



脊椎动物视神经损伤修复与再生

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摘要 视觉是脊椎动物接收外界信息的主要来源之一。视神经连通视网膜和大脑, 将视觉信息传递至大脑皮层相应区域。通常认为哺乳动物视网膜神经节细胞及视神经是无法再生的, 其损伤是导致失明的主要原因。视神经轴突的再生受到许多外在和内在因素限制, 解除外在抑制因素, 激活内源再生潜力, 能够使小部分轴突成功再生, 并改善部分视功能。此外, 通过移植体外干细胞诱导分化的视网膜神经节细胞, 或者体内重编程穆勒细胞和无长突细胞定向生成视网膜神经节细胞, 均已实现了一定程度的视网膜神经节细胞再生, 且可以恢复部分视功能, 在青光眼等神经退行性疾病的治疗方面展现出诱人的前景。本文通过调研近年来国内外相关文献, 针对脊椎动物视神经损伤后轴突再生及视网膜细胞再生相关研究进行综述。

关键词 视神经损伤, 轴突再生, 细胞再生, 视网膜神经节细胞

视觉作为重要的感官之一, 很大程度地影响着人们的生活。视觉始于视网膜, 光感受器细胞将光信号转换为电信号, 经中间神经元双极细胞将信息传递到输出神经元视网膜神经节细胞(retinal ganglion cells, RGCs)。如果RGC死亡或功能失调, 视觉编码信号将无法正常传输到大脑, 导致视觉减退甚至失明。RGC轴突汇集形成视神经, 直接连通视网膜和大脑皮层, 容易受到头部撞击等物理性损伤, 以及眼和中枢神经系统相关疾病的损害^[1]。在人类青光眼等常见视网膜神经退行性疾病中, RGC和视神经的损伤通常是进行性的和不可逆的。因此, 这类疾病的视觉修复研究集中在损伤后RGC的维持与再生, 以及促进其轴突再生长和重

建正确的神经轴突投射。

视神经损伤后RGC的存活是视功能修复的基础, 研究人员针对RGC的维持提出了多种方案, 外源神经营养因子的施用和细胞内离子稳态及代谢的平衡等, 都是维持RGC存活的重要因素。此外, 研究人员也已提出多种在体外实现RGC诱导或在体内实现RGC再生的策略。

轴突的再生对于神经损伤后神经元连接的恢复以及功能性视觉系统的重建至关重要。然而, 尽管鱼和爪蟾等低等脊椎动物中神经系统可再生, 但是哺乳动物的RGC轴突在受损后的再生能力非常有限^[2], 轴突再生始终限制在小于10%的受损RGC上^[3]。研究人员已

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经发现了部分限制或促进轴突再生的重要信号通路和分子, 可以提供新的治疗靶标, 并通过组合治疗实现更强的再生. 此外, 很多物种的中枢神经系统似乎都存在潜在的成体干细胞^[4]. 研究表明, 在某些情况下, 成熟RGC的内在生长能力可以被激活, 使这些神经元能够存活并在受损的视神经中再生轴突且进入其正确的目标区域, 从而恢复部分视力. 内在生长能力的重新激活也被认为是轴突再生的重要策略^[5].

本文旨在讨论和总结脊椎动物视觉系统中, 视神经损伤后抑制轴突再生的因素, 轴突再生的技术及其分子机制, 以及实现RGC的存活和细胞再生的最新进展.

1 轴突再生

轴突的再生和可塑性会受到诸多因素的影响, 包括由神经元表达的内在生长信号, 以及围绕神经元的外环境^[6]. 鱼和爪蟾等低等脊椎动物中神经系统可再生, 而成年哺乳动物缺乏强大的再生能力的原因可能以下几点: (i) 在低等脊椎动物中, 神经系统受到损伤后内环境中抑制因素低. 对非洲爪蟾中RGC转录组测序表明, 视神经受到损伤后, 微管蛋白(*tubulin*), *Gap43*和*Klf6*等Jak-Stat通路中与再生相关的基因表达上调, 而再生抑制因子*Klf4*表达下调^[7]. 对比鱼类和哺乳动物损伤后RGC中基因表达发现, 促凋亡因子和抗凋亡因子的表达存在显著差异, 如磷脂酰肌醇-3-激酶系统中的抗凋亡因子phospho-Akt和phospho-Bad在鱼类视神经损伤后增加, 而在大鼠中减少^[8]; 同样, 磷脂酰肌醇3-激酶系统中的关键激活因子胰岛素样生长因子1(*insulin-like growth factor-1*, IGF-1)^[9]和热休克蛋白(*heat shock protein*, HSP)也有类似的表达差异^[10], 对HSP活性的抑制作用可阻止抗凋亡蛋白Bcl-2的表达, 并增加凋亡蛋白Bax的表达水平^[11]. (ii) 哺乳动物中视神经损伤引起的神经胶质瘢痕抑制轴突再生. 少突胶质细胞分泌的髓磷脂等是哺乳动物中枢神经系统中轴突再生的抑制剂^[12-15], 而鱼类和两栖动物的少突胶质细胞没有这种抑制特性^[16]. (iii) 低等脊椎动物具有更强的细胞固有生长能力^[17]. 在蜥蜴或大鼠神经胶质细胞培养物上培养蜥蜴或大鼠神经节细胞外植体, 蜥蜴神经节细胞生长锥在培养基中生长, 而大鼠神经元则受到了少突胶质细胞的抑制^[18]. (iv) RGC存活能力

不同. 与哺乳动物不同, 低等脊椎动物中, 视神经损伤仅导致少量RGC死亡^[19]. 以上几点提示, 可以通过克服抑制性信号并激活成熟RGC的再生潜能来促进轴突再生^[20].

1.1 影响轴突再生的抑制性因素

随着中枢神经系统的成熟, 神经元的细胞外环境会以限制再生的方式发生变化^[21]. 轴突再生失败的原因复杂, 部分原因是损伤后, 细胞外基质中髓磷脂相关抑制剂(myelin associated inhibitors, MAIs)、硫酸软骨素蛋白聚糖(chondroitin sulfate proteoglycans, CSPGs)和化学驱除蛋白(semaphorin 6A和ephrin B3等)等抑制轴突再生的抑制性配体表达上调. 神经元生长锥受体与抑制性配体结合后, 激活RhoA等胞内信号传导途径, 从而扰乱肌动蛋白细胞骨架导致生长锥崩塌, 抑制轴突再生^[22,23]. 下文总结影响轴突再生常见的抑制性因素.

(1) 髓磷脂相关抑制剂. 髓磷脂相关抑制剂及其下游信号通路对轴突生长有抑制作用. Nogo, 少突胶质细胞髓磷脂糖蛋白(oligodendrocyte myelin glycoprotein, OMgp)和髓磷脂相关糖蛋白(myelin associated glycoprotein, MAG)等均被鉴定为能够使轴突生长锥塌陷并抑制神经突向外生长的髓磷脂相关抑制剂^[24]. 这些分子可以通过结合白细胞免疫球蛋白样受体B2(leucocyte immunoglobulin-like receptor B2, PirB)发挥作用. 此外, NgR家族受体NgR1也是三个髓鞘相关抑制剂的受体^[25,26]. 其配体中Nogo-A蛋白最为重要, Nogo-A蛋白由两个主要的细胞外结构域组成: Nogo-66和Nogo-A-Δ20; Nogo-66通过结合受体NgR1和PirB激活RhoA信号通路, 抑制轴突再生; Nogo-A-Δ20与S1PR2结合, 从而抑制突触可塑性并稳定神经元回路^[27], 也可以通过与EphA4相互作用来影响神经细胞的凋亡信号^[28].

抑制髓磷脂相关抑制剂及其受体的信号传导途径可有效增强中枢神经系统轴突的再生和发芽. 选择性抑制Nogo或NgR1可以提高轴突的再生能力^[29]. 在视神经损伤模型中, *Nogo-A/B/C*敲除小鼠中RGC的轴突可以再生^[30], 而在不同细胞中敲除*Nogo*, 再生效果不同, 如少突胶质细胞中特异性敲除*Nogo-A*可使视神经轴突再生显著增加, 但在RGC中特异性敲除*Nogo-A*反而会降低轴突再生效果, 这为优化轴突再生策略提

供了参考^[31]。

(2) 反应性瘢痕及相关成分。胶质瘢痕的主要成分是反应性星形胶质细胞, 其分泌的CSPG等抑制分子, 是轴突再生的有效抑制剂, 也是用于治疗颅脑损伤或脊髓损伤的重要分子靶标^[32,33]。转化生长因子 β (transforming growth factor β 1, Tgfb1)是反应性星形胶质细胞胶质化的介导者, 它能活化CSPG的上游激活物, 抑制Tgfb1信号可以减少胶质瘢痕的形成^[34]。CSPG由多种核心蛋白和与核心蛋白相连的糖胺聚糖(glycosaminoglycans, GAGs)侧链组成, GAG侧链以高亲和力与几种跨膜受体结合, 可以通过神经再生抑制性受体蛋白酪氨酸磷酸酶受体 σ (PTP σ)和白细胞共同抗原相关的蛋白酪氨酸磷酸酶(LAR), 调控RhoA, Akt, GSK-3 β 和蛋白激酶C(PKC)等通路, 因此拮抗CSPG受体是轴突再生的良好策略^[15,35]。在脊髓损伤模型中, 通过用软骨素酶ABC(ChABC)消化其GAG侧链, 已证明有利于轴突再生, 为视神经损伤修复提供有效思路^[36]。而小鼠视神经损伤刺激星形胶质细胞反应, CSPG表达持续升高^[26], 使用芳基硫酸酯酶B(ARSB)从GAG链的非还原端去除4S基团以减少CSPG的沉积, 同样可以增强轴突再生^[37]。此外, 也有研究表明, CSPG可以通过使PI3K/Akt途径失活抑制轴突再生^[38], 它可以降低Akt磷酸化水平, 并提高该途径负调节信号Eif4ebp1的表达水平^[39], CSPG还激活表皮生长因子受体(epithelial growth factor receptor, EGFR)途径^[40], 为CSPG抑制轴突再生提供理论依据和潜在靶点。

(3) 抑制性轴突导向分子。此外, 在发育过程中轴突延伸时, 其生长锥会感应到由环境表达的一系列引导分子, 从而将其引导至正确的目标。损伤后, 神经胶质细胞产生抑制性轴突导向分子, 包括Ephrin B3和A4, Semaphorin 3A, 3D和5A等。这些导向分子能被轴突生长锥表面的导向受体识别, 通过调节Rho GTPases活性介导轴突生长锥塌陷和抑制轴突的生长^[41]。抑制这些导向分子可以促进轴突再生, 例如, 从真菌菌株的发酵液中鉴定出Sema3A的强效选择性抑制剂SM-216289, 应用到脊髓损伤动物模型中可明显增强受损轴突的再生^[42]。而在小鼠中, Sema3A通过ROCK2抑制RGC轴突再生, 利用ROCK2抑制剂Y-27632可以减弱Sema3A的抑制作用, 促进视神经损伤后RGC轴突再生^[43]。

(4) 抑制性转录因子。RGC轴突生长能力的下降

也与内源基因表达变化相关。Klf(Krüppel-like transcription factor)转录因子家族是其中重要的影响因素, 17个成员中至少有15个在RGC中表达, 不同Klf转录因子的表达可以增强或抑制神经突生长。其中, Klf1, 2, 4, 5, 9, 13, 14, 15, 16等对神经突生长有抑制作用, 而Klf6, 7则可以促进神经再生^[44,45]。Klf4是其中研究较多的因子, 属于“AIN”亚家族, 在氨基末端具有酸性激活和抑制结构域, 在羧基末端具有Cys2His2锌指DNA结合结构域。其活性可受Erk1和Erk2磷酸化负调控^[46], Klf4在小鼠出生前后表达增加, 与RGC失去其固有轴突生长能力的时间重叠, 在抑制轴突生长中起重要作用。在胚胎RGC中, Klf4的过表达可以减少神经突伸长的百分比、轴突和树突的长度以及神经突分支; 早期发育中, 其过表达导致海马和皮层神经元以及RGC的神经突增生明显减少, 而其敲除导致视神经损伤后体内轴突再生增加^[44,47]。Klf4条件敲除小鼠的轴突束比对照小鼠厚, 但其敲除对视神经损伤后RGC的存活没有影响^[48]。Klf4可能是通过Jak-Stat3途径影响轴突再生^[49], Klf4通过p53和Jak-Stat3途径正向调控神经元凋亡, 而通过Jak-Stat3途径负向调控轴突再生修复^[50]。出生后, 小鼠中Klf6和Klf7的表达下降, 病毒介导的Klf7过表达可以诱导皮质脊髓束中轴突的再生^[51]。与Klf7相反, Klf9在小鼠出生后表达增加了250倍, 对轴突再生具有重要抑制作用^[44]。利用shRNA敲低Klf9的表达可促进视神经损伤后RGC轴突的再生^[52]。Klf9通过促进表达双重特异性磷酸酶14(dual specificity phosphatase 14, Dusp14)来抑制RGC轴突生长; Dusp14使MAPK(mitogen-activated protein kinase)家族成员去磷酸化, 其激活会抑制MAPK-Erk1/2, 进而降低RGC胞体和轴突的存活率, Dusp14的下调则可以促进体内视神经损伤后的轴突再生^[53]。Klf13的抑制功能部分通过抑制cAMP信号通路实现^[54-56]。该亚类的另一个抑制因子Klf16通过促进Epha5的表达, 使RGC轴突生长锥塌陷进而起到抑制轴突再生的作用^[57]。

髓磷脂相关抑制剂、反应性瘢痕及相关成分、抑制性轴突导向分子及转录因子等, 都是影响轴突再生常见的抑制性因素, 对这些因素的阻遏可以作为提高轴突再生能力的手段(表1)。

1.2 轴突再生潜能的激活

在发育过程中RGC轴突快速生长并连接大脑, 表

表 1 轴突再生的抑制因素

Table 1 Inhibitory factors for axon regeneration

轴突再生抑制分子类型	名称	作用位点	涉及通路	参考文献
髓磷脂相关抑制剂 (MAIs)	Nogo			
	少突胶质细胞髓磷脂糖蛋白(OMgp)	白细胞免疫球蛋白样受体 B2(PirB), NgR家族受体	RhoA	[24,25,27,28]
	髓磷脂相关糖蛋白(MAG)			
反应性瘢痕	硫酸软骨素蛋白聚糖 (CSPGs)	Nogo及蛋白酪氨酸磷酸酶受体σ (PTPσ)和白细胞共同抗原相关的蛋白酪氨酸磷酸酶(LAR)等	RhoA, Akt, GSK-3β, PKC, EGFR等	[15,26,37~39]
抑制性轴突导向分子	Ephrin B3/A4 Semaphorin 3A/3D/5A	Rho GTPases	Rho	[41]
抑制性转录因子	Klf家族等		Jak-Stat3, MAPK-Erk1/2, cAMP等	[49~56]

明它们具有内在的促轴突生长因素^[58]。当RGC从胚胎期过渡到出生后时,其轴突的生长速度下调了1000倍以上,这可能是由于RGC固有的分子程序所致^[59]。多条重要信号通路参与轴突再生过程(图1),操纵内在的生长控制途径可以作为促进视神经损伤后轴突再生的治疗方法^[60]。

(1) Jak-Stat3. Jak-Stat3信号通路在外周神经细胞轴突切断后被激活,对轴突损伤修复和神经元存活非常重要,由受体相关的Jak酪氨酸激酶和潜在的Stat细胞质转录因子组成。细胞因子与其同源受体的结合触发酪氨酸磷酸化和Jak的激活,然后Stat也被磷酸化。活化的Stat单体形成二聚体,迁移至细胞核并与调节基因转录的特定DNA靶序列结合^[61]。Socs分子抑制细胞因子激活的Jak-Stat信号传导,敲除Socs3可以促进轴突再生^[62]。

睫状神经营养因子(ciliary neurotrophic factor, CNTF)也可以通过激活包括RGC在内的视网膜神经元中Stat3的磷酸化激活Jak-Stat3信号通路^[63],一些研究表明CNTF对体内再生效果影响较弱^[64],可能是由于RGC成熟时Jak-Stat信号通路的阻遏物Socs3增加导致的,而Socs3的缺失则会提高CNTF的促再生能力^[62]。最近研究表明,趋化因子Ccl5(chemokine (C-C motif) ligand 5)可以介导CNTF的促再生作用。CNTF的基因疗法可以提高免疫细胞和视网膜胶质细胞中Ccl5的表达,敲低或抑制Ccl5可以抑制CNTF引发的再生作用^[65]。此外,IL-6也是成熟RGC的神经保护因子和有效的神经突生长促进因子,可通过克服髓磷脂介导的

抑制作用刺激轴突再生^[66]。通过腺相关病毒(adeno-associated virus, AAV)递送hyper-IL-6(hIL-6),也可以刺激Jak-Stat3信号通路,引发轴突再生^[67]。对视网膜胚胎发育过程至关重要的Wnt信号,也通过Jak-Stat通路影响RGC轴突再生。例如玻璃体内注射Wnt3a促进Stat3信号介导的RGC的再生和存活^[68],利用shRNA-AAV抑制IL-22也可以通过激活Stat3信号通路,促进视神经损伤后的轴突再生^[69]。

(2) PI3K-Akt. 视神经损伤后,RGC中CNTF和白白血病抑制因子(leukemia inhibitory factor, LIF)等生长因子高表达,使得受体酪氨酸激酶(receptor tyrosine kinase, RTK)激活磷脂酰肌醇3激酶(phosphoinositide 3-kinase, PI3K)以促进细胞生长和存活^[70]。PI3K进一步将磷脂酰肌醇4,5-双磷酸酯(PIP2)磷酸化为磷脂酰肌醇(3,4,5)-三磷酸酯(PIP3)。PIP3通过同源结构域将Akt募集到膜上,Akt进而被磷酸肌醇依赖性激酶1(pyruvate dehydrogenase kinase 1, Pdk1)在T308处磷酸化^[71]。Akt的激活通过抑制其下游效应因子结节性硬化复合物1和2异二聚体(tuberous sclerosis 1/2, Tsc1/Tsc2),激活mTOR(mechanistic target of rapamycin kinase)复合物1(mTOR complex 1, mTORC1)。mTORC1是PI3K-Akt在调节细胞生长过程中的关键下游信号,mTORC1的两个最具特征的底物,真核起始因子4E结合蛋白Ei-f4ebp1/2的抑制和核糖体蛋白S6激酶1(ribosomal protein S6 kinase 1, S6k1)的激活,对于mRNA生物发生以及翻译起始和延伸至关重要^[72],其活化可以促进轴突延伸^[73]。mTORC1可以被雷帕霉素特异性阻断。随

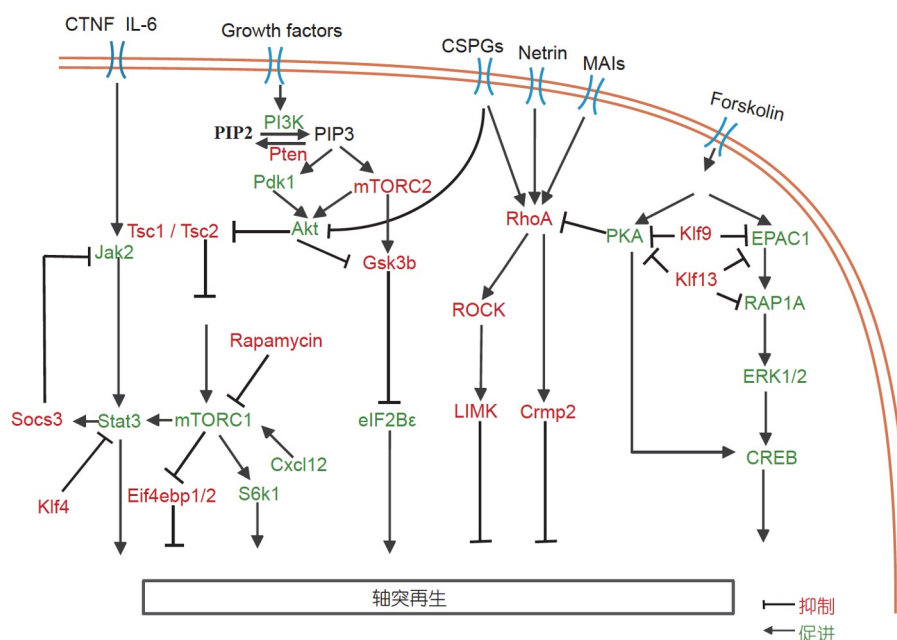


图 1 影响轴突再生的信号通路. 轴突再生过程受多条信号通路的调控, 通过对信号通路中关键分子的抑制或激活, 可以调节轴突的再生能力

Figure 1 Signaling pathways that affect axon regeneration. Multiple signaling pathways regulate axon regeneration, and its regenerative ability can be modulated by inhibiting or activating key molecules in these signaling pathways

着RGC的成熟, 哺乳动物中促进生长的分子, 雷帕霉素雷磷酸化的靶蛋白(phospho-mTOR)表达下调^[74]. 在野生型小鼠的受损RGC中, mTOR被迅速且持续地抑制, 其表达水平与轴突再生的程度和时间过程高度相关. mTOR途径的激活有效增强了神经保护和轴突再生^[75]. PI3K还以核糖体依赖性方式激活另一种mTOR复合物(mTORC2), mTORC2影响细胞存活、肌动蛋白细胞骨架动力学和/或细胞移动性和脂肪生成^[76], Akt的T308的磷酸化通过PI3K-Pdk1途径正调控轴突再生, 而其丝氨酸残基S473的磷酸化通过PI3K-mTORC2途径负调控轴突再生, 并可调节Gsk3b(glycogen synthase kinase 3 beta)的磷酸化^[77]. 磷酸化和抑制Gsk3b对于神经元极化, 轴突分支和轴突生长至关重要^[78]. mTORC1的激活和Gsk3b的抑制是Akt诱导轴突再生的两个关键平行途径.

同源性磷酸酶-张力蛋白(phosphatase and tensin homologue, Pten)是细胞生长的负调节剂^[79], 它可以拮抗PI3K, 催化从PIP3到PIP2的转化, 并抑制Akt, mTOR和S6k1的下游信号传导. Pten的失活导致PIP3途径及其下游, 包括Pdk1-Akt-T308途径和mTORC2-Akt-S473途径激活^[80]. 在皮质脊髓束(corticospinal tract,

CST)中条件敲除Pten, 增强了未受伤CST轴突的代偿性出芽, 并能使受伤的CST轴突成功再生^[81]. 但这种再生能力可能存在年龄依赖性, 随着年龄增长, Pten缺失对轴突再生影响减弱^[82]. 药物研究表明, 梓醇(catalpol)可作为一种多效神经保护剂, 对神经元有一定的保护作用, 可通过激活Akt-mTOR途径以及调节BDNF(brain-derived neurotrophic factor)和Pten的表达, 在坐骨神经挤压伤后对小鼠神经元起到保护和促进轴突生长的作用^[83]. 在RGC中, 缺失Pten使损伤后RGC存活率显著提高并大大增强轴突再生能力, 一些再生轴突可以延伸到视交叉^[74]. 此外, 最近研究表明, Pten敲除也消除了Gsk3对RGC轴突中胶原蛋白反应介质2(collapsin response mediator protein 2, Crmp2)的抑制活性, Gsk3条件敲除可通过减少Crmp2的抑制性磷酸化来促进视神经再生, 表明Gsk3-Crmp2途径也参与PI3K-Akt途径^[84].

对PI3K的研究发现, 其四个催化亚型中p110α和p110δ对轴突再生起重要作用, AAV介导的p110δ递送可作为通过PI3K途径刺激再生的新手段^[85]. 使用shRNA抑制Akt的三种亚型显著降低但并未完全消除Pten敲除诱导的视神经再生效果, 说明存在不依赖于

Akt的信号途径^[86]. 给予雷帕霉素抑制mTOR, 将减弱*Pten*缺失后带来的轴突再生影响, 表明mTOR活性在轴突再生中的重要作用. *Eif4ebp1*突变体4E-BP1-4A和*S6k1*突变体*S6k1-T229A*不能被mTORC1磷酸化, 从而大大阻碍了*Pten*敲除诱导的视神经再生^[87], 而其下游*S6k1*激活仅诱导非常弱的轴突再生^[88].

(3) cAMP-PKA. 环腺苷酸-蛋白激酶A(cAMP-PKA)信号通路也从多个方面参与中枢神经系统损伤后的轴突再生. PKA是cAMP的关键下游效应子, 由两个相同的催化亚基(PKAc)和两个不同的调节亚基(PKAr I 和 II)构成, 它们以异四聚体的形式在非活性状态下结合在一起. 升高的细胞内cAMP与PKAr I 或PKAr II结合, 导致PKAc与PKAr I 或PKAr II分离, 从而使其活化激活CREB(cAMP-response element binding protein)转录并调节*c-fos*和*BDNF*等下游基因^[89]. cAMP可以抑制Rho-ROCK信号通路的活性, 单独升高cAMP可以诱导少量的轴突再生^[90]. 电刺激小鼠小脑顶核可以通过激活cAMP-PKA途径激活轴突再生^[91]. 人参皂甙Rb1也通过该途径促进神经递质的释放, 进而促进轴突再生^[92]. 双亮氨酸拉链激酶(dual leucine zipper-bearing kinase, DLK; 又名mitogen-activated protein kinase kinase kinase 12, Map3k12)也是轴突损伤中cAMP的关键靶点和效应因子^[93]. 轴突损伤后, Map3k12作为重要损伤传感器, 可激活MAPK通路, 介导*Sox11*, *Atf2*, *P311*和*Stat3*等因子磷酸化, 影响决定神经元存活和轴突再生的基因表达程序^[94].

(4) 炎症反应. 中枢神经系统的先天免疫系统, 通过小胶质细胞、浸润的巨噬细胞和中性粒细胞介导炎症反应对神经损伤做出响应^[95], 这些巨噬细胞和中性粒细胞会释放出多种抗炎(anti-inflammatory)和促炎性(pro-inflammatory)细胞因子、趋化因子、生长因子和其他细胞因子, 尽管这些因素中有一些不利于神经元生长, 但炎症已被证实可以促进轴突再生并增强RGC的存活能力^[96]. 其中Ly6G⁺中性粒细胞可能对再生具有重要贡献, 可以通过分泌神经生长因子(nerve growth factor, NGF)和IGF-1等促进神经元的存活和轴突再生^[97]. 使用酵母聚糖等Toll样受体2(Toll-like receptor 2, Tlr2)激活剂可诱发眼内炎症, 诱导轴突再生^[98]. 酵母聚糖的主要成分是 β -葡聚糖(β -glucan), 可通过小胶质细胞上免疫受体Clec7a(C-type lectin domain family 7, member a; beta-glucan receptor)的激活

介导炎症反应, 去除炎症细胞受体Tlr2和Clec7a, 会减弱炎症引发的再生反应^[99]. 眼内炎症引发的轴突再生可能与RGC固有生长状态的改变相关, 其可导致*Spr1a*和*Gap43*等生长相关蛋白(growth-associated gene products, GAPs)表达上调, 而*Klf4*等细胞内源性抑制因子表达下调^[100]. 同时, 炎症可促进*Ocm*, *CNTF*, *BDNF*和*LIF*等信号分子的表达, 并可以激活Jak-Stat3等信号通路, 以增强轴突的再生能力^[101].

炎症等损伤可以引起如脑源性神经营养因子、胶质细胞源性神经营养因子(glial cell-derived neurotrophic factor, GDNF)、神经生长因子、睫状神经营养因子等多种神经营养因子的释放, 这些因子已被用于促进神经元存活并刺激轴突生长^[102].

此外, 衰老是神经细胞逐渐死亡和轴突无法再生的原因之一. 在青光眼模型和老龄小鼠中, *Oct4*, *Sox2*和*Klf4*基因在RGC中的异位表达可以恢复年轻的DNA甲基化模式和转录组, 促进损伤后轴突再生, 逆转视力下降^[103].

(5) 其他促轴突生长的信号通路及因子. 转录组研究鉴定出许多再生神经元与非再生神经元间的差异表达基因, 如*Klf*家族, *c-Jun*, *Atf3*, *Stat3*, *Sox11*和*Smad1*等转录因子以及*Gap43*, *Cap23*, *Arg1*, *Spr1a*, *Hspb1*, *tubulin*等与再生相关的基因在再生神经元内上调^[104], 这些均可作为视神经损伤后实现轴突再生提供参考和指导. 例如通过注射TGF β -BMP信号传导途径中细胞内介体Smads, 可以激活轴突再生^[105]. *Atf3*过表达增强了背根神经节神经元中央分支的轴突再生^[106]. 此外, 缺氧诱导因子1 α (hypoxia inducible factor 1 subunit α , Hif1 α)调控感觉神经元中的多个损伤诱导基因, 在体外或体内条件性敲除*Hif1a*会抑制感觉轴突的再生. Hif1 α 靶基因血管内皮生长因子A(vascular endothelial growth factor A, VEGFA)在受损的神经元内表达, 并有助于刺激轴突再生^[107]. RGC轴突发育需要*Sox11*和*Sox4*, 过表达*Sox11*可以诱导长距离的轴突再生^[108,109]. 除此之外, 癌调蛋白(oncomodulin, Ocm), 一种巨噬细胞系分泌的Ca²⁺结合蛋白, 以依赖于cAMP的方式与RGC结合, 通过Ca²⁺-钙调蛋白激酶依赖性途径, 中和环境抑制因素, 促进轴突再生^[101]. MicroRNA也参与神经细胞存活和凋亡的信号级联反应, 例如*miR-431*, *miR-210*, *miR-182*, *miR-34a*, *miR-127*, *miR-21*和*miR-320*等均能促进轴突再生^[110].

与单独的处理方法相比, 组合处理方法显示出更强的促再生能力。例如同时敲除PI3K-Akt信号通路抑制剂*Pten*和Jak-Stat信号通路抑制剂*Socs3*, 可以显著增强轴突再生能力, 且提高RGC存活能力^[111,112]。*Pten*与*Socs3*双敲结合CNTF施用, 能够得到更好的再生效果^[113]。*Pten*敲除结合炎症反应也能提高再生效果。在小鼠中, 酵母聚糖与cAMP类似物和*Pten*缺失结合后可提高Ocm和其他营养因子的水平, 10~12周时, 再生轴突已可以激活目标靶区^[114,115]。胰岛素样生长因子(insulin-like growth factor 1, IGF1)与骨桥蛋白(osteopontin, OPN)结合可选择性地增强 α -RGC(占RGC总数的6%左右)轴突的再生^[80]。*OPN*, *IGF1*和*CNTF*的共表达诱导了再生视网膜轴突和上丘中功能性突触的形成^[116,117]。

1.3 视网膜神经节细胞的神经保护

损伤后, RGC会逐渐凋亡, 没有细胞的支持, 轴突也无法维持。保护RGC的生存对功能恢复至关重要。细胞存活涉及多种复杂的机制, 目前对这些机制的了解仍需完善。

(1) 神经营养因子。经典的神经营养因子是一类可扩散的蛋白, 可介导神经细胞的增殖和分化、轴突和树突生长, 以及突触形成, 主要包括神经营养因子NGF、脑源性神经营养因子BDNF和神经营养蛋白NT3/4/5(neurotrophin-3/4/5), 其表达有助于RGC的存活^[118]。神经营养因子以高亲和力与特异的酪氨酸激酶受体(Trk)结合, 后者被分类为TrkA(高亲和力配体: NGF), TrkB(高亲和力配体: BDNF, NT4/5)和TrkC(高亲和力配体: NT3)。NT3还以低亲和力结合TrkA和TrkB。成熟的RGC可以表达所有三个Trk受体。BDNF和NT4/5对轴突切除的RGC有显著的神经保护作用^[119]。BDNF可以控制神经发育、调节轴突发生^[120]以及在缺氧条件下减少视网膜细胞的损伤^[121]。包含Src同源区域2的蛋白酪氨酸磷酸酶2(protein tyrosine phosphatase, non-receptor type 11, Prpn11; 又名SH2 domain-containing protein tyrosine phosphatase-2, Shp2)已被鉴定可以在BDNF-TrkB信号通路中发挥作用。Prpn11介导的TrkB去磷酸化抑制该信号通路, 从而导致RGC存活能力降低, 调节Prpn11活性可作为青光眼研究的新靶点^[122]。

除神经营养因子外, IL-6细胞因子家族成员睫状神经营养因子CNTF和白血病抑制因子LIF等也显示

出对神经保护的重要作用^[123]。CNTF是神经系统中各种神经元的营养分子, 其生物学作用需要通过与CNTF受体 α (CNTFR α)、白血病抑制因子受体 β (LIFR β)和糖蛋白130(gp130)形成的受体复合物结合^[124]。CNTF可以通过这种受体复合物激活PI3K-Akt, MAPK-Erk和Jak-Stat3等多种信号通路^[125]。CNTF可以通过包含SH2结构域的蛋白质(Shp2, Shc等)激活MAPK途径, 该途径通过抑制促凋亡分子BAD和产生抗凋亡分子Bcl2和Bcl-xL来促进损伤后神经元存活^[126]。CNTF的缺失不会导致视网膜的神经退行性疾病^[127], 但CNTF和LIF的双重缺失加速视神经损伤后的RGC死亡, 并减弱轴突再生^[128]。而玻璃体腔注射CNTF可显著提高RGC的存活率^[129], 并能够与环磷酸腺苷cAMP结合发挥作用。利用AAV载体介导*CNTF*进入眼内也可以促进视神经损伤后的轴突再生^[130], 结合应用shRNA对抑制分子Nogo和CSPG等的下游信号分子RhoA进行抑制, 可显著提高AAV-CNTF对轴突再生的治疗效果^[131]。其他与再生相关的营养因子包括趋化因子Cxcl12(chemokine (C-X-C motif) ligand 12; 又名Sdf1)以及心肌营养因子家族的几个成员等, Cxcl12的再生促进作用通过PI3K-Akt途径实现, 视神经损伤情况下, 在玻璃体腔内注射Cxcl12可以维持RGC中mTOR的活性, 并刺激轴突再生^[132]。碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)也可以保护细胞免受伤害并刺激再生, 它可上调生长促进蛋白Gap43的合成, 并促进轴突再生^[133]。IGF-1也可以促进RGC的存活, 过表达*Lin28*可以增强IGF-1介导的受损RGC的存活和轴突再生^[134]。

(2) 维持视网膜神经节细胞内离子及代谢稳态。视神经损伤后, 部分离子失衡和代谢紊乱是导致RGC细胞损伤乃至死亡的重要因素, 维持RGC内离子和代谢的稳态对RGC的存活尤为重要。

锌离子Zn²⁺对许多细胞功能包括氧化应激反应至关重要。在视神经受损后1 h内, Zn²⁺在视网膜无长突细胞中迅速增加, 并缓慢转移至RGC中。视网膜中Zn²⁺失衡可能是限制RGC存活和再生能力的主要因素。Zn²⁺聚集可以损害线粒体功能, 并产生活性氧(ROS), 与一氧化氮结合激活MAPK-p38通路, 进而介导细胞死亡^[135]。通过螯合Zn²⁺可以促进长期RGC保护和增强轴突再生^[136]。此外, 使用抗氧化剂 α -硫辛酸降低氧化应激也可以增加青光眼疾病状态下RGC的存活率^[137]。通

过AAV介导可以减少氧化应激的*Sirt1*基因表达,也可以保护RGC^[138]. 氧自由基的形成会激活凋亡信号通路,此时热休克蛋白(HSP)似乎成为RGC存活的关键. HSP是保护细胞免受各种环境和生理侵害的重要伴侣蛋白,对HSP活性的抑制可阻止抗凋亡蛋白Bcl-2的表达,并增加促凋亡蛋白Bax的水平^[11].

视神经损伤后RGC的死亡也与异常的钙离子 Ca^{2+} 活化有关. Ca^{2+} /钙调蛋白依赖性蛋白激酶 II (CaMK II) 是 Ca^{2+} 信号转导的中心协调者和执行者,在正常视网膜的RGC中高度磷酸化,小鼠体内抑制CaMK II会导致RGC死亡. 在视神经损伤模型中,使用AAV递送CaMK II可以通过CREB的激活来提高RGC存活率,并可以在兴奋性毒性损伤模型中,保护RGC轴突及其在大脑中的投射^[139].

此外,轴突再生也需要受损神经元中脂质稳态的协调变化. 3-磷酸甘油途径中的重要磷脂酸磷酸酶Lipin1,也是轴突再生的抑制因子,视神经损伤后,Lipin1的增加破坏了甘油磷酸途径中甘油酯的稳态合成,进而限制了轴突再生. 使用shRNA敲降该基因可促进损伤后轴突再生^[140].

视神经损伤后,维持RGC的存活、激活轴突再生潜能是视神经损伤修复的有效策略,可以通过影响再生相关信号通路、炎症反应、RGC神经保护等因素,或多种因素组合,促进轴突的再生(表2).

2 视网膜细胞再生

2.1 低等脊椎动物中视网膜神经元再生

虽然人类和哺乳动物视神经受到损伤时无法再

生,但两栖动物和硬骨鱼等低等脊椎动物在神经系统受到损伤后具有完全再生的能力^[141]. 斑马鱼和蝾螈在其整个生命过程中均可进行神经系统再生,而非洲爪蟾等无尾目蛙类仅在幼体阶段具有这种潜能^[142]. 低等脊椎动物可以部分再生视网膜,其再生细胞来源于位于视网膜边缘睫状缘区域(ciliary margin zone, CMZ)的视网膜干细胞和祖细胞以及视网膜色素上皮细胞(retinal pigment epithelia, RPE)^[143],其视网膜中的Müller细胞,也可以去分化生成视网膜前体细胞,进而分化为其他类型的细胞修复受损视网膜^[144]. Müller细胞是视网膜中主要的胶质细胞,占视网膜细胞总数的4%~5%,具有促进神经元分化与生存和为神经元提供营养等功能^[145]. Müller细胞与RGC、双极细胞、水平细胞和无长突细胞等视网膜神经细胞在发育上具有同源性. 此外,转录组分析发现Müller细胞也表达*Dkk3*, *Vsx2* (*Chx10*), *Pax6*, *Notch1*, *Hes1*等神经前体细胞特性的基因,揭示了该细胞具有潜在干细胞特征^[146].

研究发现,在斑马鱼视网膜受到损伤后,Müller细胞可以作为视网膜干细胞,补充受损的视网膜神经元并恢复视力^[147]. 在这个过程中,其基因表达和DNA甲基化发生明显变化. 损伤诱导的重编程过程中,Müller细胞核从内核层迁移到外核层,在那里发生不对称分裂,然后返回内核层^[148]. 糖原合酶激酶Gsk3b- β -catenin, Notch, MAPK-Erk和Jak-Stat等信号通路均参与调节斑马鱼视网膜的再生^[147]. 在两栖动物中,位于CMZ的视网膜干细胞在整个生命过程中,持续进行分化产生所有类型的视网膜细胞,损伤后,CMZ的视网膜祖细胞和RPE细胞参与视网膜再生,Müller细胞也参与这个过程^[149]. 成年鸟类不会再生受损视网膜,但在损伤

表 2 轴突再生的有利因素

Table 2 Favorable factors for axon regeneration

促进方式	作用/激活方式	参考文献
通路激活	Jak-Stat3	抑制Socs3, CNTF升高等
	PI3K-AKT	Pten失活, 递送催化亚基等
	cAMP-PKA	
	TGF β -BMP	注射Smads
炎症反应	炎症促进轴突再生	酵母聚糖
	生长因子释放	中性粒细胞可分泌NGF/IGF等生长因子
	神经营养因子释放	BDNF/GDNF/NGF/CNTF等
RGC的神经保护作用	神经营养因子释放	BDNF/GDNF/NGF/CNTF等
	维持离子及代谢稳态	螯合 Zn^{2+} , 递送CaMK II, 敲降Lipin1等

后, 其视网膜Müller细胞也能重新进入细胞周期, 并表达胚胎视网膜祖细胞转录因子*CASH-1*, *Pax6*和*Chx10*, 这些新形成的细胞中一部分分化为视网膜神经元, 少数分化成Müller胶质细胞, 大多数未分化, 并持续表达*Pax6*和*Chx10*^[150]。哺乳动物Müller细胞虽能响应损伤, 但不具有视网膜祖细胞功能。不过越来越多的研究表明, 参考低等脊椎动物视网膜再生过程, 哺乳动物Müller细胞也能够适当的条件下产生神经元^[151]。

2.2 哺乳动物中视网膜神经节细胞再生

(1) 外源性视网膜神经节细胞再生。胚胎干细胞(embryonic stem cell, ESC)作为全能干细胞可以分化为视网膜神经元和视网膜色素上皮细胞等多种视网膜细胞类型, 但由于ESC的来源具有伦理争议, 具有成瘤风险, 而且ESC的移植会造成免疫排斥反应, 故ESC在临床上的应用受到很大的限制。诱导多能干细胞(induced pluripotent stem cell, iPSC)的出现部分解决了这些问题, 但同样具有成瘤风险。外源性RGC分化方案主要是通过加入细胞外基质或者BMP, Wnt和Notch等信号通路抑制剂以及bFGF等各种不同的生长因子诱导获得RGC。如Noggin(BMP信号通路抑制剂)、Dkk1(Wnt信号通路抑制剂)和DAPT(Notch信号通路抑制剂)以及IGF1被广泛应用于干细胞诱导分化为RGC的方案中^[152~154]。通过体细胞重编程也可以诱导生成RGC, 腺病毒介导转导的*Ascl1*, *Brn3b(Pou4f2)*和*Ngn2*可以诱导小鼠成纤维细胞为类RGC^[155]。应用*Ascl1*, *Brn3b/3a*和*Isl1*三因子组合可将成纤维细胞重新编程为感觉神经节类器官, 并能诱导生成少部分RGC^[156](图2)。

移植干细胞衍生的RGC是潜在的疾病治疗策略。向视神经病变小鼠模型玻璃体腔注射的iPSC衍生RGC, 可存活长达1个月, 并整合至宿主细胞中^[157], 但RGC的轴突无法长距离再生至中央靶标^[158]。此外, 基于神经保护原理, 移植神经胶质细胞和间充质干细胞, 可以起到支持和营养的功能, 进而保护青光眼模型中的RGC^[159]。通过3D类视网膜培养也能得到RGC, 但得到的是包含RGC在内的类器官, 而非定向分化的RGC, 在这里不详细讨论。体外定向诱导RGC方案普遍存在效率较低、移植后在宿主视网膜中生存率和整合率很差、不能长久存活等诸多问题。

(2) 内源性视网膜神经节细胞再生。基于对低等脊椎动物视网膜再生的研究, 研究人员尝试对哺乳动物

Müller细胞进行重编程, 生成多能性Müller细胞来源的祖细胞, 来分化补充受损神经元。在P0小鼠视网膜前体细胞中过表达*Klf4*, 可以诱导视网膜晚期前体细胞向RGC方向发育^[160]。基于脊椎动物的Müller细胞修复时*Ascl1*基因表达上调的研究, 在体外发现*Ascl1*会促进Müller细胞转化为具有某些视网膜祖细胞性质的细胞^[161]。在此基础上, 在年轻小鼠损伤视网膜Müller细胞中过表达*Ascl1*, 能够使其再生分化为无长突细胞、双极细胞和感光细胞^[162]。但是, 单独表达*Ascl1*在成年小鼠中产生的诱导效果很差, 主要是由于染色体遗传水平的改变所致。在成年小鼠中过表达*Ascl1*结合应用组蛋白去乙酰化酶抑制剂, 可以使Müller细胞增殖分化为各种神经元^[163]。

研究发现, 损伤刺激视网膜穆勒细胞重新增殖主要是通过Wnt途径实现的, 抑制Wnt信号途径能显著减少穆勒细胞的增殖; 进一步实验发现, 在视网膜没有受损的情况下, 通过腺相关病毒转染 β -catenin也能使正常成年小鼠的Müller细胞重新进入细胞周期, 实现Müller细胞增殖1~2代, 表达干细胞特性蛋白^[164]。随后, 在增值的基础上进一步在Müller细胞中过表达视杆细胞发育相关因子*Otx2*, *Crx*和*Nrl*, 可以促使增殖的Müller细胞表达视杆细胞特异性蛋白, 并向外核层移动, 实现哺乳动物中视杆细胞的原位再生, 且能恢复疾病小鼠模型的部分视力^[165]。

最近研究表明, 即使Müller细胞没有增生, 也可以通过重编程定向转分化为RGC(图2)。Ptbp1对于神经元的命运决定和成熟过程起着重要作用^[166]。Zhou等人^[167]敲低Müller细胞内Ptbp1的表达, 可以在视网膜中将Müller细胞转化为RGC。但Fu等人^[168]利用同样的技术敲低Müller细胞内Ptbp1的表达, 却发现Müller细胞大多转分化为感光细胞而非RGC。这种实验结果的不一致性需要更多的观察。根据RGC发育过程中的基因调控网络特性, 利用AAV携带RGC命运决定的关键转录因子*Math5*和*Brn3b*成功重编程Müller细胞并将其定向分化为RGC^[169]。

除Müller细胞外, 视网膜内还有另外一种成体干细胞, 即表达Lgr5的一小群无长突细胞^[170]。利用类似的策略, AAV携带转录因子*Ascl1*, *Brn3b*和*Sox4*成功将Lgr5⁺无长突细胞转分化为RGC^[171]。但由于Lgr5⁺无长突细胞数量较少, 该转分化方案得到的RGC数量相对较少(图2)。

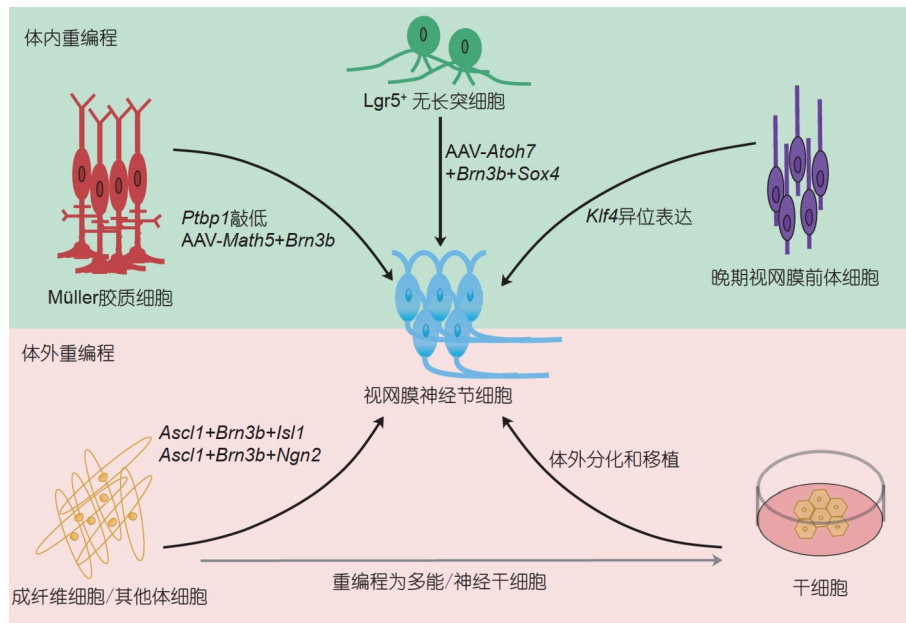


图 2 视网膜神经节细胞的重编程和再生

Figure 2 Reprogramming and regeneration of retinal ganglion cells

和外源性RGC移植相比,所有上述内源性RGC再生方案具有共同的优点:不存在免疫排斥,新生的RGC轴突可以沿着既有的神经束生长,容易投射到大脑视功能区,似乎受到抑制轴突生长因素的影响很小.因而再生的成熟RGC都具有和原生RGC相同的功能,能有效恢复青光眼等疾病小鼠模型的部分视力.

3 讨论

成年哺乳动物RGC及其轴突再生能力的丧失,使得哺乳动物视神经损伤不可逆而导致视力丧失,迄今为止,尚无有效的治疗手段.近年来,视神经修复研究的基本策略是损伤后维持RGC的存活,促进轴突的再生,并使其重建突触连接.然而,神经元的细胞外环境存在许多限制轴突再生的因素,越来越多的研究揭示了这些因素.通过对细胞外基质中硫酸软骨素蛋白聚糖、髓磷脂相关抑制剂、化学驱除蛋白(semaphorin 6A和eprhrin B3等)以及抑制性转录因子等抑制因素的阻遏,能够提高轴突再生能力.然而,单纯降低某一类再生抑制因素并不能有效促进神经系统再生和视觉重建,轴突内在生长能力的增强也必不可少,激活PI3K-Akt和Jak-Stat等轴突再生相关的信号通路,可以实现轴突再生和突触形成^[172,173].研究表明组合的方式可

能更加有效,一方面激活神经再生内源动力,另一方面调整外部环境,可能得到更好的轴突再生效果.

此外,RGC在视神经损伤后死亡的问题,也是视神经损伤修复的关键问题,有三种策略可以缓解.第一种是通过施用神经营养因子或降低氧化应激等方法,保护原有的RGC,再促使这些神经细胞本身的轴突重新延伸.第二种是干细胞移植,应用细胞疗法,通过体外诱导RGC移植到视网膜的方法进行治疗,但可能存在免疫排斥等问题.第三种是胶质细胞转分化,研究人员提出了通过诱导内源性细胞,尤其是Müller细胞重编程以替代丢失的神经元.研究人员通过操纵如*Ptp1b*等关键调控因子的表达,实现了功能性神经元的再生.然而,针对这一策略,存在诸多争议,Zhang团队^[174]使用谱系追踪发现,在胶质细胞中高表达*NeuroD1*或敲低*Ptp1b*,均无法成功转分化为神经元细胞,并认为这可能与病毒载体启动子胶质细胞特异性丧失有关.但启动子特异性丢失原因尚不清楚,而胶质细胞是否能够转分化为神经元细胞有待进一步探究.

总之,视神经损伤后视觉重建需要从多方面入手,如何保护已有神经元,如何获得安全有效的神经元再生,如何获得高效的轴突再生,以及如何将其应用于临床疾病,实现视网膜退行性疾病功能的改善,还有待更多的研究.

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Optic nerve repair and regeneration in vertebrates

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Vision is one of the major pathways for receiving external information in vertebrates. The optic nerve connects the retina to the brain and relays visual coding signals to the corresponding cortical regions. It is widely believed that the retinal ganglion cells (RGCs) and optic nerve cannot regenerate in mammals, and their injuries are the most common causes of blindness. Several extrinsic and intrinsic factors limit axonal regeneration in the optic nerve. Elimination of extrinsic inhibitory factors and activation of intrinsic pro-regenerative factors enable a small number of damaged axons to regenerate and improve visual functions in a limited manner. Furthermore, transplantation of stem cell-derived RGCs or *in vivo* reprogramming of Müller and amacrine cells into RGCs can restore part of the vision, which provides insights into the future therapy for various neurodegenerative disorders, such as glaucoma. This review summarizes the recent studies on axon and retinal cell regeneration after optic nerve injury in vertebrates.

optic nerve injury, axon regeneration, cell regeneration, retinal ganglion cell

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