# 基于诱导多能干细胞的基因编辑和细胞治疗

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**摘要** 诱导多能干细胞(induced pluripotent stem cells, iPSCs)是指分化细胞重编程恢复到类似胚胎干细胞, 具有自我更新、多向分化等潜能的一类细胞。基因编辑是对基因组特定位点进行的遗传操作, 可方便快捷地实现靶向遗传修饰。随着重编程效率提高和安全性等的完善, 对患者来源的iPSCs进行基因编辑、校正致病突变并用于细胞治疗正成为转化医学研究的热点。该文在简单介绍基因编辑原理和方法的基础上, 重点综述了近年来基于iPSCs的基因编辑和细胞治疗的研究进展, 对存在的问题进行了讨论, 并对其在再生医学领域的发展前景进行了展望。

**关键词** 诱导多能干细胞; 基因编辑; 细胞治疗

# Gene Editing and Cell Therapy Based on Induced Pluripotent Stem Cells

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**Abstract** Induced pluripotent stem cells (iPSCs) are embryonic stem cell-like cells with self-renewal and differentiation capacity, which are reprogrammed from adult cells. Gene editing for the specific sites of genome can achieve the targeted genetic modification conveniently and efficiently. With improvement of efficacy and safety in cell reprogramming, gene editing and genetic correction for the pathogenic mutation of iPSCs derived from patients are attracting increasing attention on translational medicine, which open a remarkable avenue for cell therapy. In this review, we summarized the principles of gene editing, and then introduced the advance of gene editing and cell therapy in iPSCs. Occurring challenges and perspectives were also discussed.

**Keywords** induced pluripotent stem cells (iPSCs); gene editing; cell therapy

诱导多能干细胞(induced pluripotent stem cells, iPSCs)是指分化细胞重编程恢复到类似胚胎干细胞的具有自我更新、多向分化等潜能的一类细胞。2006年, Yamanaka等[1]利用4个转录因子*Oct4*、*Sox2*、*Klf4*和*c-Myc*的组合将小鼠成纤维细胞重编程为iPSCs, 阐释了高等动物体细胞的全能型, 开拓了再生医学和药物筛选的新领域, 荣获2012年度诺贝尔生理学或医学奖。

不同的载体在诱导iPSCs过程中有不同的效果。逆转录病毒载体在重编程后期会发生沉默, 内源基因无法激活导致重编程不完全, 而且病毒基因的激活会导致肿瘤的发生[2-4]。腺病毒比逆转录病毒具有更高的感染效率, 而且能将外源基因以非插入的方式导入细胞, 在细胞中游离表达, 降低插入突变激活

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癌基因的风险[5]。慢病毒载体构建的多顺反子体系将4个转录因子连接在一个慢病毒载体上, 降低了外源基因插入突变带来的不利影响[6]。

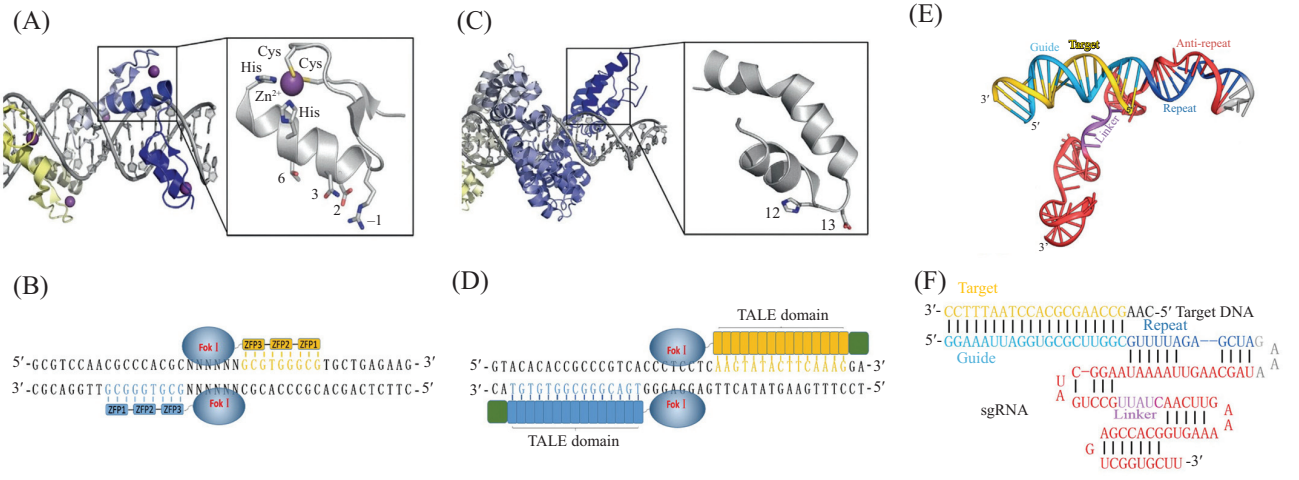
某些小分子化合物可以提高重编程效率, 如丙戊酸(valproic acid, VPA)[7]、维生素C(vitamin C, VC)[8]、aza-cytidine(AZA)[4]、iDot1L[9]、BIX/BayK[10]和Parnate[11]等通过改变表观遗传水平来提高重编程效率或速率, VPA甚至可以在不使用*c-Myc*的前提下将重编程效率提高100倍[7]。Kenpaullone[12]、LIF[13]、BIM-0086660[14]、RepSox[15]和Rapamycin[16]等则通过调控TGFβ受体、GSK3和MAPK等细胞信号通路来提高重编程效率。邓宏魁等[17]报道小分子化合物组合可使重编程效率达到0.2%。由于小分子化合物在重编程过程中并不引入外源基因, 极大地降低了iPSCs的致癌风险。另外, 不同的细胞重编程效率也有所不同[18-19]。

随着重编程效率提高和安全性等的完善, 对患者来源的iPSCs进行基因编辑、校正致病突变并用于细胞治疗正成为转化医学研究的热点。本文在简单介绍基因编辑原理的基础上, 重点综述了近年来基于iPSCs的基因编辑和细胞治疗的研究进展, 阐述了其中存在的一些问题并对其进行了展望。

## 1　基因编辑

基因编辑是对基因组特定位点进行的遗传操作。早期, 人们基于细胞自主的DNA损伤修复机制, 通过同源重组(homologous recombination)对小鼠等模式生物进行基因编辑[20], 但其效率低下, 难以广泛应用。近年来, 锌指核酸酶(zinc finger nucleases, ZFN)[21]、转录激活因子样效应物核酸酶(transcription activator-like nucleases, TALEN)[22-23]和常间回文重复序列丛集关联蛋白系统(clustered regularly interspaced palindromic repeats/CRISPR-associated proteins, CRISPR)[24-25]等新一代基因编辑技术快速发展, 使得方便快捷地实现靶向遗传修饰成为可能。它们的结构与作用机制如图1所示[26-27]。

ZFN由一个DNA位点特异性锌指结构域和一个非特异性核酸内切酶*Fok* I组合形成。锌指结构域为锌指蛋白(zinc finger protein, ZFP), ZFP能够特异性识别DNA序列。非特异性核酸内切酶*Fok* I能够切割双链DNA, 与ZFP相结合可以特异性切割DNA双链。ZFN技术已经应用于斑马鱼[28]、大鼠和小鼠[29]




A: 锌指蛋白与靶DNA(灰色)结合。–1、2、3和6位氨基酸残基与DNA直接作用。$Zn^{2+}$与半胱氨酸和组氨酸结合; B: 锌指核酸酶二聚体作用于DNA示意图。ZFN靶序列包括锌指蛋白结合序列以及5~7 bp *Fok* I结合序列; C: TALE蛋白与靶DNA(灰色)结合。每个TALE蛋白12、13位特异性识别一个碱基; D: TALEN二聚体作用于DNA示意图。TALEN靶序列包括TALE蛋白结合位点以及12~20 bp *Fok* I结合序列; E: sgRNA与靶NDA结合结构图; F: sgRNA与靶NDA结合示意图。天蓝色为crRNA导向序列, 蓝色部分为重复序列。红色部分为tracrRNA, 连接部分用紫色表示。黄色部分为目的序列。

A: ZFP combines with target DNA (grey). Amino acid residues of –1, 2, 3 and 6 sites interact with DNA directly. $Zn^{2+}$ combines with Cys and His residues; B: diagram of zinc-finger nuclease (ZFN) dimer interact with target DNA. ZFN target sequence including two ZFP binding sites and a 5~7 bp *Fok* I binding site; C: TALE protein combines with target DNA (grey). Amino acids at 12 and 13 site of each TALE protein recognize one base pair of target sequence; D: diagram of TALEN dimer interact with target DNA. TALEN target sequence including two TALE protein binding sites and a 12~20 bp *Fok* I binding site; E: sgRNA combines with target DNA; F: diagram of sgRNA interacts with target DNA. Sky blue and blue indicate the guide and repeat sequence of crRNA respectively. Red represents tracrRNA sequence, and violet represents the linker region. Yellow represents target DNA.

**图1　ZFN、TALEN和CRISPR/Cas的结构及作用机制(根据参考文献[26-27]改编)**

**Fig.1　Structure and mechanisms of ZFN, TALEN and CRISPR/Cas (modified from references [26-27])**

等模式生物的基因编辑。

TALEN是一种人工核酸酶，由转录激活因子样效应物(transcription activator-like effector, TALE)蛋白在C-端加上核酸内切酶*Fok* I改造而成。TALE蛋白包含一段1.5~35.5个能够与DNA特异性结合的氨基酸串联重复序列，重复序列的第12位和第13位的可变双残基(repeat variable di-residues, RVD)能够特异性结合一个碱基。TALE蛋白与*Fok* I核酸内切酶结合形成的融合蛋白能够特异性识别并且切割DNA。TALEN技术已经成功应用于小鼠[30]和斑马鱼[31]等各种模式生物的基因修饰。

CRISPR/Cas系统广泛存在于细菌及古生菌中，是经过长期进化形成的抵御病毒或噬菌体入侵的免疫系统，在RNA的指导下可以降解病毒或噬菌体等外源DNA。CRISPR由一系列高度保守的重复序列(repeats)与间隔序列(spacers)组成，转录形成的RNA称为CRISPR RNAs(crRNAs)，CRISPR相关基因(CRISPR associated genes, Cas genes)编码形成的Cas蛋白具有核酸酶的性质，与crRNAs共同作用可以特异性切割DNA。自2012年Jinek等[32]首次报道用于切割外源DNA靶序列后，相关研究日渐增多，目前已经报道应用于小鼠[33-34]、斑马鱼[35]等多种模式生物以及人的细胞[36-38]。

## 2 利用ZFN对iPSCs进行基因编辑

ZFN技术已经成功应用于人iPSCs的基因编辑，包括疾病特异性iPSCs和正常人的iPSCs(表1)，疾病特异性iPSCs包括血液疾病、神经系统疾病等遗传病。Chang等[39]建立了α-地中海贫血病人特异性的iPSCs，并利用ZFN技术在安全位点插入野生型*globin*，获得包含正常*globin*的iPSCs，基因编辑后的iPSCs分化形成的红细胞表达正常的β-球蛋白。Zou

**表1 利用ZFN编辑iPSCs**

**Table 1 Editing iPSCs with ZFN**

| 细胞来源<br>Cell sources | 遗传背景<br>Genetic backgrounds | 修饰基因<br>Modified genes | 作用<br>Functions | 参考文献<br>References |
|---|---|---|---|---|
| hiPSCs | α-thalassemia | Insert a globin transgene in the *AAVS1* site | Correct globin chain imbalance in erythroid cells differentiated from the corrected iPS cells | [39] |
| hiPSCs | Sickle cell disease with 2 mutated *β*-globin alleles ($\beta^S/\beta^S$) | Correct point mutation in *β*-globin ($\beta^S/\beta^S \rightarrow \beta^S/\beta^A$) | Express wild-type *β*-globin when differentiated into erythrocytes after gene correction | [40] |
| hiPSCs | PD *α*-Synuclein A53T (G209A) | Correct *α*-Synuclein mutation A53T (G209A) | Generate sets of isogenic disease | [43] |
| hiPSCs | Tauopathy carrying a TAU-A152T mutation | Correct TAU-A152T mutation | Restore neuronal and axonal morpho-logies, decrease TAU fragmentation and phosphorylation | [44] |
| hiPSCs | X-linked chronic granulomatous disease (X-CGD) | Insert $gp91^{phox}$ in the *AAVS1* site | Sustained expression of gp91 (phox) and substantially restored neutrophil ROS production | [45] |
| hIMR90/ CBMNCs | Normal | Modify chemokine (C-C motif) receptor 5 (*CCR5*), insert *tdTomato* and *CFTR* transgenes | CFTR is expressed efficiently | [46] |
| hiPSCs hESCs | Normal | Insert red fluorescent protein, firefly luciferase and herpes simplex virus thymidine kinase reporter genes in the *AAVS1* site | Successfully track the survival of ZFN-edited human embryonic stem cells and their differentiated cardiomyocytes and endothelial cells in murine models | [47] |
| hESCs hiPSCs | Normal | Insert *eGFP* and TetO-eGFP in the *Oct4* and *AAVS1* site respectively, target the *PITX3* gene | Monitor the pluripotent state of hESCs, generate a robust drug-inducible overexpression system in hESCs and reporter cells | [48] |

等[40]利用ZFN技术直接在原位点将镰刀型细胞贫血致病突变*β-globin*($\beta^S/\beta^S$)修复为*β-globin*($\beta^S/\beta^A$), 基因修复后的iPSCs分化形成的红细胞表达正常的β-球蛋白。利用ZFN技术进行基因编辑的疾病特异性iPSCs还有神经系统疾病如帕金森氏病等。除了用于校正疾病特异性iPSCs, ZFN技术还可以用于编辑正常人的iPSCs, 在特定位点插入荧光蛋白来监控、追踪细胞的存活情况或基因的表达情况, 或在安全位点插入抗生素基因建立人类胚胎干细胞药物诱导过表达体系。

ZFN技术提供了一种快速对基因进行编辑的方法, 但是其发展还存在一定局限。首先, ZFN存在上下文依赖效应[41], 因此ZFN设计和筛选效率大大降低, 高昂的成本及繁琐的程序限制了其成为实验室常规技术; 其次, ZFN的脱靶效应会导致其他DNA位点的错误切割, 产生较强的细胞毒性, 造成细胞的死亡[42], 使得其在基因治疗领域中的应用受到限制。

## 3　利用TALEN对iPSCs进行基因编辑

利用TALEN技术对iPSCs进行基因编辑, 包括疾病特异性iPSCs致病基因的修复和在正常iPSCs中插入荧光蛋白基因或者抗性基因等(表2)。Ma等[49]和Sun等[50]先后报道建立了地中海贫血特异性iPSCs细胞系和镰刀型细胞贫血特异性iPSCs细胞系, 并利用TALEN技术修复了致病基因*β-globin*上的突变, 修复后的iPSCs依然保持干性以及正常的核型, 最重要的是修复后的iPSCs分化形成β-球蛋白表达正常的成红细胞。TALEN的另一重要应用是可以在细胞中插入各种标记基因对细胞进行进一步深入的研究, 如在细胞中插入绿色荧光蛋白等标记基因来监控细胞特定基因的表达情况, 或者在细胞中插入抗性基因对细胞进行筛选等。2011年, Hockemeyer等[51]报道, 在*Oct4*位点插入*eGFP*及puromycin, 其中绿色荧光蛋白eGFP可以用来指示*Oct4*的表达情况, puromycin可以用于调控干性基因的表达, 利用两者的结合建立了药物诱导监控细胞干性基因表达系统。 2014年, Zhu等[52]报道, 利用荧光蛋白mCherry和不同神经转录因子LMX1a/FOXA2/OTX2的组合, 可以优化多能干细胞向神经细胞分化所需要的转录因子。

与ZFN技术相比, TALEN技术效率更高, 而且

**表2　利用TALEN编辑iPSCs**

**Table 2　Editing iPSCs with TALEN**

| 细胞来源<br>Cell resources | 遗传背景<br>Genetic backgrounds | 修饰基因<br>Modified genes | 作用<br>Functions | 参考文献<br>References |
|---|---|---|---|---|
| hiPSCs | *β*-Thalassemia (*β*-Thal) with a mutation in *β*-globin | Correct point mutation in *β*-globin | Gene-corrected *β*-Thal iPS cell lines can be induced to differentiate into erythroblasts expressing normal *β*-globin | [49] |
| hiPSCs | Sickle cell disease with a mutation in *β*-globin | Correct point mutation in *β*-globin | The corrected hiPSCs retain full pluripotency and a normal karyotype | [50] |
| hESCs, hiPSCs | Normal | Insert *eGFP* and puromycin in the *Oct4* site, target *PITX3* and *PPP1R12C* | Establish drug induced gene expression system to monitor cell pluripotency, and evaluate off target frequency of TALEN | [51] |
| hESCs, hiPSCs | Normal | Insert mCherry and combination of the neural transcription factors *LMX1a*, *FOXA2* and *OTX2* in the safe locus | Offer rapid, efficient and precise gene insertion in ESC and iPSCs and is particularly well suited for repeated modifications of the same locus | [52] |
| hESCs, hiPSCs | Normal | Generate mutant alleles of 15 genes | Analyze off-target efficiency of TALEN and changes of cell phenotype | [54] |
| hiPSCs | Normal | Insert *EGFP* in the safe locus | Optimize the reporter and transgene constructs, express the transgene persistently | [55] |
| hiPSCs | Normal | Transfect the targeting vector carrying the homology arms, *EGFP* gene, and a drug-selection marker in the *AAVS1* site | Evaluate the behavior of iPS-derived hepatic cells, purify hepatic cells | [56] |

对细胞的毒性也更小。TALEN技术中的TALE蛋白的两个氨基酸残基对应一个碱基, 识别DNA特异性位点比ZFN要高。TALEN技术也存在一定缺陷, 经TALEN处理以后的细胞克隆与克隆之间单核苷酸多态性位点存在一定程度的差异, 虽然不影响TALEN的使用, 但是也不可忽视[53]。

## 4 利用CRISPR/Cas对iPSCs进行基因编辑

CRISPR/Cas技术多用于正常人ESCs和iPSCs的基因编辑, 如在内源基因中产生特定的突变用于建立罕见疾病特异性的细胞模型、插入药物诱导调控的启动子来调控细胞的分化或者在安全位点插入报告基因用以测定同源重组修复效率等(表3)。Li等[57]利用CRISPR/Cas9技术成功的修复了杜兴氏肌营养不良(Duchenne muscular dystrophy, DMD)致病基因。Horii等[58]利用CRISPR/Cas技术成功地在*DNMT3B*基因中引入突变, 构建了ICF(immunodeficiency, centromeric region instability, facial anomalies syndrome)综合征iPSCs模型, 其引入突变效率高达63%。ICF综合征全球发病率较低, 对其发病机制的研究受到实验材料短缺的障碍, 利用CRISPR/Cas技术构建的ICF细胞模型为其发病机制的研究提供了珍贵的实验材料。Matsunaga等[59]利用CRISPR技术成功地在小鼠iPSCs *VEGFR2/Flk1*启动子区上游插入四环素诱导调控的启动子tet-OFF/TRE-CMV。*VEGFR2/Flk1*是内皮细胞分化的一个关键基因, 内源Flk1的表达受到TRE-CMV启动子的干扰, 随着强力霉素的消耗, Flk1的表达恢复正常, 利用该系统可以重现内皮细胞分化的表型变化。

CRISPR/Cas系统的构建比ZFN和TALEN系统的构建要简单和廉价, 普通实验室也可自行构建, 大大提高了基因操作的简便性。然而CRISPR/Cas系统碱基配对序列较短, 可能导致CRISPR/Cas系统切割特异性降低[60], 还需要进一步的改进与完善。

## 5 基于iPSCs的细胞治疗

iPSCs用于移植主要有两种方法, 一种为直接移植iPSCs, 移植后在体内周围环境的影响下分化成特定种类的细胞; 另一种是在体外将iPSCs分化形成某种特定类型的细胞或者组织器官后再进行移植。两种方法在模式生物中的应用均有报道(表4)。

Wang等[64]将iPSCs注射到脑缺血大鼠左侧脑室, 注射后的治疗反应通过正电子发射断层摄影术及神经功能测试来评估。细胞移植后的第一周和第二周, 大鼠葡萄糖代谢水平恢复正常, 神经功能测试结果显示, 大鼠神经功能得到改善。免疫组化结果显示, 移植的细胞存活下来, 迁移到缺血区域附近并且表达相关细胞蛋白标志物。这项研究表明, iPSCs可以用于细胞移植治疗。

Asgari等[65]将hiPSCs诱导形成肝细胞样细胞(hepatocyte-like cells, HLCs), 诱导形成的HLCs表达肝细胞特异性标志物, 并且表现出储存糖元、脂质

**表3 利用CRISPR/CAS编辑iPSCs**

**Table 3 Editing iPSCs with CRISPR/CAS**

| 细胞来源<br>Cell resources | 遗传背景<br>Genetic backgrounds | 修饰基因<br>Modified genes | 作用<br>Functions | 参考文献<br>References |
|---|---|---|---|---|
| hiPSCs | Normal | Obtain iPSCs with mutations in both alleles of DNA methyltransferase 3B (*DNMT3B*) | Manipulate genome of human iPSCs efficiently | [58] |
| miPSCs | Normal | Insert a tetracycline-regulated inducible gene promoter (tet-OFF/TRE-CMV) upstream of the endogenous promoter region of vascular endothelial growth factor receptor 2 (*VEGFR2/Flk1*) gene | Reproduce endothelial cell differentiation | [59] |
| hESCs, hiPSCs | Normal | Insert *tdTomato* in the safe site and *EGFP* at the *Oct4* locus | Target hPSCs accurately and efficiently | [61] |
| hESCs, hiPSCs | Normal | Target 6 genes | Measure mutation frequency after genome editing | [62] |
| 293T, K562, hiPSCs | Normal | Insert reporter gene in safe loci | Measure CRISPR targeting frequency and homologous recombination efficiency | [63] |

**表4 细胞治疗**

**Table 4 Cell therapy**

| 供体细胞/组织<br>Donor cells/tissues | 受体<br>Recipients | 移植物种及特征<br>Characters of transplanted species | 效果<br>Effects | 参考文献<br>References |
|---|---|---|---|---|
| hiPSCs | Brain | Rat (intracerebral hemorrhage, ICH) | Improve neurological function | [68] |
| hiPSCs | Myocardial | Pig (myocardial infarction) | hiPSC-derived endothelial cells contributed to vascularization | [69] |
| miPSCs | Striatum | Rat (Huntington's disease, HD) | Preserve motor function | [70] |
| miPSCs | Cerebral cortex | Rat (cerebral ischemia/ stroke) | Improve the motor function, reduce infarct size, attenuate inflammation cytokines and mediate neuroprotection | [64,71] |
| miPSCs | Knee-joint | Mouse (joint defect) | Regenerate a joint, repair bone and cartilage | [72] |
| miPSCs | Retinal | Rat (retinal ischemia and reperfusion injury) | Ameliorate retinal morphological changes, attenuate I/R-induced loss of retinal ganglion cells, and suppress the I/R-induced reduction in the ERG a- and b-wave ratio | [73] |
| miPSCs | Cochlear | Mouse (normal) | Regenerate neurons 1 week after transplantation, and express vesicular glutamate transporter 1 | [74] |
| iPSCs/hepatocyte-like cells (mouse) | Caudal vein | Mouse ($CCl_4$ injection) | Increase the numbers of proliferating hepatocytes, and express more IP-10 mRNA | [75] |
| iPSCs/hepatocyte-like cells (mouse) | Intraperitoneal | Mouse (carbon tetrachloride ($CCl_4$)-injured) | Reduce hepatic necrotic areas, and improve hepatic functions and survival rate | [76] |
| Neuroepithelial-like stem cells (human) | Spinal cord | Mouse (spinal cord injury) | Restore mouse motor function | [77] |
| Neuroepithelial-like stem cells (human) | Brain | Mouse (intracerebral hemorrhage) | Recovery of neurological dysfunction | [78] |
| Dopaminergic neurons (human) | Striatum | Rat (6-OHDA-lesioned) | Improvement of the motor behavior, without tumor formation | [79] |
| Hepatocyte-like cells (human) | Intraperitoneal | Mouse (carbon tetrachloride ($CCl_4$)-injured) | Enhancement in total serum ALB, reduction of total serum LDH and bilirubin, and improve liver general condition | [65] |
| Liver bud (human) | Cranial window | Mouse (drug-induced lethal liver failure) | Format functional vasculatures, and rescue the drug-induced lethal liver failure model | [66,80] |
| Oligodendrocyte progenitors (human) | Optic chiasm | Rat (lysolecithin-induced demyelinated optic chiasm) | Recover from symptoms and integrate and differentiate into oligodendrocytes | [81] |
| Photoreceptors (human) | Retina | Mouse (normal) | Integrate into a normal mouse retina and express photoreceptor markers | [82] |
| Dopaminergic neurons (*Cynomolgus macaque*) | Striatum | Rat (Parkinson's disease) | Integrate into the striatum and promote behavioral recover without tumor formation and inflammatory reactions | [83] |
| Hematopoietic progenitors (mouse) | Intraperitoneal | Mouse (sickle cell anemia) | Mice can be rescued after transplantation | [67] |
| Fetal liver kinase 1 positive ($Flk1^+$) cells (mouse) | Hind limb | Athymic nude mice (ischemic hind limbs) | Accelerate ischemic hind limb revascularization, increase *VEGF* mRNA expression | [84] |
| Pancreatic beta cells (mouse) | Kidney | Mouse (diabetic) | Respond to glucose by secreting insulin, normalizing blood glucose levels | [85] |

等活性, 将hiPSCs-HLCs移植到$CCl_4$致肝损伤小鼠模型后发现, hiPSCs-HLCs已经融入到小鼠肝脏中, 一周后总的血清白蛋白含量明显提升, 低密度脂蛋白和胆红素的含量比对照组低。Takebe等[66]利用hiPSCs在体外诱导形成的肝芽基(iPSCs-liver buds, iPSCs-LBs)移植到小鼠体内后产生功能性肝脏。肝细胞通过模拟器官形成过程中内皮细胞与间充质干细胞间的相互作用, 自我组装形成具有三维结构的iPSCs-LBs, 将iPSCs-LBs移植到肝坏死小鼠模型后形成功能性脉管系统及肝脏。这是人类首次报道将多能干细胞诱导形成功能性器官。要将这些技术应用于病人疾病的治疗还有很长的一段路要走, 但是器官芽基移植技术为再生医学展现了光明的应用前景。

对遗传病和基因突变导致的疾病最好的方法是校正基因的致病突变, 此方法在人的细胞中尚未

见报道, 但是在小鼠中已有报道。2007年, Hanna等[67]将小鼠的成纤维细胞重编程为iPSCs后, 再用同源重组的方法用人野生型βa-珠蛋白基因代替βs-珠蛋白基因, 最后诱导遗传修饰后的iPSCs定向分化为造血祖细胞(hematopoietic progenitors, HPs), 并将其移植到镰刀型细胞贫血小鼠体内。结果显示, HPs可有效抑制镰刀性红细胞贫血症症状。对疾病特异性iPSCs进行基因编辑校正其致病基因, 进一步分化成特定类型的细胞并用于细胞治疗, 为遗传病患者带来了新的希望。

## 6 存在的问题与展望

iPSCs可以用来建立疾病特异性的细胞模型[86]和药物筛选[87], 在再生医学领域展示了广阔的发展前景, 但是iPSCs真正应用于临床治疗还有一系列的问题需要解决。首先是安全问题, 虽然基因编辑效率越来越高, 但是脱靶效应依然存在, 脱靶效应会产生细胞毒性, 超过一定范围会造成细胞死亡[26,88]。因此, 对于需要进行遗传校正的疾病特异性iPSCs来说, 需要进一步完善基因编辑技术来降低脱靶效应的产生。其次, 若iPSCs没有完全分化为特定类型的细胞, 则残存的多能干细胞有可能导致肿瘤的形成[89]。因此, 需要建立更高效的定向分化的方法确保没有残存的干细胞, 或者筛选出已经完全分化的细胞, 来降低肿瘤形成的风险。基于iPSCs的基因编辑及细胞治疗为疾病的治疗提供了一种新的思路, 在医学领域将产生深远的影响。

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