC lines using a non-viral, non-integrating method (Figure 2A). The generated iPSC clones displayed clear edges and close-packed cells (Figure 2B) and exhibited a normal karyotype (Figure 2C). More than 90% of cells expressed stem cell markers SSEA4 and TRA-1-81 (Figure 2D). Both iPSC lines were subjected to immunofluorescence staining for pluripotency markers SSEA4, OCT4, SOX2, and NANOG (Figure 2E and F). *In vitro* differentiation demonstrated that the iPSCs from the patient and control were capable of differentiation into three germ layers (Figure 2G and H). These results demonstrated the successful construction of patient-specific iPSCs with a homozygous *MERTK* mutation.

iPSCs with the homozygous MERTK mutation exhibited altered junction and reduced differentiation efficiency

Despite their similar appearance, *MERTK*-mutated iPSCs showed slight deviations from the control iPSCs under the same culture conditions. On the 2nd to 3rd d after passage, the robust proliferative capability of the stem cells resulted in gradual expansion of the cell clones. On the VTN-coated culture plate, the majority of iPSC clones from the control group adhered tightly to the plate and exhibited extensive growth, with only a few small clones or individual cells floating on the surface of the culture medium (Figure 3A). However, iPSCs with the *MERTK* mutation showed both large and small clones floating on the surface of the culture medium (Figure 3B). Furthermore, during the directed induction of epithelial cell differentiation, control cells displayed neat and close arrangement on the culture plate (Figure 3C), whereas cells with a *MERTK* mutation tended to float on the surface of the culture medium, resulting in large voids and reduced differentiation efficiency (Figure 3D). CCK-8 detection confirmed that there was no significant difference in the proliferation ability of the two groups (Figure 3E). The cadherin family is a group of transmembrane proteins that play an important

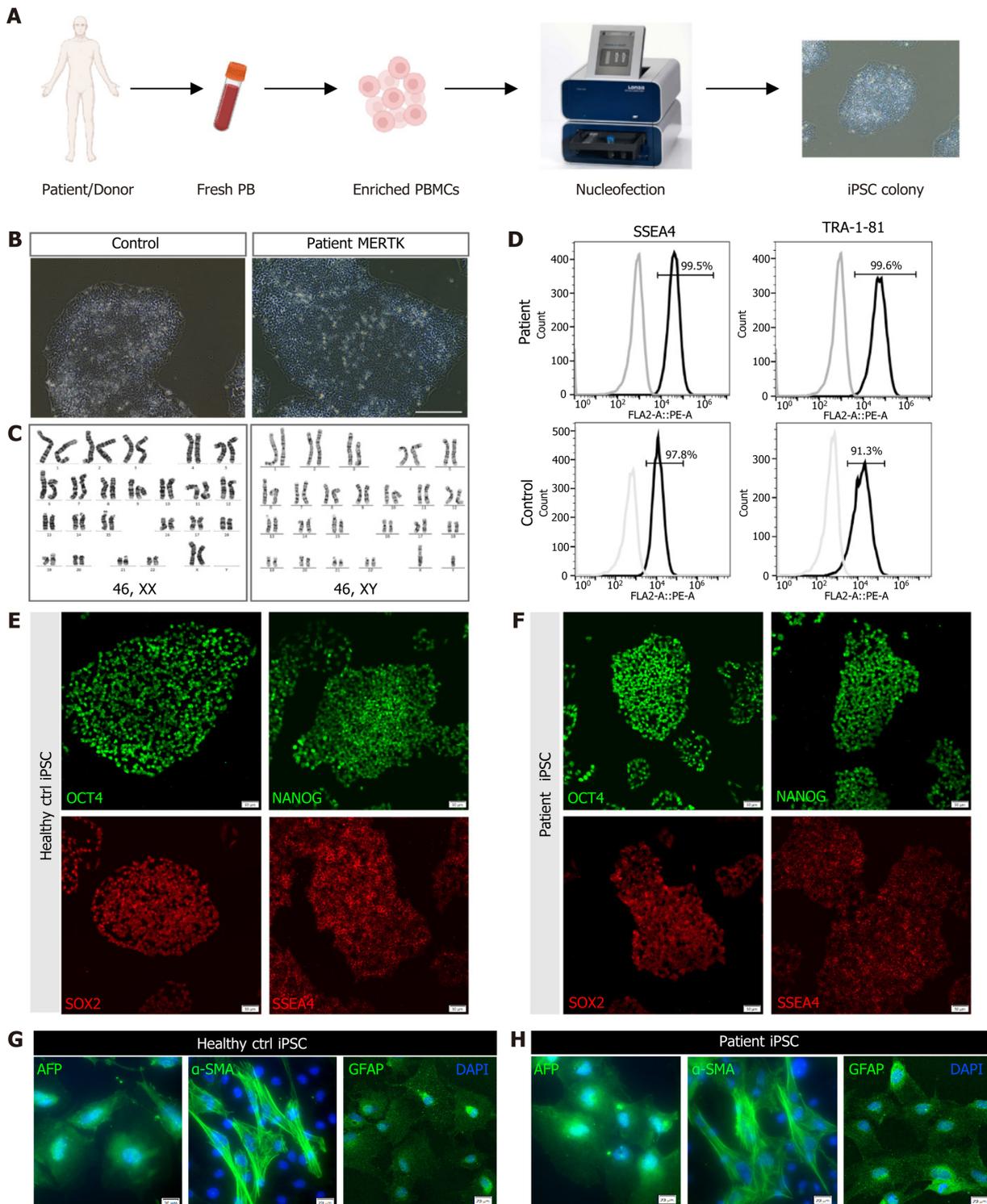


Figure 2 Generation and identification of the human induced pluripotent stem cells derived from the retinitis pigmentosa patient. **A:** Timeline of human induced pluripotent stem cell generation; **B:** Imaging by phase-contrast microscopy. Scale bar = 200 μm ; **C:** Karyotype analysis of the healthy control (left) and retinitis pigmentosa patient (right); **D:** Flow cytometry of pluripotency markers SSEA-4 and TRA-1-81; **E** and **F:** Immunostaining of pluripotency markers OCT4, NANOG, SOX2, and SSEA4 of the healthy control and retinitis pigmentosa patient. Scale bar = 20 μm ; **G** and **H:** *In vitro* differentiation of control (**G**) and patient (**H**) induced pluripotent stem cells into three germ layers, endoderm (AFP+), mesoderm (α -SMA+) and ectoderm (GFAP+). Scale bar = 20 μm . PB: Peripheral blood; PBMC: Peripheral blood mononuclear cell; iPSC: Induced pluripotent stem cell.

role in cell junction or adhesion[26]. RNA-seq data suggested that the expressions of the cadherin subfamily members DCHS1 and CELSR2 were downregulated in iPSCs with a *MERTK* mutation, and quantitative PCR approved this result (Figure 3F, Supplementary Figure 2A).

***MERTK*-mutated iPSCs displayed gene expression differences in cell adhesion and differentiation**

Principal component analysis displayed certain differences between the gene expressions of two iPSC lines (Figure 4A).

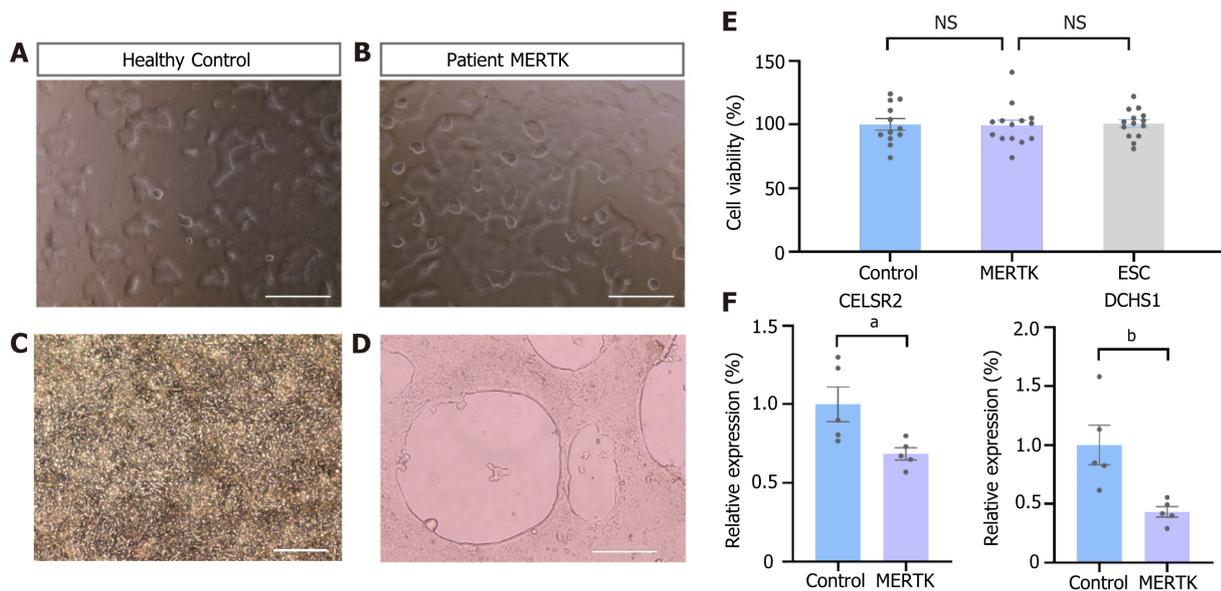


Figure 3 Abnormal adherent capability and aberrant differentiation potential. A and B: Imaging of clones floating on the surface of the culture medium by phase-contrast microscopy. Scale bar = 1000 μ m; C and D: Imaging of cells during epithelial cell differentiation. Scale bar = 200 μ m (C), scale bar = 400 μ m (D); E: Cell counting kit-8 detection of human induced pluripotent stem cell proliferation ability. mean \pm SEM, $n = 12-14$; F: Quantitative polymerase chain reaction analysis of representative cadherin subfamily members CELSR2 and DCHS1. mean \pm SEM, $n = 5$. Two-tailed t -test. ^a $P < 0.05$, ^b $P < 0.05$. NS: No significance. ESC: Embryonic stem cell.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of differentially expressed mRNA revealed differences in cell differentiation and cell adhesion (Figure 4B-D). A total of 121 differentially expressed miRNAs were identified, and GO and KEGG enrichment analyses of their predicted target genes indicated their involvement in cell adhesion, cell-cell junction, and cell differentiation (Figure 4E-G). Additionally, these target genes were significantly associated with central nervous system development, the extracellular matrix, and extracellular exosome. Similarly, GO and KEGG enrichment analyses of the predicted target genes of differentially expressed lncRNA showed similar results (Supplementary Figure 3). The number of DEPs in iPSCs was significantly fewer than that of RNA (Figure 4H). GO and Reactome pathway enrichment analyses of these DEPs suggested that *MERTK*-mutated iPSCs exhibited reduced mesodermal development potential and lower protein expressions of gap junction trafficking and regulation compared to the control (Figure 4I). These findings indicated that *MERTK*-mutated iPSCs had reduced cell adhesion capacity and differentiation potential.

***MERTK*-mutated iPSC-derived EVs helped regulate cell adhesion and differentiation**

EVs played a crucial role in regulating the extracellular microenvironment, and the transcriptomics and proteomics profiles indicated that *MERTK*-mutated iPSCs displayed different gene and protein expressions in the extracellular matrix and extracellular exosomes compared to the control (Figure 4C, Supplementary Figure 3D). Compared to the intracellular RNA components, the amount of differentially expressed RNA cargos in the EVs was significantly lower (Figure 5A and C). GO-cellular component enrichment analysis revealed that the differentially expressed mRNA cargo in *MERTK*-mutated iPSC-derived EVs was significantly enriched in the adherens junction (Figure 5B). GO enrichment analysis of the predicted target genes of differentially expressed miRNAs and lncRNAs indicated that *MERTK*-mutated iPSC-derived EVs might regulate cell junctions (Figure 5D, Supplementary Figure 4).

Using a 4D-label free proteomics platform, we detected more than 800 proteins in the two types of vesicles and found an overlap between some of the top 10 and bottom 10 expressed proteins between patient iPSCs and control iPSCs (Figure 5E). DEPs were more abundant compared to RNA cargos in the two types of EVs (Figure 5F). GO analysis revealed that these DEPs were significantly enriched in cell-cell junctions, cell junction organization, regulation of cell junction assembly, and cell adhesion activity (Figure 5G-I). Additionally, these DEPs potentially impact epithelial cell differentiation (Figure 5I). KEGG and Reactome pathway enrichment analyses revealed that DEPs were significantly enriched in tight junction, adherens junction, and signaling by Rho GTPases (Figure 5J and K). Gene set enrichment analysis revealed that patient-specific iPSC-derived EVs might be involved in downregulated tight junction, gap junction, and epithelial cell differentiation potential (Figure 5L).

DISCUSSION

The invention of iPSC technology has provided new opportunities for the treatment of degenerative diseases. Patient-specific iPSCs facilitate personalized treatment. In this study, iPSCs were generated from fresh peripheral blood rather than skin fibroblasts to minimize UV exposure-associated DNA damage[27]. The non-integrating plasmid repro-

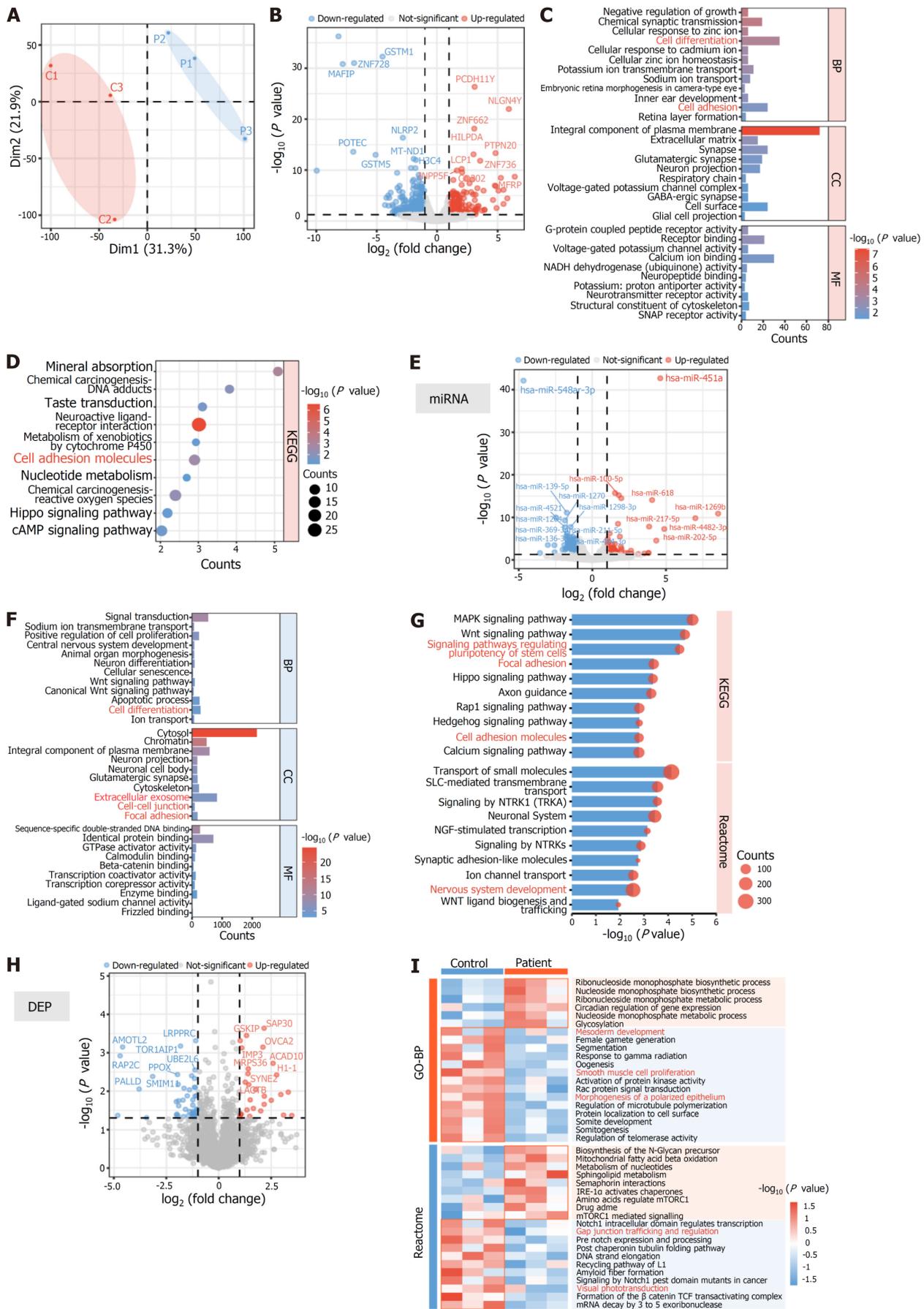


Figure 4 Transcriptomic and proteomic analyses identifying defects of cell adhesion and differentiation in patient-derived human induced pluripotent stem cells. A: Principal component analysis of mRNA expression; B: Differentially expressed mRNAs between patient human induced

pluripotent stem cells (iPSCs) and control iPSCs; C: Gene Ontology (GO) enrichment analysis for differentially expressed genes; D: Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis for differentially expressed genes; E: Differentially expressed microRNAs (miRNAs) between patient iPSCs and control iPSCs; F: GO enrichment analysis for differentially expressed miRNA target genes; G: KEGG and Reactome enrichment analyses for differentially expressed miRNA target genes; H: Differentially expressed proteins between patient iPSCs and control iPSCs; I: GO and Reactome enrichment analyses for differentially expressed proteins. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: Biological process; CC: Cellular component; MF: Molecular function; miRNA: MicroRNA; DEP: Differentially expressed protein.

gramming method increased the preclinical relevance of this study. The two generated iPSC lines exhibited similar morphological appearance, presence of normal karyotypes, and expression of pluripotency markers (Figure 2). Sanger sequencing confirmed that the obtained iPSCs retained the same genetic mutations as those found in the somatic cells from which they were derived.

Strict criteria and specific standards are required for validating iPSCs as genuine and appropriate entities for research and clinical purposes. These characteristics are crucial for ensuring the dependability, replicability, and security of iPSC-derived products. In previous publications, researchers typically control the quality of iPSCs through several aspects. First, iPSCs need possess the ability to differentiate into all three germ layers, ectoderm, endoderm, and mesoderm, which is typically confirmed by *in vitro* differentiation experiments or by the establishment of teratoma formation *in vivo*. Here, we performed *in vitro* differentiation and completed immunofluorescence staining for typical markers AFP, α -SMA, and GFAP of three germ layers[20], which demonstrated the pluripotency of generated iPSCs (Figure 2G and H). Second, iPSCs must express pluripotency markers, such as OCT4, SOX2, SSEA-4, TRA-1-81, and NANOG, which have been confirmed in this study by immunofluorescence staining or flow cytometry. Third, iPSCs need display similar biological and functional characteristics to ESCs, such as similar morphology, proliferation ability, gene expression patterns, or typical markers. In this study, CCK-8 analysis demonstrated similar division and proliferation under the same laboratory circumstance. This study lacked a more in-depth comparison between iPSCs and ESCs. G-banding chromosome karyotype analysis was commonly used for evaluating the genetic stability of PSCs, and detecting structural and numerical variations in chromosomes. However, it has limited detection sensitivity, which makes it difficult to identify variations below the threshold through the microscope. Therefore, pre-clinical or clinical study requires whole exome sequencing or whole genome sequencing to reach a higher diagnostic efficiency[28-30]. Episomal iPSC reprogramming plasmids used in this study are a non-viral, non-integrating system that allows reprogramming somatic cells into iPSCs in feeder-free conditions. After about 10 cell cycles, most episomal plasmids are lost, leading to the generation of iPSCs free of genomic integration or genetic alterations. Generated iPSCs by this system even can be utilized for pre-clinical research and human gene therapy according to manual (catalog# SC900A-1). Chemical reprogramming provides an innovative approach, with higher controllability and ease of standardization, holding promising prospects for future clinical applications, although it has not been widely applied[31]. Furthermore, we are exploring the directed differentiation into several cell types in a controlled setting using these iPSC lines.

The establishment of iPSCs is a prerequisite for subsequent directed differentiation, requiring iPSCs to have good proliferative capabilities and multidirectional differentiation potential. However, there is no clear assessment index for the differentiation potential of stem cells. During the differentiation process of retinal epithelial cells, in the early stage of directional induction, large-scale detachment of *MERTK* mutant cells led to the appearance of a large space at the bottom of the culture plate, and the cells along the edge of the space exhibited morphology alterations, from tightly connected round cells to spindle cells with irregular edges, which were similar to the characteristics of epithelial-mesenchymal transition (Figure 3). These cells usually cannot be further induced into terminal cells. Our statistics demonstrated that the probability of differentiation failure is approximately 50%, although this phenomenon has never been reported to have occurred in healthy iPSCs or ESCs. A previous study indicated that cell adhesion and cell junctions influence differentiation and development[27,32-34]. Furthermore, cell differentiation and cell-cell adhesion are influenced by disparities in protein expressions[35,36]. We investigated this phenomenon by examining RNA and protein expression. Our systematic transcriptomics and proteomics analyses revealed that iPSCs carrying a *MERTK* mutation exhibited differential expression of genes that are significantly involved in the regulation of cell junction, adherens junction, tight junction, gap junction, focal adhesion, and cell differentiation (Figure 4). Additionally, a recent study reported that defects in mitochondrial gene expression led to reduced stem cell differentiation potential[18]. In our study, the expression of representative mitochondrial genes, MT-ND1, MT-ND2, and MT-ND4, were significantly reduced in *MERTK* mutant cells (Supplementary Figure 2), which might have caused aberrant differentiation potential. However, the specific mechanism by which energy metabolism affects cell differentiation potential through epigenetics is the focus of attention in the field and requires further exploration.

iPSCs transcriptomics analysis suggested differences in gene expression related to EV secretion between the two types of iPSCs. EVs contain various bioactive substances and play an important role in regulating cell junction, adhesion, cell growth, development, and differentiation. Xie *et al*[37] reported that EVs from nasopharyngeal carcinoma cells containing miR-455 could target reducing zonula occludens 1 expression. Chai *et al*[38] found that BCR-ABL1-driven exosome-miR130a-3p interacted with gap-junction Cx43, thereby affecting gap junctional intercellular communications. Moreover, circulating EVs from sickle cell patients have been shown to regulate endothelial gap junctions[39]. EVs act as important paracrine mediators, providing biochemical cues for inducing stem cell differentiation[40,41]. Therefore, we collected the supernatants of the two types of iPSCs to isolate EVs and conducted transcriptomic and proteomic analyses. The systematic analysis revealed that differential RNA expression was significantly enriched in neural system development, adhesion connection, and cell cycle regulation. Using 4D label-free high-throughput sequencing technology, we found that the DEPs in EVs were more abundant and prominently clustered in cell connections, adhesion connections, as well as

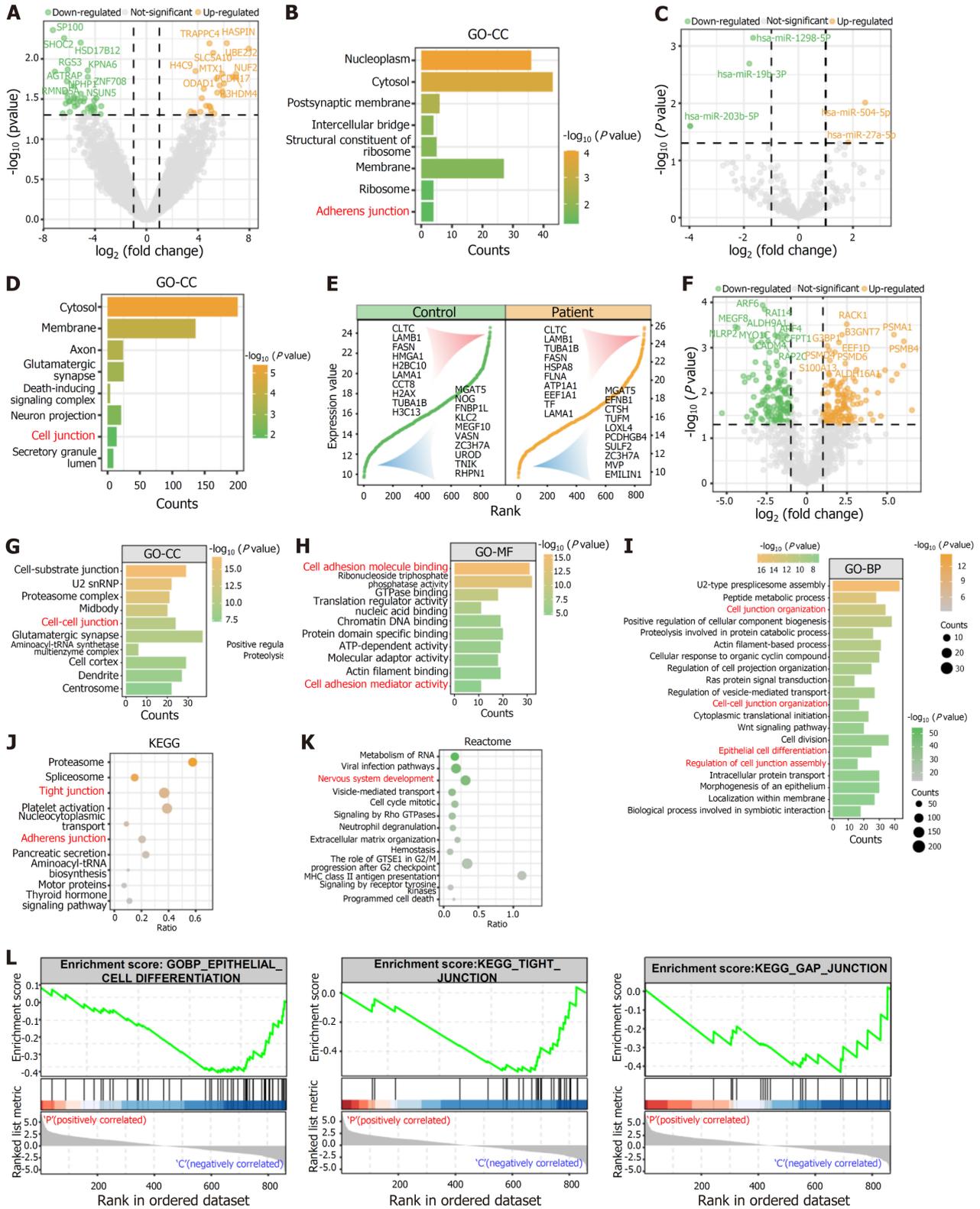


Figure 5 Transcriptomic and proteomic analyses indicating that human induced pluripotent stem cell-derived extracellular vesicles play a crucial role in regulating cell adhesion and differentiation. A: Differentially expressed mRNAs between patient human induced pluripotent stem cell (iPSC)-derived extracellular vesicles (EVs) and control iPSC-derived EVs; B: Gene Ontology (GO)-cellular component enrichment analysis of differentially expressed mRNAs; C: Differentially expressed microRNA between patient iPSC-derived EVs and control iPSC-derived EVs; D: GO-cellular component enrichment analysis of differentially expressed microRNA target genes; E: Abundance of proteins from patient iPSC-derived EVs and control iPSC-derived EVs. The abscissa represents the protein number, and the ordinate represents the normalized protein expression score. The top 10 and bottom 10 expressed proteins are displayed; F: Differentially expressed proteins between patient iPSC-derived EVs and control iPSC-derived EVs; G-I: GO enrichment analysis for differentially expressed proteins; J and K: Kyoto Encyclopedia of Genes and Genomes and Reactome enrichment analyses for differentially expressed proteins; L: Gene set enrichment analysis of patient iPSC-derived EVs and control iPSC-derived EVs. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: Biological progress; CC: Cellular

component; MF: Molecular function.

cell cycle and programmed cell death. While there were fewer differential RNAs in the exosomes, there were more differential proteins compared to cells. EVs derived from iPSCs with a *MERTK* mutation exhibited poorer ability to regulate cell adhesion, greater ability to regulate apoptosis or programmed cell death, and poorer ability to regulate cell differentiation. These results suggested that proteins in EVs play a more important role in regulating stem cell proliferation and differentiation, and the biological property of EVs is an important dimension for more fully characterizing iPSCs.

However, this research has some limitations. The phenotypic differences among iPSCs, such as differentiation potential and cell morphology, have a 5%-46% possibility of being attributed to individual variations[42]. In addition to genetic background, donor-specific epigenetics after reprogramming may influence stem cell variability[36], and the expression of quantitative trait loci might also contribute to the maintenance of iPSC lines and their differentiation capability[36,43]. Due to the extremely low incidence of this homozygous mutation on the second exon, we lacked the additional samples necessary for validating the phenotype. Nevertheless, this research provides evidence indicating that iPSCs carrying a *MERTK* gene mutation may exhibit altered junctions and aberrant differentiation potential.

In this study, we reported for the first time a homozygous mutation in the *MERTK* gene (c.296_297delCA, p.T99Sfs*8) and established an iPSC line associated with this mutation. We also reported, for the first time, that iPSCs with a *MERTK* mutation show impaired adhesion capabilities, tight junctions, and directional differentiation potential. By combining cellular RNA and protein expression with EV components, we revealed that intracellular components and EVs collectively regulate cell tight junctions and differentiation. Our findings suggest that repairing pathogenic gene mutations before conducting stem cell therapy in patients carrying gene mutations is a favorable strategy.

CONCLUSION

We first reported a homozygous mutation in the *MERTK* gene (c.296_297delCA, p.T99Sfs*8) and established an iPSC line associated with this mutation. We demonstrated that iPSCs with a *MERTK* mutation exhibited impaired adhesion capabilities, tight junctions, and directional differentiation potential. Our findings elucidated the collective regulation of intracellular components and EVs on cell tight junctions and cellular differentiation. These findings highlighted the importance of repairing the pathogenic gene mutations prior to stem cell therapy.

FOOTNOTES

Author contributions: Zhang H and Wu LZ contributed equally to this work. Zhang H and Jin ZB designed experiments and drafted the manuscript; Zhang H and Liu ZY performed experiments; Zhang H and Wu LZ analyzed and interpreted data; Jin ZB conceived the study, supervised the project, revised the manuscript and provided the financial support; and the manuscript was approved by all authors.

Supported by the National Natural Science Foundation of China, No. 82125007; and Beijing Natural Science Foundation, No. Z200014.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of Beijing Tongren Hospital, Capital Medical University (Approval No. TRECKY2021-088).

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: All data needed to evaluate the conclusions in the paper are present in the paper and the supplementary materials. The RNA-seq and proteomics data reported in this paper are available from the corresponding author on reasonable request.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <https://creativecommons.org/licenses/by-nc/4.0/>

Country/Territory of origin: China

ORCID number: Hang Zhang 0000-0002-5836-8225; Ling-Zi Wu 0000-0002-7554-629X; Zi-Bing Jin 0000-0003-0515-698X.

S-Editor: Wang JJ

L-Editor: A

P-Editor: Zhang YL

REFERENCES

- 1 **Doss MX**, Sachinidis A. Current Challenges of iPSC-Based Disease Modeling and Therapeutic Implications. *Cells* 2019; **8** [PMID: 31052294 DOI: 10.3390/cells8050403]
- 2 **Yan YW**, Qian ES, Woodard LE, Bejoy J. Neural lineage differentiation of human pluripotent stem cells: Advances in disease modeling. *World J Stem Cells* 2023; **15**: 530-547 [PMID: 37424945 DOI: 10.4252/wjsc.v15.i6.530]
- 3 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]
- 4 **Yamanaka S**. Pluripotent Stem Cell-Based Cell Therapy-Promise and Challenges. *Cell Stem Cell* 2020; **27**: 523-531 [PMID: 33007237 DOI: 10.1016/j.stem.2020.09.014]
- 5 **Mandai M**, Watanabe A, Kurimoto Y, Hiram Y, Morinaga C, Daimon T, Fujihara M, Akimaru H, Sakai N, Shibata Y, Terada M, Nomiyama Y, Tanishima S, Nakamura M, Kamao H, Sugita S, Onishi A, Ito T, Fujita K, Kawamata S, Go MJ, Shinohara C, Hata KI, Sawada M, Yamamoto M, Ohta S, Ohara Y, Yoshida K, Kuwahara J, Kitano Y, Amano N, Umekage M, Kitaoka F, Tanaka A, Okada C, Takasu N, Ogawa S, Yamanaka S, Takahashi M. Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. *N Engl J Med* 2017; **376**: 1038-1046 [PMID: 28296613 DOI: 10.1056/NEJMoa1608368]
- 6 **Liang Y**, Sun X, Duan C, Tang S, Chen J. Application of patient-derived induced pluripotent stem cells and organoids in inherited retinal diseases. *Stem Cell Res Ther* 2023; **14**: 340 [PMID: 38012786 DOI: 10.1186/s13287-023-03564-5]
- 7 **Xiong Y**, Mahmood A, Chopp M. Mesenchymal stem cell-derived extracellular vesicles as a cell-free therapy for traumatic brain injury via neuroprotection and neurorestoration. *Neural Regen Res* 2024; **19**: 49-54 [PMID: 37488843 DOI: 10.4103/1673-5374.374143]
- 8 **Kalluri R**, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* 2020; **367** [PMID: 32029601 DOI: 10.1126/science.aau6977]
- 9 **Manai F**, Smedowski A, Kaarniranta K, Comincini S, Amadio M. Extracellular vesicles in degenerative retinal diseases: A new therapeutic paradigm. *J Control Release* 2024; **365**: 448-468 [PMID: 38013069 DOI: 10.1016/j.jconrel.2023.11.035]
- 10 **Chen N**, Wang YL, Sun HF, Wang ZY, Zhang Q, Fan FY, Ma YC, Liu FX, Zhang YK. Potential regulatory effects of stem cell exosomes on inflammatory response in ischemic stroke treatment. *World J Stem Cells* 2023; **15**: 561-575 [PMID: 37424949 DOI: 10.4252/wjsc.v15.i6.561]
- 11 **Gao X**, Gao H, Shao W, Wang J, Li M, Liu S. The Extracellular Vesicle-Macrophage Regulatory Axis: A Novel Pathogenesis for Endometriosis. *Biomolecules* 2023; **13** [PMID: 37759776 DOI: 10.3390/biom13091376]
- 12 **Keshkar S**, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem Cell Res Ther* 2018; **9**: 63 [PMID: 29523213 DOI: 10.1186/s13287-018-0791-7]
- 13 **Bi Y**, Qiao X, Liu Q, Song S, Zhu K, Qiu X, Zhang X, Jia C, Wang H, Yang Z, Zhang Y, Ji G. Systemic proteomics and miRNA profile analysis of exosomes derived from human pluripotent stem cells. *Stem Cell Res Ther* 2022; **13**: 449 [PMID: 36064647 DOI: 10.1186/s13287-022-03142-1]
- 14 **DeRyckere D**, Huelse JM, Earp HS, Graham DK. TAM family kinases as therapeutic targets at the interface of cancer and immunity. *Nat Rev Clin Oncol* 2023; **20**: 755-779 [PMID: 37667010 DOI: 10.1038/s41571-023-00813-7]
- 15 **Zhou S**, Li Y, Zhang Z, Yuan Y. An insight into the TAM system in Alzheimer's disease. *Int Immunopharmacol* 2023; **116**: 109791 [PMID: 36738678 DOI: 10.1016/j.intimp.2023.109791]
- 16 **Mikolajczyk A**, Mitula F, Popiel D, Kaminska B, Wiczorek M, Pieczykolan J. Two-Front War on Cancer-Targeting TAM Receptors in Solid Tumour Therapy. *Cancers (Basel)* 2022; **14** [PMID: 35626092 DOI: 10.3390/cancers14102488]
- 17 **Huang H**, Jiang J, Chen R, Lin Y, Chen H, Ling Q. The role of macrophage TAM receptor family in the acute-to-chronic progression of liver disease: From friend to foe? *Liver Int* 2022; **42**: 2620-2631 [PMID: 35900248 DOI: 10.1111/liv.15380]
- 18 **Meshrkey F**, Scheulin KM, Littlejohn CM, Stabach J, Saikia B, Thorat V, Huang Y, LaFramboise T, Lesnefsky EJ, Rao RR, West FD, Iyer S. Induced pluripotent stem cells derived from patients carrying mitochondrial mutations exhibit altered bioenergetics and aberrant differentiation potential. *Stem Cell Res Ther* 2023; **14**: 320 [PMID: 37936209 DOI: 10.1186/s13287-023-03546-7]
- 19 **Li YP**, Liu H, Jin ZB. Generation of three human iPSC lines from a retinitis pigmentosa family with SLC7A14 mutation. *Stem Cell Res* 2020; **49**: 102075 [PMID: 33207307 DOI: 10.1016/j.scr.2020.102075]
- 20 **Deng WL**, Gao ML, Lei XL, Lv JN, Zhao H, He KW, Xia XX, Li LY, Chen YC, Li YP, Pan D, Xue T, Jin ZB. Gene Correction Reverses Ciliopathy and Photoreceptor Loss in iPSC-Derived Retinal Organoids from Retinitis Pigmentosa Patients. *Stem Cell Reports* 2018; **10**: 1267-1281 [PMID: 29526738 DOI: 10.1016/j.stemcr.2018.02.003]
- 21 **Gao ML**, Zhang X, Han F, Xu J, Yu SJ, Jin K, Jin ZB. Functional microglia derived from human pluripotent stem cells empower retinal organ. *Sci China Life Sci* 2022; **65**: 1057-1071 [PMID: 35451725 DOI: 10.1007/s11427-021-2086-0]
- 22 **Xu ZH**, Zhang H, Zhang CJ, Yu SJ, Yuan J, Jin K, Jin ZB. REG1A protects retinal photoreceptors from blue light damage. *Ann N Y Acad Sci* 2023; **1527**: 60-74 [PMID: 37531162 DOI: 10.1111/nyas.15045]
- 23 **Huang XF**, Huang F, Wu KC, Wu J, Chen J, Pang CP, Lu F, Qu J, Jin ZB. Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. *Genet Med* 2015; **17**: 271-278 [PMID: 25356976 DOI: 10.1038/gim.2014.138]
- 24 **Jones DT**. Protein secondary structure prediction based on position-specific scoring matrices. *J Mol Biol* 1999; **292**: 195-202 [PMID: 10493868 DOI: 10.1006/jmbi.1999.3091]
- 25 **Hudson JD**, Tamilselvan E, Sotomayor M, Cooper SR. A complete Protocadherin-19 ectodomain model for evaluating epilepsy-causing mutations and potential protein interaction sites. *Structure* 2021; **29**: 1128-1143.e4 [PMID: 34520737 DOI: 10.1016/j.str.2021.07.006]
- 26 **Zhang H**, Jin ZB. A rational consideration of the genomic instability of human-induced pluripotent stem cells for clinical applications. *Sci China Life Sci* 2023; **66**: 2198-2200 [PMID: 37296348 DOI: 10.1007/s11427-023-2354-6]
- 27 **Goffinet AM**, Tissir F. Seven-span Cadherins CELSR1-3. *Semin Cell Dev Biol* 2017; **69**: 102-110 [PMID: 28716607 DOI: 10.1016/j.semdb.2017.07.014]
- 28 **Zhang H**, Su B, Jiao L, Xu ZH, Zhang CJ, Nie J, Gao ML, Zhang YV, Jin ZB. Transplantation of GMP-grade human iPSC-derived retinal pigment epithelial cells in rodent model: the first pre-clinical study for safety and efficacy in China. *Ann Transl Med* 2021; **9**: 245 [PMID: 33708872 DOI: 10.21037/atm-20-4707]
- 29 **Sun L**, Li X, Tu L, Stucky A, Huang C, Chen X, Cai J, Li SC. RNA-sequencing combined with genome-wide allele-specific expression patterning identifies znf44 variants as a potential new driver gene for pediatric neuroblastoma. *Cancer Control* 2023; **30**: 10732748231175017

- [PMID: 37161925 DOI: 10.1177/10732748231175017]
- 30 **Sampson JK**, Sheth NU, Koparde VN, Scalora AF, Serrano MG, Lee V, Roberts CH, Jameson-Lee M, Ferreira-Gonzalez A, Manjili MH, Buck GA, Neale MC, Toor AA. Whole exome sequencing to estimate allelic reactivity potential between donors and recipients in stem cell transplantation. *Br J Haematol* 2014; **166**: 566-570 [PMID: 24749631 DOI: 10.1111/bjh.12898]
 - 31 **Liuyang S**, Wang G, Wang Y, He H, Lyu Y, Cheng L, Yang Z, Guan J, Fu Y, Zhu J, Zhong X, Sun S, Li C, Wang J, Deng H. Highly efficient and rapid generation of human pluripotent stem cells by chemical reprogramming. *Cell Stem Cell* 2023; **30**: 450-459.e9 [PMID: 36944335 DOI: 10.1016/j.stem.2023.02.008]
 - 32 **Li MY**, Miao WY, Wu QZ, He SJ, Yan G, Yang Y, Liu JJ, Taketo MM, Yu X. A Critical Role of Presynaptic Cadherin/Catenin/p140Cap Complexes in Stabilizing Spines and Functional Synapses in the Neocortex. *Neuron* 2017; **94**: 1155-1172.e8 [PMID: 28641114 DOI: 10.1016/j.neuron.2017.05.022]
 - 33 **Lodge EJ**, Xekouki P, Silva TS, Kochi C, Longui CA, Faucz FR, Santambrogio A, Mills JL, Pankratz N, Lane J, Sosnowska D, Hodgson T, Patist AL, Francis-West P, Helmbacher F, Stratakis C, Andoniadou CL. Requirement of FAT and DCHS protocadherins during hypothalamic-pituitary development. *JCI Insight* 2020; **5** [PMID: 33108146 DOI: 10.1172/jci.insight.134310]
 - 34 **Moore KS**, Moore R, Fulmer DB, Guo L, Gensemer C, Stairley R, Glover J, Beck TC, Morningstar JE, Biggs R, Mukherjee R, Awgulewitsch A, Norris RA. DCHS1, Lix1L, and the Septin Cytoskeleton: Molecular and Developmental Etiology of Mitral Valve Prolapse. *J Cardiovasc Dev Dis* 2022; **9** [PMID: 35200715 DOI: 10.3390/jcdd9020062]
 - 35 **Mirauta BA**, Seaton DD, Bensaddek D, Brenes A, Bonder MJ, Kilpinen H; HipSci Consortium, Stegle O, Lamond AI. Population-scale proteome variation in human induced pluripotent stem cells. *Elife* 2020; **9** [PMID: 32773033 DOI: 10.7554/eLife.57390]
 - 36 **Volpato V**, Webber C. Addressing variability in iPSC-derived models of human disease: guidelines to promote reproducibility. *Dis Model Mech* 2020; **13** [PMID: 31953356 DOI: 10.1242/dmm.042317]
 - 37 **Xie L**, Zhang K, You B, Yin H, Zhang P, Shan Y, Gu Z, Zhang Q. Hypoxic nasopharyngeal carcinoma-derived exosomal miR-455 increases vascular permeability by targeting ZO-1 to promote metastasis. *Mol Carcinog* 2023; **62**: 803-819 [PMID: 36929868 DOI: 10.1002/mc.23525]
 - 38 **Chai C**, Sui K, Tang J, Yu H, Yang C, Zhang H, Li SC, Zhong JF, Wang Z, Zhang X. BCR-ABL1-driven exosome-miR130b-3p-mediated gap-junction Cx43 MSC intercellular communications imply therapies of leukemic subclonal evolution. *Theranostics* 2023; **13**: 3943-3963 [PMID: 37554265 DOI: 10.7150/thno.83178]
 - 39 **Gemel J**, Mao Y, Lapping-Carr G, Beyer EC. Gap Junctions between Endothelial Cells Are Disrupted by Circulating Extracellular Vesicles from Sickle Cell Patients with Acute Chest Syndrome. *Int J Mol Sci* 2020; **21** [PMID: 33255173 DOI: 10.3390/ijms21238884]
 - 40 **Roballo KCS**, Ambrosio CE, da Silveira JC. Protocol to Study the Role of Extracellular Vesicles During Induced Stem Cell Differentiation. *Methods Mol Biol* 2021; **2273**: 63-73 [PMID: 33604844 DOI: 10.1007/978-1-0716-1246-0_3]
 - 41 **Wu Y**, Gu S, Cobb JM, Dunn GH, Muth TA, Simchick CJ, Li B, Zhang W, Hua X. E2-Loaded Microcapsules and Bone Marrow-Derived Mesenchymal Stem Cells with Injectable Scaffolds for Endometrial Regeneration Application. *Tissue Eng Part A* 2024; **30**: 115-130 [PMID: 37930721 DOI: 10.1089/ten.TEA.2023.0238]
 - 42 **Kilpinen H**, Goncalves A, Leha A, Afzal V, Alasoo K, Ashford S, Bala S, Bensaddek D, Casale FP, Culley OJ, Danecek P, Faulconbridge A, Harrison PW, Kathuria A, McCarthy D, McCarthy SA, Melekyte R, Memari Y, Moens N, Soares F, Mann A, Streeter I, Agu CA, Alderton A, Nelson R, Harper S, Patel M, White A, Patel SR, Clarke L, Halai R, Kirton CM, Kolb-Kokocinski A, Beales P, Birney E, Danovi D, Lamond AI, Ouwehand WH, Vallier L, Watt FM, Durbin R, Stegle O, Gaffney DJ. Common genetic variation drives molecular heterogeneity in human iPSCs. *Nature* 2017; **546**: 370-375 [PMID: 28489815 DOI: 10.1038/nature22403]
 - 43 **Carcamo-Orive I**, Hoffman GE, Cundiff P, Beckmann ND, D'Souza SL, Knowles JW, Patel A, Papatsenko D, Abbasi F, Reaven GM, Whalen S, Lee P, Shahbazi M, Henrion MYR, Zhu K, Wang S, Roussos P, Schadt EE, Pandey G, Chang R, Quertermous T, Lemischka I. Analysis of Transcriptional Variability in a Large Human iPSC Library Reveals Genetic and Non-genetic Determinants of Heterogeneity. *Cell Stem Cell* 2017; **20**: 518-532.e9 [PMID: 28017796 DOI: 10.1016/j.stem.2016.11.005]



Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: <https://www.f6publishing.com/helpdesk>
<https://www.wjgnet.com>

