# 介导诱导性少突胶质细胞祖细胞生成的重编程因子的研究进展

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**摘要** 少突胶质细胞(oligodendrocytes, OLs)是中枢神经系统(central nervous system, CNS)中主要的成髓鞘细胞, 其功能障碍会引发一系列的神经性疾病, 例如: 多发性硬化症(multiple sclerosis, MS)和脑白质营养不良。少突胶质细胞祖细胞(oligodendrocyte precursor cells, OPCs)的移植是治疗髓鞘相关疾病的一种潜在方法。在脑损伤后, OPCs可向OLs方向分化并对损伤部位的轴突进行髓鞘化, 但是, OPCs在大脑中仅占5%~8%, 这种髓鞘修复作用十分有限。通过体外重编程技术生成诱导性少突胶质细胞祖细胞(induced oligodendrocyte precursor cells, iOPCs)的策略可为髓鞘损伤疾病的治疗提供大量的细胞资源。但是该方法仍存在一系列亟待解决的问题, 包括iOPCs生成效率较低、体外培养周期较长等。因此, 该文从限定性转录因子、miRNA以及小分子物质等方面阐述了iOPCs的生成方法, 并分析了iOPCs的现存问题和应用前景, 旨在为其在疾病模型构建、药物开发和再生医学等方面的应用提供理论和技术参考。

**关键词** 诱导性少突胶质细胞祖细胞; 少突胶质细胞; 转录因子; miRNA; 重编程

# Reprogramming Factors Involved in Generation of Induced Oligodendrocyte Precursor Cells

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**Abstract** Oligodendrocytes (OLs) are the major type of myelin-generatingcells in the central nervous system (CNS), dysfunction of them contributes to a variety of neurological diseases, such as multiple sclerosis (MS) and congenital leukodystrophies. Transplantation of oligodendrocyte precursor cells (OPCs) is a promising therapeutic strategy for those demyelinating diseases. After the brain tissue is injured, OPCs will be differentiated into OLs which can remyelinate the axon, but this kind of repairability is very limited because there are only 5% to 8% OPCs in the brain. Reprogramming of mammalian cells into induced OPCs (iOPCs) *in vitro* is a promising strategy for remyelination, but some problems such as lower productive efficiency and long-time culture for generation of them *in vitro* need to be solved or improved. Therefore, different induced factors (including defined transcription factors, miRNAs and small molecules), problems and application prospects of iOPCs were reviewed

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in this paper in order to provide the theoretical and technological guidances for disease modeling, new drug development and regenerative medicine research.

**Keywords** induced oligodendrocyte precursor cells (iOPCs); oligodendrocytes (OLs); transcription factor; miRNA; reprogramming

神经细胞轴突外的髓鞘对于神经系统的信号传导和脑内稳态的维持至关重要。髓鞘的缺失或功能障碍会导致众多疾病, 例如儿童的脑瘫和先天性白血病、成人的多发性硬化症(multiple sclerosis, MS)和脑白质营养不良。由少突胶质细胞祖细胞(oligodendrocyte precursor cells, OPCs)分化而成的少突胶质细胞(oligodendrocytes, OLs)是中枢神经系统(central nervous system, CNS)中的主要成髓鞘细胞。由于体内OPCs的数量较少且在成人脑中维持缓慢增殖或静止的状态, 因此, 在脑损伤后, 通过OPCs分化成为OLs进而修复损伤髓鞘的能力十分有限。Yang等[1]通过体外过表达3种转录因子: 少突胶质细胞转录因子2(oligodendrocyte transcription factor 2, Olig 2)、性别决定区域Y 10 [(sex determining region Y)-Box10, Sox 10]和锌指蛋白536(zinc finger protein 536, Zfp 536)将小鼠胚胎成纤维细胞(mouse embryonic fibroblasts, MEFs)和大鼠胚胎成纤维细胞(rat embryonic fibroblasts, REFs)转化成为诱导性少突胶质细胞祖细胞(induced oligodendrocyte precursor cells, iOPCs), 为髓鞘再生机制研究和细胞移植治疗等提供了细胞来源。Fan等[2]通过体外过表达miRNA成功地将小鼠胚胎干细胞(mouse embryonic stem cells, mESCs)转化成为iOPCs, 经移植至缺髓鞘模型小鼠的脑中后, 其可以分化成为OLs, 后者可进一步成熟、生成髓鞘并重新包裹至神经轴突 上, 改善小鼠的认知能力。此后, 人子宫内膜基质细胞(human endometrial-derived stromal cells, hEnSCs)[3]、人诱导性多能干细胞(human induced pluripotent stem cells, hiPSCs)[4]、神经干细胞(neural stem cells, NSCs)[5]等也被作为生成iOPCs的细胞启动源(图1)。但是, iOPCs的生成仍存在一些亟待解决的问题, 例如重编程的整体效率较低、体外培养周期较长等。因此, 有必要进一步探讨重编程因子的作用机制, 优化重编程因子的组合, 提高iOPCs的生成效率。为此, 本文综述了iOPCs生成过程中所涉及的诱导因子(包括转录因子、miRNA和小分子物质等)、现存问题和前景展望, 旨在为脱髓鞘疾病的细胞代替治疗、疾病模型构建和药物开发等方面提供理论和技术参考。

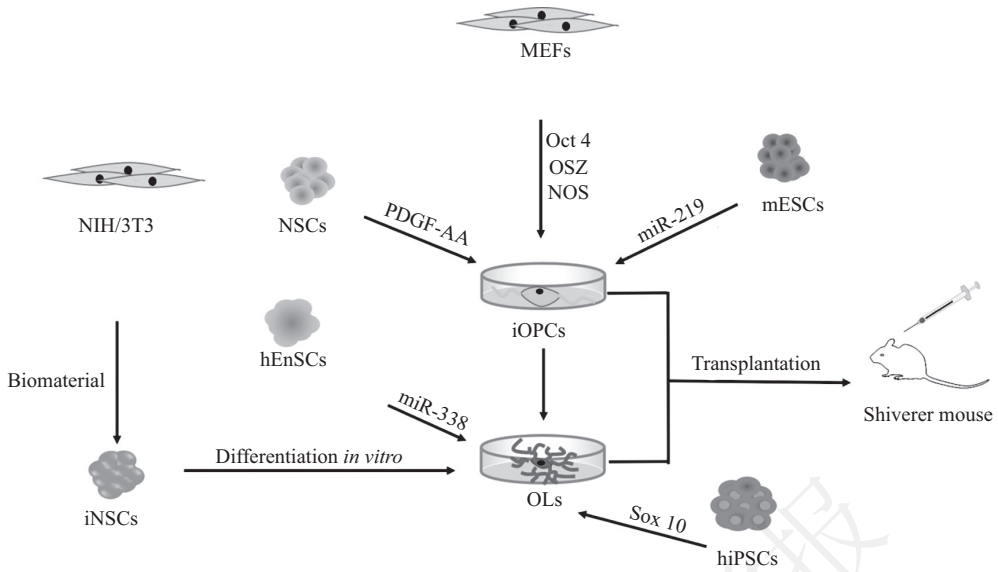
## 1 转录因子

### 1.1 Olig 2

Olig属于碱性螺旋−环−螺旋(basic helix-loop-helix, bHLH)转录因子家族, 其中Olig 1和Olig 2在脊髓和前脑中稳定表达, 而Olig 3在CNS中呈特异性且短暂性表达, 然后在发育过程中消失[6]。研究表明, Olig 2主要与OPCs的早期定向分化有关[7]。在胚胎的早期发育过程中, Olig 2通过激活*Ngn1*和*Ngn2*基因, 建立起运动神经元祖细胞的腹侧区, 决定着运动神经元的命运且促进了祖细胞向运动神经元的分化。在OPCs生成期间, Olig 2结构域中的丝氨酸147发生去磷酸化, 在OPCs向运动神经元分化过程中丝氨酸147被磷酸化。如果将丝氨酸147突变为丙氨酸, 运动神经元则无法形成, 但这并不会影响OPCs的形成[8]。p53能够提高恶性胶质瘤细胞对辐射和基因毒性药物的敏感性[9], 高水平的Olig 2能够拮抗p53的这一作用, 致使上述敏感性降低或丧失; 当Olig 2缺乏时, 恶性胶质瘤细胞经γ射线辐射后生长停止且诱发细胞凋亡[10]。Olig 2还可以激活转录因子Nkx 2.2, 促使脊髓中OPCs的形成[11]。体外研究表明, 过表达Olig 2可以加速mESCs分化成为iOPCs的进程, 经过30天的培养后, 神经/胶质抗原2(neural/glial antigen 2, NG 2)$^+$iOPCs的生成效率可达80%[12]; iOPCs还能在体外分化成为成熟少突胶质细胞(mature oligodendrocytes, mOLs)[13]; Copray等[14]通过过表达Olig 2能将NSCs体外分化成为mOLs, 移植后者至长期饲喂脑白质脱髓鞘、空泡样变性诱导剂——双环己酮草酰二腙的小鼠的胼胝体中, 可以修复该部位的髓鞘异常。因此, 过表达Olig 2可使ESCs和NSCs等重编程成为mOLs, 参与体内损伤髓鞘的再生。

### 1.2 Sox 10

哺乳动物的Sox家族包括Sox A、B、C、D、E、F、




MEFs: 小鼠胚胎成纤维细胞; mESCs: 小鼠胚胎干细胞; hiPSCs: 人诱导性多能干细胞; hEnSCs: 人子宫内膜基质细胞; NSCs: 神经干细胞; iNSCs: 诱导性神经干细胞; NIH/3T3: 小鼠胚胎成纤维细胞系; iOPCs: 诱导性少突胶质细胞祖细胞; OLs: 少突胶质细胞; PDGF-AA: 血小板源生长因子AA; OSZ: Olig 2、Sox 10和Zfp 536转录因子; NOS: Nkx 6.2、Olig 2和Sox 10转录因子。

MEFs: mouse embryonic fibroblasts; mESCs: mouse embryonic stem cells; hiPSCs: human induced pluripotent stem cells; hEnSCs: human endometrial-derived stromal cells; NSCs: neural stem cells; iNSCs: induced neural stem cells; NIH/3T3: mouse embryonic fibroblast line; iOPCs: induced oligodendrocyte precursor cells; OLs: oligodendrocytes; PDGF-AA: platelet-derived growth factor AA; OSZ: transcription factor Olig 2, Sox 10 and Zfp 536; NOS: transcription factor Nkx 6.2, Olig 2 and Sox 10.

**图1 iOPCs生成的体外重编程策略**

**Fig.1 Strategies for generation of iOPCs via reprogramming *in vitro***

G、H、I和J, 由20种蛋白质组成, Sox家族成员具有高迁移率族(high mobility group, HMG)保守盒, 其编码的蛋白质可以与DNA的特定序列结合, 从而发挥诱导、维持或抑制细胞状态的作用[15]。其中, Sox E调控神经嵴和OPCs的分化; Sox D调节心脏、骨骼肌和红细胞的分化; Sox F调节三胚层的正常形成; Sox H调控精原细胞的分化。因此, Sox家族是哺乳动物发育过程中的一个基本调节器[16]。

Sox 10属于Sox E家族, 为髓鞘特异性调控网络中的重要转录因子。髓鞘作为脊椎动物神经系统的重要组成部分, 由CNS中的OLs和外周神经系统(peripheral nervous system, PNS)中的Schwann细胞产生, 维持整个神经系统中电信号的快速传导。髓鞘的形成依赖于Sox 10, Sox 10的缺乏会导致小鼠神经嵴主干多个细胞宗系的缺损以及人类Waardenburg综合症等。在OPCs生成的早期阶段就已经开始表达Sox 10, Sox 10与Sox 9的结合保证了OPCs的存活与迁移[17]; 在髓鞘形成前期, Schwann细胞中的Sox 10能够与Oct 6协同诱导锌指转录因子Krox 20的表达, Krox 20与Sox 10的相互结合进一步促进了髓鞘的形成[18]。过表达Sox 10和Olig 2可使hiPSCs转化成为iOPCs, 后者与大鼠皮质神经元共培养时, 能够包裹神经元轴突形成髓鞘[19]; Wang等[20]通过分析神经前体细胞和OPCs中转录因子的表达情况和功能, 从中鉴定出了10个OPCs所特有的转录因子, 其中只有Sox 10能够使人神经前体细胞转变成为iOPCs; García-León等[21]仅过表达Sox 10就可以在22天内使hiPSCs生成$O4^+$细胞, 且表达髓鞘碱性蛋白(myelin basic protein, MBP)。当诱导生成的OLs与神经元共培养时, 发现在神经细胞轴突周围存在$MBP^+$细胞, 且出现多层致密髓鞘; 在OLs移植后, 提高Sox 10的表达有望促进髓鞘修复的治疗效果[22]。因此, Sox 10能够激活髓鞘形成的相关基因, 是决定OPCs终末分化和髓鞘形成的主控开关。

### 1.3 Oct 4

Oct 4又名POU5F1(POU domain, Class 5, transcription factor 1), 属于POU家族的同源域转录因子[23], 在维持ESCs的多能性方面起着至关重要的作用, 且常被作为未分化细胞的标志基因[24]。在原肠胚形成前, Oct 4就已经存在于卵母细胞、囊胚和外胚层中, 在胚胎期大

约为8.5天时, *Oct 4*在神经管中的表达水平下调, 随后*Oct 4*在原始生殖细胞中表达[25]。在桑葚胚发育为囊胚的过程中, *Oct 4*基因的缺失会破坏内细胞团特异性识别上皮细胞或原始内胚层细胞的能力, 从而无法启动胎儿的发育[26]。因此, Oct 4是起始、维持和分化ESCs的主要调控因子, 对于调节早期胚胎细胞的多能性和分化过程尤为重要。

此外, Oct 4还是神经祖细胞(neural progenitor cells, NPCs)的关键调控因子[27], Okuda等[28]发现, 通过腺病毒载体下调Oct 4的表达可以加速神经分化的进程, 而Oct 4的持续表达则可以抑制神经分化。然而, Shimozaki等[29]在缺乏白血病抑制因子(leukemia inhibitory factor, LIF)的无血清培养液中持续上调Oct 4的表达可使ESCs有效地形成神经外胚层, 且能够正常分化。因此, 不同幅度地上调或下调Oct 4的表达水平可以改变细胞的命运。Lee等[30]发现, 过表达Oct 4且结合Sma和Mad同源物(Sma and Mad homologue, SMAD)以及糖原合成酶激酶-3(glycogen synthasekinase-3, GSK-3)小分子抑制剂(SB431542、LDN-193189、Noggin和CHIR99021)的作用, 可将新生儿和成人外周血$CD34^+$细胞直接转化成为诱导性神经祖细胞(induced neural precursor cells, iNPCs), iNPCs在体内可分化成为OLs和星形胶质细胞; Kim等[31]也证明了Oct 4能够介导MEFs重编程为iOPCs, 后者表现出典型的双极形态, 且能够维持"自我更新"和表达OPCs特异性表面蛋白: A2B5、血小板源生长因子受体α(platelet-derived growth factor receptor alpha, PDGFRα)和NG 2。iOPCs在体外可以传至31代, 经小鼠体内移植可以分化生成mOLs和星形胶质细胞, 修复受损髓鞘且不会形成肿瘤。

### 1.4 NK 6同源盒转录因子2(NK 6 homeobox transcription factor 2, Nkx 6.2)

Nkx 6.2作为一种反式作用因子, 与脊椎动物神经系统的分化以及睾丸生殖细胞的发育等密切相关[32]。在早期神经发育中, 脊索信号和神经底板分泌的音猬因子(sonic hedgehog, SHH)可诱导腹侧神经管内Nkx 6.2的表达[33], 而骨形成蛋白-7(bone morphogenetic protein 7, BMP-7)则可抑制Nkx 6.2的表达。在9.5天的小鼠胚胎中, Nkx 6.2于中脑和后脑的基底部呈纵向表达, 参与中间神经元和运动神经元的调控[34]。到了胚胎发育后期, Nkx 6.2的表达更具有细胞特异性, 其主要存在于mOLs中, 在促进OLs成熟、髓鞘形成、神经元轴突−胶质细胞连接和维持髓鞘稳定等方面具有重要作用[35]。

### 1.5 Zfp 536

Zfp 536是一种高度保守的C2H2型锌指蛋白[36], 在神经系统的发育和分化中起着关键调控作用。Zfp 536在大脑皮层、海马区和下丘脑等区域均有表达, 尤其是在CNS和背根神经节中表达丰富。研究表明, 在维甲酸(retinoic acid, RA)诱导小鼠畸胎瘤P19细胞的神经分化过程中, Zfp 536能够抑制RA应答元件(RA response element, RARE)的转录活性, 进而负调控P19细胞形成神经元, 即过表达Zfp 536可抑制神经元分化, 反之则促进神经元分化[37]。此外, Zfp 536还能够促进OLs的晚期分化, 其具体机制有待进一步研究。

在哺乳动物细胞体外重编程为iOPCs的研究中, 单个转录因子虽然也能够实现谱系转化, 但是, 存在耗时长、效率低等问题。因此, 通常采用多种转录因子诱导iOPCs的生成。Najm等[38]采用慢病毒载体介导8个(Olig 1、Olig 2、Nkx 2.2、Nkx 6.2、Sox 10、ST18、Gm98/Myrf和Myt1)或3个(Nkx 6.2、Sox 10和Olig 2, 即NSO)限制性转录因子的过表达, 成功地将MEFs和小鼠肺成纤维细胞(mouse lung fibroblasts, MLFs)重编程成为iOPCs, 后者能在体外增殖5代和分化成熟。将iOPCs移植到Shiverer小鼠的前脑, 宿主轴突会产生多层致密髓鞘; 将iOPCs移植至Shiverer小鼠的背部脊柱时, 背侧脊柱白质增多, 并在背部脊柱周围产生致密髓鞘; Yang等[1]使用慢病毒载体仅过表达Olig 2、Sox 10和Zfp 536(OSZ)则可将MEFs和REFs重编程成为iOPCs, 后者不仅在形态、基因表达等方面与原代OPCs相似, 还能够在体外分化形成mOLs。同样, 将iOPCs移植到Shiverer小鼠脑中, 可形成髓鞘; Lee等[39]发现, NSO比OSZ更能重编程MEFs成为iOPCs, 前者的转化效率大约为80%。OSZ可用于REFs的重编程, 其iOPCs的生成效率大约为90%; Li等[40]采用非病毒载体聚(β-氨基酯)[Poly(β-aminoesters), PBAE]纳米颗粒介导Olig 1和Olig 2的表达, 可将人神经干细胞(human neural stem cells, hNSCs)有效分化成为OLs, 这为髓鞘形成障碍性疾病的治疗提供了一种更为安全、可靠的细胞来源。因此, 转录因子的适宜组合和转基因载体的安全性对于iOPCs的生成和应用具有重要的影响。

## 2 miRNA

miRNA是一类内源性、小分子(约22 nt)、非编码RNA, 具有转录后沉默特定mRNA的作用[41]。miRNA能够影响细胞中蛋白质表达的所有阶段, 调节细胞发育和体内平衡, 对细胞的命运决定、生长、分化、增殖和凋亡等产生广泛的影响。在OLs的发育过程中, miRNA可调节OLs的迁移、增殖和髓鞘形成等过程。除限定性转录因子外, 过表达特定的miRNA也能成功地将哺乳动物细胞重编程成为iOPCs或OLs。

### 2.1 miR-219

miR-219是OLs分化过程中高表达的miRNA, 为调控OPCs增殖及OLs分化所必需的因子。miR-219可以直接抑制促进OPCs增殖的4个靶点: PDGFRα、Sox 6、叉头框J3(forkhead box J3, FoxJ3)和锌指蛋白238(Zinc Finger Protein 238, ZFP 238), 从而促进OPCs分化成为mOLs[42]。此外, miR-219能够在一定程度上修复OPCs中miRNA缺失所引起的分化缺陷[43]。miR-219的下调可以靶向性地激活Tau通路, 通过抑制神经元的分化进而导致神经退化[44-45]。

hEnSCs是一种可大量获得、具有低免疫原性的成体干细胞。Ebrahimi-Barough等[3]使用慢病毒载体过表达miR-219将hEnSCs重编程为iOPCs; Nazari等[46]也通过过表达miR-219成功地将hiPSCs分化成为iOPCs, 后者可表达Olig 2、Sox 10、PDGFRα和A2B5等OPCs特异性蛋白, iOPCs经三碘甲状腺氨酸(triiodothyronine, T3)体外诱导可分化成为早期的OLs; Fan等[2]发现, miR-219能够通过锚定Foxj3和Zbtb18诱导mESCs向神经谱系细胞分化生成iOPCs, 将iOPCs移植到经双环己酮草酰二腙诱导出现慢性脱髓鞘小鼠的胼胝体后, iOPCs可以向胼胝体中迁移并进一步分化成熟, 表达MBP, 改善小鼠的认知能力。因此, 经miR-219诱导产生的iOPCs不仅可以促进髓鞘再生, 而且, 还能在慢性髓鞘脱失时增强内源性NPCs的增殖, 从而改善神经功能, 提高动物和人的认知能力。

### 2.2 miR-338

miR-338在mOLs、脊髓和视神经中高度表达, 参与OLs的分化以及神经系统的髓鞘修复, 在脊椎动物中高度保守。在脊髓中, 启动OLs形成的关键miRNA是miR-338, 而在大脑中更多的则是miR-219发挥作用。miR-338可以通过直接抑制OLs分化的负调控基因*PDGFRα*、*ZFP 238*、*FGF 2*、*Sox 6*和*Hes5*的表达而促进OLs的分化[47]。在*Dicer1*或*Olig 1*突变的髓鞘中, miR-338的表达显著下调, 在MS患者的脑内缺乏miR-338和miR-219的表达。虽然miR-338在CNS形成髓鞘的过程中是非必需的因子, 但是, miR-338的缺乏会加剧miR-219突变型低髓鞘的表型, 大大降低髓鞘修复能力[48]。Ebrahimi-Barough等[49]采用慢病毒载体介导hEnSCs过表达miR-338, 转染6天后细胞表达OLs谱系标志蛋白例如A2B5、PDGFα和Sox 10。因此, miR-338的下调会阻断OLs的体外成熟过程, 反之则可以促进OPCs向OLs的分化[50]。

### 2.3 其他miRNA

miR-138能够特异性地促进OPCs向OLs分化的早期阶段, 使得新生成的OLs能够充分伸展突起, 包裹轴突, 进而形成mOLs[51]。miR-138在小鼠海马体中表达较高, 且与小鼠的记忆行为相关。Tatro等[52]研究表明, miR-138可抑制乙酰蛋白硫脂酶1(acyl protein thioesterase 1, APT1)的表达, 使小鼠的记忆力减弱。Liu等[53]发现, 体内轴突的再生可通过miR-138及其下游分子靶点SIRT1进行调控: miR-138可促进哺乳动物的轴突再生, 而SIRT1可通过抑制miR-138的功能进而阻止轴突的再生。因此, miR-138和SIRT1形成了互为负反馈的轴突再生调节回路。此外, miR-7a也在OPCs中高度表达, 小鼠胚胎皮质或NPCs中过表达miR-7a会促进OL谱系细胞的生成; 抑制miR-7a的功能则使OLs的数量减少且增加了神经元的数量, 因此, miR-7a的过表达能够促进OPCs的成熟[54]。miR-9的表达水平与其靶蛋白——外周髓鞘蛋白22(peripheral myelin protein 22, PMP22)的表达呈负相关。而产生PMP22蛋白的Schwann细胞中则缺乏miR-9, 因此, 很有可能是miR-9与PMP22的3′未翻译区域相互作用并下调其表达[55]。miR-23是OLs中富含的另一种miRNA, miR-23可以通过调节丝氨酸/苏氨酸蛋白激酶(serine-threonine protein kinase, STK)和丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)通路来促进髓鞘相关蛋白的表达[56]。miR-23的上调可促进OLs的成熟, 反之可抑制OLs成熟基因的表达; 在OLs发育成为mOLs的过程中, miR-23a和miR-23b的表达量大约增加了5倍, 过表达其中之一均可促进OLs的成熟[57]。在MS病变中, miR-145的表达量异常高, 其过度表达可能会破坏髓鞘形成基因, 这可能是MS患者髓鞘修复失败的一个重要原因[58]。

目前, 体外重编程体细胞或多能干细胞生成iOPCs的策略主要采用逆转录病毒或慢病毒载体介导转录因子或miRNA的表达(表1)。然而, 外源性病毒载体和重编程因子在受体细胞基因组中的整合, 使得生成的iOPCs具有潜在的致瘤性, 从而限制了其在临床上的应用。非病毒载体因其相对安全且易于大规模生产而受到人们的广泛关注。常用的非病毒载体包括聚乙烯亚胺(polyethyleneimine, PEI)[59]、脂质体[60]和纳米颗粒[61]等, 但是其转染效率相对较低。Arsianti等[62]发现, 采用PEI包被的磁性氧化铁纳米颗粒转染乳仓鼠肾细胞(baby hamster syrian kidney cells, BHK21)时, PEI的氨基可以通过质子化作用使溶酶体的pH发生改变, 从而导致溶酶体囊泡不稳定, 避免了溶酶体对载体和外源基因的捕获, 提高其转染效率。因此, 寻求一种安全的体外重编程技术对于脱髓鞘疾病的再生治疗非常重要, 小分子物质有望提供新的重编程策略。

## 3 小分子物质

### 3.1 血小板源生长因子AA(PDGF-AA)

PDGF-AA是OPCs增殖的一个强有力的有丝分裂原, 它能够调控NSCs向OLs方向分化。研究发现, 采用神经母细胞瘤B104细胞制备的条件培养基(B104 neuroblastoma cell conditioned medium, B104CM)可以促进NSCs的分化和OPCs的增殖[63]。但是, B104CM中究竟是哪些因子促进了OPCs的增殖, 至今仍不完全清楚。Hu等[64]发现, B104细胞中存在高水平的*PDGF-AA* mRNA, 且B104CM中有高浓度的PDGF-AA蛋白。AG1295是PDGFR信号通路的一种抑制剂, 能够阻断B104CM诱导NSCs分化成为OPCs的过程。此外, PDGF-AA蛋白在体外可激活细胞外信号调节激酶1/2(extracellular signal-regulated kinases 1 and 2, Erk1/2)、磷脂酰肌醇激酶3(phosphatidylinositol-3 kinase, PI3K)和p38信号通路, 当B104CM、PDGF-AA与NSCs共培养时, 可促进其向OPCs谱系分化。因此, PDGF-AA可以通过激活Erk、PI3K、p38等信号通路调节NSCs分化成为OPCs。

### 3.2 SHH

SHH是脊髓祖细胞的有丝分裂原, 定位在神经上皮祖细胞的顶膜。SHH通过激活SHH信号通路, 促进脑源性神经营养因子(brain derived neurotrophic factor, BDNF)的表达, 进而促进神经元的增殖和分化。在OPCs谱系细胞的发育过程中, SHH是促进OPCs形成和增殖的关键因子, 当*SHH*基因下调或SHH信号失活时, OPCs将从增殖状态转变成为分化状态[65]。胚胎腹侧脊髓中OPCs的生成依赖于硫酸酯酶1(sulfatase 1, Sulf 1)的活性, Sulf 1能够激活SHH信号通路, SHH可以诱导脊髓腹侧的运动神经元祖细胞(progenitor of motor neuron, pMN)域表达Olig 2转录因子, 从而生成第一波OPCs, 以后在很长一段时间内OPCs的生成仍然取决于SHH的活性[66]。RA也能促进OPCs的维持[67]。此外, 在胚胎发育时期, SHH能够调控OPCs中Olig 1/2的表达; 在整个成年期, SHH信号对OPCs的产生和招募以及由溶血卵磷脂引起的局部脱髓鞘病变的修复都发挥着重要作用。Bian等[68]发现, 当SHH与成纤维细胞生长因子2(fibroblast growth factor 2, FGF-2)和PDGF-AA联合使用时, 可诱导神经上皮样干细胞(neural epithelia-like stem cells, NESCs)重编程成为iOPCs, 后者能在体外进一步分化成为具有迁移能力的OLs。

### 3.3 成纤维细胞生长因子(fibroblast growth factor, FGF)

FGF在调控OPCs的增殖和分化等方面具有重要作用。FGF能够诱导NSCs中*Olig 2*基因的表达, 使得NSCs向OPCs谱系分化, 在腹侧脊索生成第一波OPCs之后, FGF信号开始被激活, 激活的FGF信号在受体酪氨酸激酶(receptor tyrosine kinases, RTKs)的介导下激活MAPK和Erk1/Erk2激酶, 进而促进了OPCs的增殖与迁移能力。此外, FGF还可以通过抑制BMP信号通路使得背侧脊索相继生成OPCs[69], 而抑制Erk1/Erk2信号通路则会出现OLs的缺失, 这表明FGF对于OLs谱系的分化至关重要。虽然FGF和SHH均能调控OPCs的形成、增殖和分化, 但是, 体外实验表明, 当FGF作用于神经球时, 即使有环巴胺(SHH信号通路的有效抑制剂)存在也无法阻止神经球向OLs方向分化。体内实验也发现即使是SHH缺失的小鼠, 当存在FGF时, 其神经球也会分化生成OLs。因此, 依赖于FGF的OLs谱系细胞生成过程独立于SHH的作用[70]。Chen等[71]发现, 由Schwann细胞分泌的FGF-2和PDGF-AA可诱导OPCs的增殖和迁移。因此, 深入了解PDGF-AA、SHH和FGF等一系列小分子物质的作用, 不仅有助于探讨OPCs的增殖和迁移机制, 而且为脱髓鞘疾病的再生治疗提供新的靶点。

**表1 重编程因子和载体介导细胞体外重编程生成iOPCs及神经谱系细胞的策略**

**Table 1 Strategies for generation of iOPCs and nerve lineage cells via *in vitro* programming mediated by reprogramming factors and vectors**

| 载体类型 Carrier types | | 重编程因子 Reprogramming factors | 初始细胞 Initial cells | 目的细胞 Target cells | 参考文献 References |
|---|---|---|---|---|---|
| Viral vector | Lentiviral vector | Olig 2 | mESCs | iOPCs | [13] |
| | | Sox 10 | hiPSCs | OLs | [21] |
| | | Olig 2 and Sox 10 | hiPSCs | iOPCs | [19] |
| | | Oct 4 | Human blood $CD34^+$ cell | iNPCs | [30] |
| | | Nkx 6.2, Sox 10 and Olig 2 | MEFs/MLFs | iOPCs | [38] |
| | | Olig 2, Sox 10 and Zfp 536 | MEFs/REFs | iOPCs | [1] |
| | | miR-219 | hEnSCs | iOPCs | [3] |
| | | | hiPSCs | | [2] |
| | | | mESCs | | |
| | | miR-338 | hEnSCs | OLs | [49] |
| | Retrovirus vector | Oct 4 | MEFs | iOPCs | [31] |
| Non-viral vector | Poly (β-amino ester) (PBAE)-based nanoparticle | Olig 1 and Olig 2 | hNSCs | OLs | [40] |
| | Electroporation | Olig 2 | NSCs | mOLs | [14] |
| Free-carrier | / | Biological materials (gelatin, nano-HA, pig brain extract) | NIH/3T3 cell | iNSCs | [72] |
| | / | Small molecules (SHH, FGF-2, PDGF-AA) | NESCs | iOPCs | [68] |

Nano-HA: 纳米羟基磷灰石; SHH: 音猬因子; FGF: 成纤维细胞生长因子; PDGF-AA: 血小板源生长因子; mESCs: 小鼠胚胎干细胞; hiPSCs: 人诱导性多能干细胞; MEFs: 小鼠胚胎成纤维细胞; REFs: 大鼠胚胎成纤维细胞; MLFs: 小鼠肺成纤维细胞; hEnSCs: 人子宫内膜基质细胞; hNSCs: 人神经干细胞; NSCs: 神经干细胞; NIH/3T3 cells: 小鼠胚胎成纤维细胞系; NESCs: 神经上皮样干细胞; iOPCs: 诱导性少突胶质细胞祖细胞; OLs: 少突胶质细胞; iNPCs: 诱导性神经祖细胞; mOLs: 成熟少突胶质细胞; iNSCs: 诱导性神经干细胞。

Nano-HA: nano-hydroxyapatite; SHH: sonic hedgehog; FGF: fibroblast growth factor; PDGF-AA: platelet-derived growth factor AA; mESCs: mouse embryonic stem cells; hiPSCs: human induced pluripotent stem cells; MEFs: mouse embryonic fibroblasts; REFs: rat embryonic fibroblasts; MLFs: mouse lung fibroblasts; hEnSCs: human endometrial-derived stromal cells; hNSCs: human neural stem cells; NSCs: neural stem cells; NIH/3T3 cells: mouse embryonic fibroblast cells line; NESCs: neural epithelia-like stem cells; iOPCs: induced oligodendrocyte pecursor cells; OLs: oligodendrocytes; iNPCs: induced neural progenitor cells; mOLs: mature oligodendrocytes; iNSCs: induced neural stem cells.

## 4 问题与展望

由OPCs分化而来的OLs是CNS中重要的成髓鞘细胞, 它能够包绕神经元轴突形成髓鞘, 保证神经信号的快速传导。由于脑内OPCs的数量十分有限, 原代OPCs的获得具有一定的挑战性。通过过表达重编程因子, 现已成功地将NSCs、mESCs、REFs、MEFs和hEnSCs等细胞转化成为iOPCs谱系细胞, iOPCs的体内移植试验也表明, 其可以进一步分化成熟, 形成多层致密髓鞘, 改善缺髓鞘小鼠的认知能力[2], 促进大鼠脊髓损伤后的功能恢复[72]。此外, 体外培养的OPCs或iOPCs能够用于构建糖氧剥夺模型, 这为探讨机体OLs的缺血、缺氧损伤分子机制和细胞凋亡机制提供了良好的研究方法[73], 而且它们还能为促髓鞘修复、抗精神病等的药物筛选建立高通量模型, 加速药物研发速度[74]。因此, iOPCs在再生医学、疾病模型构建以及药物开发等方面具有广阔的应用前景。

但是, 目前iOPCs的生成和应用仍存在一些问题有待解决: (1)利用重编程技术生成功能性iOPCs的效率整体较低, 仅有25%左右; (2)iOPCs在体外培养周期较长, 增加了基因突变的风险, 而且, 生成的iOPCs中通常还混杂有神经细胞, 不适用于细胞的移植治疗; (3)鉴定iOPCs分化阶段的技术比较单一, 大多依赖于细胞表面抗原的表达; (4)在体内损伤微环境中, 如何实现iOPCs的高效增殖和定向分化? (5)如何促进iOPCs来源OLs的体内成熟和功能化? (6)在过表达Olig 2、Sox 10、Nkx 6.2以及miRNA等重编程因子时, 大多采用了病毒载体介导iOPCs的生

表2 iOPCs生成所涉及主要重编程因子的作用机理及功能

**Table 2 Action mechanisms and functions of major reprogramming factors involved in generation of iOPCs**

| 重编程因子<br>Reprogramming factors | 作用机理<br>Action mechanisms | 功能<br>Functions | 参考文献<br>References |
|---|---|---|---|
| Olig 2 | Activating *Ngn1* and *Ngn2* genes | Regulating the proliferation and differentiation of OPCs | [11] |
| Sox 10 | Activating MCS5-Sox 10 enhancer region Combining with its target Oct 6 to induce the expression of Krox 20 | Inducing the myelination | [21] |
| Oct 4 | Regulating SMAD and GSK-3β signal pathway | Promoting the proliferation and self-renewal of OPCs | [30] |
| Zfp 536 | Binding to RARE in the promoters of downstream genes | Negatively regulating the differentiation of neuron | [37] |
| miR-9 | Interacting with 3'untranslated region of PMP22 and down-regulating its expression | Inhibiting the differentiation of OLs | [55] |
| miR-23 | Activating the STK and MAPK pathways which promote the expression of myelin genes | Promoting the maturity of OLs | [56] |
| miR-219 | Inhibiting *Foxj3* and *Zbtb18* genes | Regulating the proliferation and differentiation of OPCs | [42] |
| miR-338 | Inhibiting *Sox6* and *Hes5* genes | Regulating the proliferation and differentiation of OPCs | [50] |
| PDGF-AA | Activating *PI3K*and *Erk1/2/P38* genes | Promoting the proliferation and self-renewal of OPCs | [63] |
| SHH | Activating SHH signal pathway and promoting the expression of *BDNF* gene | Regulating the proliferation and differentiation of nerve cells and axonal formation | [64] |
| Sulf 1 | Promoting the expression of *Olig 1/2* genes | Activating the motor neuron-to-OPCs fate switch | [66] |
| FGF | Activating MAPK kinase and Erk1/Erk2 kinase | Promoting the proliferation and migration of OPCs | [69] |

SHH: 音猬因子; FGF: 成纤维细胞生长因子; PDGF-AA: 血小板源生长因子AA; MCS: 多物种保守序列; SMAD: Sma和Mad同源物; GSK-3β: 糖原合成酶激酶-3β; RA: 维甲酸; RARE: RA应答元件; PMP22: 外周髓鞘蛋白; STK: 丝氨酸/苏氨酸蛋白激酶; FoxJ3: 叉头框J3; Erk1/2: 细胞外信号调节激酶1/2; PI3K: 磷脂酰肌醇激酶3; BDNF: 脑源性神经营养因子; MAPK: 丝裂原活化蛋白激酶; Sulf 1: 硫酸酯酶1; OPCs: 少突胶质细胞祖细胞。

SHH: sonic hedgehog; FGF: fibroblast growth factor; PDGF-AA: platelet-derived growth factor AA; MCS: multi-species conserved sequences; SMAD: Sma and Mad homologue; GSK-3β: glycogen synthasekinase-3β; RA: retinoic acid; RARE: RA response element; PMP22: peripheral myelin protein 22; STK: serine-threonine protein kinase; FoxJ3: forkhead box J3; Erk1/2: extracellular signal-regulated kinases 1 and 2; PI3K:phosphatidylinositol-3 kinase; BDNF: brain derived neurotrophic factor; MAPK: mitogen-activated protein kinase; Sulf 1: sulfatase 1; OPCs: oligodendrocyte precursor cells.

成, 存在较为严重的生物安全隐患, 限制了其在临床中的应用。最近Kantawong等[75]采用由明胶、纳米羟基磷灰石(nano-hydroxyapatite, Nano-HA)和猪脑提取物制成的生物材料, 在无病毒载体介导重编程因子表达、不添加特殊化学诱导剂的情况下, 实现了NIH/3T3细胞向神经谱系细胞iNSCs的转化, 后者可以进一步分化成为OLs, 这为修复髓鞘缺失提供了新思路。因此, 在未来的研究中, 需要进一步探讨重编程因子的功能和作用机制(表2), 优化重编程因子的组合, 特别是探寻完全基于小分子物质的谱系转化方法, 提高非病毒载体介导的重编程效率, 加大iOPCs体内移植后的微环境影响、分化过程跟踪和功能研究等, 为脱髓鞘疾病的再生治疗提供更为安全、高效的新策略。

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