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评 述

干细胞与再生医学

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摘要 多能干细胞(PSCs)具有发育的多潜能性, 可以分化为机体各种细胞类型, 是再生医学领域进行细胞替代治疗以及组织/器官再生的基础. 如何由终末分化的体细胞重编程获得病人特异的 PSCs, 是再生医学领域的核心问题之一, 目前主要采取两种重编程策略: 借助核移植技术由早期胚胎体外建系获得, 或通过诱导重编程技术获得. 本文将综述不同多能性等级 PSCs 的获得方法以及其在多能性机制研究中的应用, 并讨论 PSCs 通过异种嵌合实现组织/器官再造的潜在应用价值. PSCs 的研究不仅推动了基础生物学研究的发展, 同时也为再生医学走向临床开辟了道路.

关键词 多能干细胞, 再生医学, 重编程, 异种嵌合

从细胞水平上讲, 一个生物体从受精卵到成年的发育过程, 是按照既定的“程序”由全能性状态逐渐降低多能性等级, 直到终末分化状态的过程. 终末分化细胞的衰老死亡或意外创伤, 会对机体造成不可逆转的损害, 而机体不能有效地替换这些“罢工”的细胞, 就造成了各种疾病以及衰老死亡. 传统医学通过药物或手术等传统治疗手段并不能从根本上征服“罢工”的细胞. 再生医学是通过改造或替换受损或衰老的细胞、组织、甚至器官来重建那些“罢工”细胞的功能, 在一些传统医学力不能及的疾病(如脊髓损伤)治疗方面是革命性的. 可以预见, 在未来的几十年里再生医学将掀起广泛而深入的医学革命.

多能干细胞(pluripotent stem cells, PSCs)是再生医学领域最具潜力的种子细胞, 其具有发育的多潜能性, 不仅可以不断维持自我更新, 而且可以分化为机体各种类型的细胞. 从不同阶段的早期胚胎或流产胎儿或成体组织中, 可以分离获得多种不同潜能的 PSCs, 但显然这受到资源的限制, 而且免疫排斥因素极大地限制了这些 PSCs 的临床价值.

重编程是细胞从一种基因表达谱转换为另一套不相关表达谱的过程, “滚落”模型[1]描述得很贴切: 沿着既定轨道逆行或者跳转到其他轨道都是重编程. 通过重编程可以获得病人特异的具有较高多能性等级的 PSCs, 这是再生医学走向临床的基础之一. 目前主要的重编程策略有两种, 一种是核移植, 一种是转录因子过表达(包括转分化及诱导多能干细胞(induced pluripotent stem cells, iPSCs)技术).

疾病模型制备, 包括多种疾病/治疗动物模型的

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构建以及获得具有“器官巢”(“organ niche”[2], 给异种细胞提供某个器官发育的“巢穴”)的动物模型, 是再生医学领域关注的另一热点. 随着功能基因组学的发展及基因组边际技术的不断革新, 各种疾病/治疗动物模型陆续获得, 然而只有很少一部分模型具有临床应用价值. 人类器官再造依然是个科学难题, 无论是通过异种嵌合技术还是通过 3D 生物打印技术.

1 多能干细胞: 更高多能性, 更接近人类

胚胎干细胞(embryonic stem cells, ESCs)具有很高的多能性水平, 是细胞替代治疗以及体外器官再造的良好细胞来源, 在多种疾病的治疗方面很具价值, 如帕金森(Parkinson’s disease)、阿尔兹海默病(Alzheimer’s disease)和糖尿病(diabetes). 自从第一株小鼠(*Mus musculus*) ESCs 于 1981 年被建立[3,4], 多个物种的 ESCs 相继问世, 包括人(*Homo sapiens*)[5]、恒河猴 (*Macaca mulatta*)[6,7] 、大鼠 (*Rattus norvegicus*)[8~10]和兔(*Leporidae*)[11,12]等. 相比其他物种, 人 ESCs 具有更高的临床应用价值. 随着越来越多的人 ESCs 相关工作的开展[13,14], 国际上相继出台了《关于用于研究的人胚胎干细胞的存储及使用的国际共识指南》(the international consensus guidance for banking and supply of human ESCs for research purposes[15])及《用于临床的多能干细胞的保种工作的发展的考虑要点》(the points to consider in the development of seed stocks of pluripotent stem cells for clinical applications[16]).

更高的多能性以及更低的免疫源性的 PSCs 是再生医学领域孜孜以求的. 四倍体补偿是证实干细胞具有全能性的黄金标准. 目前只有小鼠 ESCs 和小鼠 iPSCs[17]通过了四倍体补偿黄金标准. 从临床应用的角度, 在没有完美的人 PSCs 的现阶段, 将低多能性等级的 PSCs 采用合适的方法应用于合适的临床疾病是符合实际需求的. 临床级的可移植的神经前体细胞已经相继由大鼠 ESCs[18]和恒河猴 ESCs[19,20]定向分化获得, 基于神经前体细胞移植的细胞治疗策略的临床效果也在非人灵长类动物上进行了评估[19,20]. 能嵌合的恒河猴 ESCs 的建系成功, 推动了非人灵长类干细胞研究的进展. 此外, 人孤雌胚胎干细胞(human parthenogenetic ESCs)[21]及人纯合孤雄胚胎干细胞(human androgenetic ESCs)[22,23]细胞系的建立, 又为细胞治疗提供了良好的材料.

2 重编程: 病人特异多能干细胞的源泉

重编程解决了病人特异 PSCs 资源问题, 大大缩短了干细胞临床之路.

2.1 核移植

最早的核移植(nuclear transfer, NT)实验设计是 1952 年由 Briggs 和 King[24]在非洲爪蟾(*Xenopus*)中建立的, 1958 年 Gurdon 等人[25]首次成功实现了核移植重编程并获得了成年核移植非洲爪蟾. 中国科学家童第周先生另辟蹊径, 于 1963 年获得了世界上第一条克隆鲫鱼(*Carassius auratus*), 并于 1973 年实现了鲫鱼和鲤鱼(*Rhodeus sinensis*)的异种核移植, 这是世界首例异种核移植案例. 1997 年 Wilmut 等人[26]获得了世界上第一只克隆羊“多莉”(Dolly), 随后小鼠[27]、牛(*Bovine*)[28,29]、山羊(*Capra aegagrus* hircus)[30]、猪(*Sus scrofa domestica*)[31~33]、猫(*Felinae*)[34]、兔子[35]、骡子(*Equus ferus* × *asinus*)[36]、马(*Equus caballus*)[37]、大鼠[38]、狗(*Canis lupus* familiaris)[39]、雪貂(*Mustela putorius* furo)[40]、骆驼(*Camelus bactrianus*)[41]等相继被克隆. 虽然早在 2006 年就已经可以稳定获得猴(*Primates*)克隆囊胚[42], 但目前尚未有克隆猴出生.

2004 年, Hwang 等人[43]建立了第一株核移植胚胎干细胞系(nuclear transfer ESCs, ntESCs), 是治疗性克隆的里程碑事件. 此外, 人卵母细胞的形态分级[44]及异种克隆的尝试[45], 都促进了治疗性克隆的发展. 最近, Kit^{w}/Kit^{wv} 基因型 ntESCs 经基因修饰及体内分化, 使 Kit^{w}/Kit^{wv} 不育雄性小鼠获得了正常可育子代[46], 以及小鼠 ESCs 体外诱导分化为可育精细胞样细胞(spermatid-like cells)[47], 为再生医学治疗男性不育指明了方向[46].

提高核移植重编程效率是促进治疗性克隆发展的潜在动力, 关于如何提高核移植效率的文献层出不穷. 表观重编程的缺陷是大部分核移植胚胎发育异常的主要内因[48~51]. 用组蛋白去甲基化酶抑制剂处理可提高核移植重编程效率[52~55]. 另一方面, 核移植胚胎体外培养体系的进一步完善[56], 也是提高核移植重编程效率的关键. 核移植胚胎胎盘异常是普遍存在的发育缺陷, *HSPC117*[57]等基因的异常表达是部分原因. 减Ⅱ中期卵母细胞的蛋白质谱分析, 为

研究核移植重编程因子提供了良好的材料[58].

2.2 转录因子过表达

早在 1987 年, Davis 等人[59]通过过表达 MyoD 将成纤维细胞有效诱导转分化为肌细胞, 这是转分化重编程领域的开拓性研究. 相似的, 过表达 C/EBPα 可将前 T 细胞[60]或前 B 细胞[61]诱导转分化为巨噬细胞; 将胰腺外分泌腺泡细胞诱导转分化为肝细胞[62]. 2010 年, Vierbuchen 等人[63]通过过表达 19 个神经特异的转录因子, 将小鼠成纤维细胞诱导转分化为功能性神经细胞. 2012 年, 睾丸支持细胞被诱导转分化为神经干细胞, 后者被移植到小鼠大脑齿状回区后可有效存活并形成突触. 这是首次将成体细胞直接转分化为另一类群细胞的前体干细胞, 前体干细胞可以扩增和分化从而获得大量的转分化细胞, 这在某种意义上克服了转分化效率低的问题, 对于诱导转分化的临床应用具有一定的推动作用[64,65]. 值得注意的是, 2008 年, Zhou 等人[66]通过腺病毒在体感染, 将胰腺腺泡细胞诱导转分化为具有正常胰岛素分泌功能的胰岛β细胞, 对于Ⅱ型糖尿病的再生医学治疗意义重大.

2006 年, Takahashi 和 Yamanaka[67]通过过表达 4 个转录因子, OCT4, SOX2, KLF4 和 c-Myc, 成功将小鼠成纤维细胞变为 iPSCs, 这无疑是重编程领域的里程碑. 通过四倍体补偿技术获得的全 iPSCs 小鼠“小小”[17,68~70], 以及通过核移植证明 iPSCs 的发育全能性[71], 为 iPSCs 用于再生医学铺平了道路. 虽然全 iPSCs 小鼠在发育特性上与全 ESCs 小鼠没有显著差异, 但却更容易罹患肿瘤[72], 因而 iPSCs 真正大规模走向临床之前还有大量基础工作需要进行. 猪 iPSCs 近几年来颇受关注, 因为猪是重要的人类器官再生宿主, 而且尚无猪 ESCs 建系成功. 虽然通过改善体外培养条件可以将猪 iPSCs 调整为小鼠样形态[73], 但是能有效嵌合的猪 iPSCs 依然没有建系成功.

转入外源基因是限制 iPSCs 走向临床的壁垒之一. 为了避免外源基因对基因组的破坏, 多个研究组采用 4 因子的蛋白进行 iPS 诱导, 取得了可喜的成果[74~77]. 同时, 邓宏魁研究组[78,79]采用小分子化合物诱导 iPSCs, 也完全避免了外源基因的插入, 大大降低了 iPS 诱导造成基因突变的风险. 不经过基因修饰的 iPS 细胞的获得是 iPS 走向临床应用的关键之一.

提高 iPSCs 诱导效率是推进 iPSCs 走向临床的另一关键. 添加表观修饰相关小分子以提高重编程效率是最常规的思路, 如组蛋白去乙酰化酶抑制剂丙戊酸(valproic acid, VPA)[80], DNA 甲基转移酶抑制剂[80,81], 组蛋白甲基转移酶抑制剂[82], 组蛋白去乙酰化酶抑制剂[83], 精氨酸甲基转移酶抑制剂[84], 以及维生素 C[85]等均被报道可提高 iPSCs 重编程效率. 另外一条思路来自核移植重编程, 即母源因子促进重编程, 如组蛋白变体 TH2A, TH2B[86]可有效促进 iPSCs 重编程. 此外, 有报道称内源逆转录病毒 HERVH 的活性与人 iPSCs 的多能性密切相关[87]. 值得注意的是, 母源非甲基化的 Dlk1-Dio3 印迹区域在 iPSCs 诱导重编程过程中, 会因为 c-Myc[88]和(或)Gtl2[89]的表达异常而被异常甲基化沉默, 这是大部分 iPSCs 不能通过四倍体补偿黄金标准的根本原因之一[90,91]. 随着研究的深入, RNA 修饰对重编程的影响也开始慢慢被了解, 例如, 提高 N(6)-甲基腺苷(N(6)-methyl-adenosine, m^6A)的丰度可促进小鼠成纤维的诱导重编程效率, 且受到多种 miRNA 的调控[92], 而降低其丰度则会降低诱导重编程效率[93].

2.3 配子来源的单倍体胚胎干细胞

胚胎来源的单倍体胚胎干细胞技术是核移植技术的延伸技术之一, 已经成为哺乳动物遗传学研究及再生医学的新兴技术[94]. 早在 20 世纪 70 年代就已经有获得小鼠单倍体胚胎的尝试[95], 但直到 2011 年, 在流式细胞分选技术的辅助下, 小鼠孤雌单倍体胚胎干细胞系(parthenogenetic haploid ESCs, phESCs)才被真正建立起来[96,97]. 2012 年, 小鼠孤雄单倍体胚胎干细胞系(androgenetic haploid ESCs, ahESCs)也被建立了, 并被证明可以替代精子获得可育后代[98,99]. 紧接着猴 phESCs[100]、大鼠 ahESCs、大鼠 phESCs[101]也相继被建立了.

单倍体 ESCs 不仅是遗传筛选、药物筛选的良好模型[94], 也是再生医学领域的重要资源, 原因主要有: (ⅰ) 小鼠、大鼠的单倍体 ESCs 与二倍体 ESCs 具有相似的多能性状态, 可以嵌合进入所有组织器官, 包括生殖系统[102]; (ⅱ) 单倍体 ESCs 可替代配子, ahESCs 及 phESCs 均可借助显微操作技术获得可育后代[98,101,103]; (ⅲ) 由小鼠 ahESCs 和 phESCs 融合得到的 ESCs 可通过四倍体补偿技术获得可育动物[104]; (ⅳ) 单倍体 ESCs 可分化为单倍体上胚层干细胞[23]

及单倍体体细胞[105], 通过转录因子过表达或小分子处理很可能体外诱导分化为可育配子; (v) 单倍体 ESCs 更容易实现双等位基因突变修复.

将小鼠 ahESCs 与大鼠 phESCs 融合, 或将小鼠 phESCs 与大鼠 ahESCs 融合, 可获得小鼠-大鼠杂合二倍体胚胎干细胞系(mouse-rat allodiploid ESCs, AdESCs), AdESCs 基因型稳定, 并具有与小鼠 ESCs 相似的多能性状态, 可嵌合入所有组织器官, 并可形成早期生殖细胞[106]. AdESCs 不仅是研究基因调控进化的良好模型, 而且对于研究物种间多能性差异的分子基础非常有价值. 小鼠-猴 AdESCs 甚至小鼠-人 AdESCs 的建系及多能性研究, 将会推动人 ESCs 多能性机制研究的进展.

3 动物模型: 疾病/治疗模型和器官缺陷模型

动物疾病/治疗模型及含“器官巢”的器官缺陷模型的构建是再生医学领域的另一重要方向. 在过去很长的一段时期, 基因编辑主要依赖正向遗传学筛选, 这从本质上讲就是随机的、低效的[107,108]. 随着人工核酸酶技术的发展, 锌指核酸酶(zinc finger nucleases, ZFN)、转录激活因子样效应物核酸酶(transcription activator like effector nucleases, TALEN)、CRISPR(clustered regularly interspaced short palindromic repeat)/Cas9(CRISPR-associated)系统相继问世, 实现了核苷酸水平的精确基因组修饰, 极大地推动了动物模型的构建工作. 利用 TALEN 或 CRISPR/Cas9 技术, 许多人类疾病的动物模型在小鼠[109]、大鼠[110]、猪[111,112]、猴[113~115]等动物上被建立起来.

在器官再造方面, 最初的工作可追溯到 1993 年, Chen 等人将正常小鼠 ESCs 注入 $Rag2^{-/-}$的小鼠囊胚($Rag2^{-/-}$情况下缺乏成熟 T 细胞及成熟 B 细胞), 弥补了 $Rag2^{-/-}$小鼠的免疫缺陷[116]. 虽然这仅是细胞层面的替代, 但为器官再造指明了方向. 2010 年, Kobayashi 等人[117]用大鼠 ESCs 囊胚嵌合弥补了 $Pdx1^{-/-}$小鼠的胰腺缺陷, 获得了长有大鼠胰腺的小鼠, 掀起了器官再造的热潮. 2012 年, Usui 等人[118]利用小鼠 mESCs 囊胚嵌合成功弥补了 Sall1 小鼠的肾脏缺陷; 2013 年, Matsunari 等人[119]利用猪正常卵裂球弥补了 Pdx1-Hes1 猪的胰腺缺陷.

3D 生物打印技术的出现为器官再生提供了新的思路[120], 是再生医学领域很具潜力的技术. 3D 生物打印技术离真正走向临床还很远, 需要生物材料的革新以及基础生物学研究的夯实.

4 展望

2012 年诺贝尔生理学或医学奖颁发给了 Shinya Yamanaka 和 John Gurdon, 以表彰他们在体细胞重编程领域的贡献. 体细胞重编程解决了病人特异干细胞的来源问题, 推动了再生医学领域的发展. 重编程机制研究目前依然是基础研究的热点之一, 深入而全面地了解重编程机制将有助于更高多能性状态的人 PSCs 的获得, 也是为更安全、更有效的细胞替代治疗夯实基础.

在未来的几十年里, 再生医学领域将一如既往地致力于建立大动物及人的更高多能性状态的 PSCs; 建立更接近临床应用的动物疾病/治疗模型及含“器官巢”的器官缺陷模型; 开发新的生物材料, 完善体外培养体系, 推动 3D 生物打印技术走向临床.

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